INFLUENCE OF FERTILIZERS, HARVEST MATURITY, POLYETHYLENE BUNCH COVERS AND POSTHARVEST TREATMENT WITH 1-METHYLCYCLOPROPENE ON PHYSICAL, PHYSIOLOGICAL AND BIOCHEMICAL QUALITY OF TISSUE-CULTURED BANANAS (*Musa* spp.)

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(Food Science and Postharvest Technology)

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2012

Influence of fertilizers, harvest maturity, polyethylene bunch covers and postharvest treatment with 1-Methylcyclopropene on physical, physiological and biochemical quality of tissue-cultured bananas (*Musa* spp.)

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A thesis submitted in fulfillment for the degree of Doctor of Philosophy in Food Science and Postharvest Technology in the Jomo Kenyatta University of Agriculture and Technology

2012

DECLARATION

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

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This thesis is dedicated to my husband James Muchui Munyiri and children, Eva Wambui Muchui and Brian Munyiri Muchui. Thanks for being there for me when I needed you most. God bless you always and help you to achieve your dreams.

ACKNOWLEDGEMENT

First and foremost I give thanks to the Almighty God for giving me the opportunity to come this far in my profession. Further, I express my sincere thanks to my supervisors Prof. F.M. Mathooko, Prof. C.K. Njoroge and Prof. E.M. Kahangi for their invaluable advice, guidance and academic counsel throughout this study. Special gratitude goes to Mr P. Karanja, Mr E. Kimani and Mr D. Votha for their invaluable assistance and guidance in laboratory analyses. I acknowledge the Director, Kenya Agricultural Research Institute (KARI) for sponsoring the major part of this study under the Kenya Agricultural Productivity Project (KAPP) and also for granting me a study leave. Special thanks also go to Jomo Kenyatta University of Agriculture and Technology for sponsoring part of this study through a research grant to Prof. F.M. Mathooko, Prof. E.M. Kahangi and Dr. C. A. Onyango. I acknowledge the Tropical Soil Biology and Fertility (TSBF) Institute of CIAT for sponsoring part of this study. The family of Eng. Gitoho is highly acknowledged here for their approval to use their banana orchard in Maragua as the experimental field. I also thank the Centre Director KARI-Thika, Dr. Charles Waturu for providing a conducive environment for my study through assistance in logistics whenever the need arose. Special thanks also go to the staff and students of the Department of Food Science and Technology for participating in the sensory evaluation of bananas. I also thank my mothers Mrs Bellah Ndarai Mahinda and Mrs Trufena Wambui Munyiri for being very supportive in family matters during the period of my study and always. My brothers and sisters are also acknowledged for their support during my study. For those of you whom I could not mention by name

and you assisted in one way or another, you are kindly acknowledged and may God bless you too.

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LIST OF ABBREVIATIONS AND ACRONYMS

1-MCP	1-methylcyclopropene
ACC	Aminocyclopropane -1-carboxylic acid
AEZ	Agro ecological zones
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
ATP	Adenosine triphosphate
CAN	Calcium ammonium nitrate
CIAT	Centro International de Agriculltura Tropical
CRD	Completely randomized design
FID	Flame ionization detector
FW	Fresh weight
GC	Gas chromatograph
HPLC	High performance liquid chromatography
ISAAA	International Service for the Acquisition of Agri-biotech Applications
KARI	Kenya Agricultural Research Institute
SE	Standard error
Micros	Micronutrients
MOP	Muriate of potash
PG	Polygalacturonase
PL	Pectate lyase
PME	Pectin methyl esterase
RCBD	Randomised complete block design

RH	Relative humidity
SAM	S-adenosyl methionine
TSP	Tri super phospshate
UAE	United Arab Emirates
UM3	Upper midland 3
USA	United States of America

ABSTRACT

This study aimed at determining the effect of inorganic fertilizers on yield and postharvest quality characteristics of tissue-cultured bananas in order to establish the limiting nutrients. The study also aimed at establishing the proper maturity indices and the effect of pre-harvest polyethylene bunch covers alone and in combination with postharvest treatment with 1-Methylcyclopropene on physical, physiological and biochemical characteristics of banana fruit at harvest and during ripening.

The experimental site was in Maragua Ridge, Maragua District, Agro-Ecological Zone (AEZ) upper midland zone 3 (UM3). For the experiment on effect of inorganic fertilizers on yield and postharvest quality, nutrients under investigation were, nitrogen at 400kg/ha, phosphorous at 50kg/ha, and potassium at 600kg/ha. Micronutrients were supplied as a combined treatment as follows; magnesium at 60 kg/ha, zinc at 6kg/ha, molybdenum at 0.5 kg/ha and boron at 1kg/ha. The treatments included all above nutrients applied in such a way as to omit one nutrient, where all nutrients were applied and a control where no nutrients were applied. A Randomised Complete Block Design (RCBD) with four replications was used. The other experiments consisted of studies carried out to establish clear harvest indices, the effect of pre-harvest bunch bagging on fruit quality and postharvest response to 1-MCP using a completely randomized design (CRD) with three replications. Fruits were analysed for selected postharvest quality parameters at harvest and during ripening. Data were examined for normality using R software and outliers by scatter plot using MS Excel software. Data were then subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS statistical

programme (SAS, 2001). The means were compared according to Student Newman Keul's (SNK) test and Least Significant difference (LSD) (α = 0.05) to test for significant effects. Correlations among maturity indices were tested using MS Excel software.

Application of inorganic fertilizers significantly ($p \leq 0.05$) affected bunch weight, number of hands, number of fingers, grade, finger weight, finger length, pulp to peel ratio of green fruit, pulp and peel objective firmness, starch content, total soluble solids, vitamin C, pulp crude fibre, lightness (L*) of green peel, L* raw pulp, hue angle of green peel and raw and ripe pulp, pulp Ca, Mg and P content and peel P content. Green life and shelf life were not significantly ($p > 0.05$) affected. Sensory evaluation of the fruits from all treatments showed significant ($p \leq 0.05$) differences for preference, aroma and texture but not for sweetness. Phosphorous and micronutrients were found to limit postharvest quality of tissue-cultured bananas in Maragua Ridge region. Fruits harvested at ¾ mature, light full ¾, full ¾ and full mature stages generally had similar postharvest qualities especially at the eating ripe stage, except for green life where fruits harvested at the fully mature stage had significantly ($p \leq 0.05$) short green life for both banana cultivars (cv.) Grand Nain and Williams. Shelflife was not influenced by the stage of maturity at harvest for both banana cultivars. It may be concluded here that the optimum harvest maturity stage is three quarter, light full ¾, full ¾ corresponding to 22, 24 and 26 weeks from flowering for cv. Grand Nain and 24 to 28 weeks for cv. Williams, as the fruits had acceptable grades and kept well while attaining optimum postharvest quality. Both banana cultivars showed a positive correlation (R) between bunch age and finger

grade, weight, length, pulp/peel ratio, total soluble solids (TSS) and total titratable acidity (TTA). Finger grade correlated very well with such postharvest qualities as TSS, TTA and pulp/peel ratio at R=+0.85, +0.72, +0.98 for cv. Grand Nain and R=+0.75, +0.87, +0.86 for cv. Williams, respectively. The results indicate that the best maturity indices for both banana cultivars may be a combination of bunch age and grade as they correlated very well with postharvest characteristics such as TSS, pulp to peel ratios, TTA, green life and shelflife and are not destructive.

Bunch covers did not influence significantly ($p>0.05$) the finger grade, finger length and bunch weight for banana cv. Williams. However, for cv. Grand Nain only grade was not significantly ($p>0.05$) affected by the bunch covers. Pulp/peel ratio for banana cv. Williams was not significantly affected while that cv. Grand Nain was affected ($p\leq0.05$). Starch content, total soluble solids (TSS), pulp and peel moisture content, weight loss, chlorophyll content, peel and pulp firmness, peel and pulp colour, lightness (L*) and hue angle at harvest and during ripening were not influenced by bunch covers for both cultivars. Total sugar content was similar in all treatments for cv. Williams but differed in cv. Grand Nain. Bunch covers did not influence green life and shelflife of both banana cultivars. Peak ethylene production differed for cv. Grand Nain in all treatments but not for cv. Williams while respiration was influenced slightly by bagging for cv. Grand Nain during ripening but not for cv. Williams. The covered fruits were more visually appealing, cleaner and had minimal bruises compared to the unbagged fruits. However, few fingers from top hands of few bunches grown covered suffered sunburn irrespective of the bunch colour. 1-MCP application delayed and reduced the ethylene peak, respiratory

peak, starch degradation, TSS and TTA accumulation. Decrease in fruit firmness, green colour loss and chlorophyll degradation were also delayed considerably by 1-MCP application. Green life was significantly ($p \leq 0.05$) extended by 1-MCP application. However, the unbagged control fruits ripened faster than the bagged control fruits while bagged fruits treated with 1-MCP started to ripen earlier compared to unbagged fruits treated with 1-MCP. Degreening of the peel and loss of firmness were also disrupted by 1-MCP irrespective of the growing condition. In this study 1-MCP was not effective in extending the green life of bananas.

Key words: Banana, tissue-cultured, inorganic fertilizer, maturity indices, bunch covers, 1-methylcyclopropene, fruit quality.

CHAPTER ONE

1.0. INTRODUCTION

1.1 Origin of bananas and their production in Kenya

Edible bananas (*Musa* spp.) are believed to have originated from Asia and were distributed throughout the world during early migration of Polynesians (Simmonds, 1962; Arvanitoyannis *et al*., 2008; Lorenzen *et al*., 2010). They were probably brought into East Africa by the Portuguese in the fifteenth century (Purseglove, 1975). They are divided into two broad categories depending on their end uses as either a dessert or for cooking (Marriot, 1980; Njuguna *et al*., 2010). The most important group of banana cultivars are the AAA-triploid cultivars originating from *Musa acuminata* and are mainly consumed as desserts (Israeli and Lahav, 1986). Bananas can be cultivated under tropical and subtropical climates (Marriot, 1980; Panis and Thinh, 2001).

Major banana growing areas of the world are geographically situated in the tropics between the equator and latitudes 20˚North and 20˚South (Stover and Simmonds, 1987; Robinson, 1993). However, bananas also grow in the subtropical regions situated between 20˚ and 30˚N and S (Stover and Simmonds, 1987). They are starch staple crops of major importance in the developing world (Lescot, 2000) and are ranked 4th as the world's most valuable food crop after rice, wheat and potatoes (Africa Harvest, 2007). They are an important item in the world trade and in relation to other fresh products, the value of banana exports well outranks those of other fruits, such as apples and oranges as well as vegetables, such as tomatoes and potatoes in the world (Frison and Sharrock, 1999).

Bananas are consumed for their high nutritive and therapeutic values (Stover and Simmonds, 1987). The cooking and dessert bananas are a rich source of energy of about 128 kcal and 116 kcal/ 100g, respectively (Gowen, 1995). They provide carbohydrates and are low in cholesterol and salt and are, therefore, recommended for obese and geriatric patients (Stover and Simmonds, 1987). They are also recommended for persons with peptic ulcer, coeliac disease, and colitis and for treatment of infant diarrhoea (Stover and Simmonds, 1987). Bananas are also rich in potassium, vitamins A, C and B6 (Chandler, 1995; Arvanitoyannis *et al.*, 2008). The banana fruit has a very high content of potassium (K) and a wide potassium/sodium (K/Na) ratio thus imparting a protective effect of K against excessive Na intake in diets (Srinivas *et al.*, 2006). Infact, the low sodium and high potassium contents of the fruit is of significance in dietary terms and bananas are recommended for low-sodium diets (Frison and Sharrock, 1999). With the above qualities, bananas should form a major part of diets for people living with HIV and AIDS, children and the elderly. The year-round fruiting habit of the crop would ensure a constant supply of fruits in the market throughout the year.

In Kenya, banana is an important food and cash crop grown mainly in Central, Eastern, Western, Nyanza and Coast provinces, although it is currently growing in almost all parts of the country (MOA, 2008). In fact, it provides 25% of the total calorie intake of Kenya's consumers (Acharya and Mackey, 2008). Banana is a widely grown crop in areas where rainfall occurs throughout the year and where the prevailing soil types are ferralssols and nitosols (Kwach *et al.*, 2000). In a priority setting exercise for horticultural crops research division of Kenya Agricultural Research Institute (KARI) (Anon, 1996a and b; Anon, 2006), banana was ranked as the most important fruit crop. Indeed, by end of year 2006,

Kenya had 82,518 hectares under bananas which is about 2% of the total arable land, from which 1,058,018 metric tonnes valued at over 9 billion Kenya shillings (US $ 0.15 billion) were produced (MOA, 2006) while in 2007, 1.2 million MT valued at 14 billion were produced (MOA, 2008). Bananas therefore play a key role in the economy and food security of Kenya (MOA, 2006). The continuous availability of harvestable bunches from a banana orchard contributes to the year-round food and income security of banana growers and other players in the banana value chain.

Despite the importance of the crop to Kenya, the yield is still very low (4.5-10 tons/ha) compared to the potential 30-40 tons/ha (Qaim, 1999). The production has been declining over the past two decades with the realised yields falling below potential each year. Farmers attributed the yield decline to several constraints which included high incidence of banana diseases and pests attack since traditional methods of propagating bananas using suckers perpetuate the problem of diseases and pests (Kung'u *et al*., 1995; Genze and Gathumbi, 2004; Nguthi, 2008). Other constraints included poor crop husbandry, low soil fertility, high postharvest losses and poor marketing infrastructure (Acharya and Mackey, 2008). However, public institutions such as KARI (Nguthi, 1999), Jomo Kenyatta University of Agriculture and Technology (JKUAT) (Kahangi, 2003) and some private institutions came up with disease free plantlets using the tissue culture technology which has helped alleviate the disease problem. With the introduction of this technology, banana production has recently increased with a resultant increase in its value (MoA, 2008). Factors such as local tastes, eating habits, market demand and environmental conditions have tended to influence cultivar distribution (Nguthi, 1999).

Previously, banana production in the country was in the backyard mainly produced by women while currently it has become commercial (Wambugu and Kiome, 2001; Technoserve, 2009) and is also now mainly a 'man's' crop (Nguthi, 1999). Recently, farmers have been uprooting coffee and planting banana orchards mainly in Central and Eastern provinces due to low coffee prices and the high returns from banana. Also, farmers in the Rift Valley have been uprooting citrus due to the greening disease and replacing it with banana (Acharya and Mackey, 2008). This has translated into large volumes of the crop being produced at a commercial level (Technoserve, 2009). Establishing a tissue culture banana orchard is expensive compared to establishing one using conventional suckers (Qaim, 1999) and hence the need to produce high quality fruits in order to make profit. Technologies that enhance production and help realize the benefits of tissue culture technology would lead to higher incomes earned by the farmers. However, banana is highly perishable and postharvest losses at farm level and those incurred in transporting and marketing are estimated at about 50% (Chege *et al.*, 1995; MOA, 2006). Therefore, whereas commercial prospects for banana production are improving in Kenya from successful adoption of the tissue culture technology, projections point to a technology backlash unless urgent measures are taken to reduce the high postharvest losses.

1.2 Problem statement

In Kenya, the bulk of bananas are produced by smallholder farmers. They are produced mainly under subsistence farming although recently, commercial banana orchards have been established. There is a need to continually apply production technologies that result in increased banana production. These include the use of tissue culture plantlets and inorganic fertilizers that have been shown to increase

yield (Nguthi, 1999). In Kenya, the yield is still very low (4.5-10 tons/ha) compared to the potential 30-40 tons/ha. There is a general belief among the small-scale farmers and traders that growing bananas using inorganic fertilizers renders them unpalatable as they do not soften on turning yellow. However, the physiological basis of this has not been established. Further, in Kenya, only blanket fertilizer recommendations for bananas are in place (Anon, 2000). This is a combination of both inorganic and organic fertilizers where 2 *debes* (40 litres) of manure mixed with 200g calcium ammonium nitrate (CAN) are applied per stool every year. The banana plant is a heavy feeder that removes mineral elements in large amounts. It is estimated that a crop harvest of 25 tonnes per hectare per year of fresh fruits removes 28 kg of N, 7 kg of P and 78 kg of K from the soil (Purseglove, 1985). However, there is lack of knowledge on how much nutrients the bananas require and the limiting nutrients and their impact on maturation and fruit quality.

Bananas are harvested at the mature green stage (Turner, 1997) thus allowing for handling before ripening. It is, therefore important to establish optimum maturity indices in order to reduce postharvest losses and poor eating quality resulting from harvesting at the improper maturity stages. In Kenya, most banana fruits reach the market with blemishes that are acquired during the growing period from bird drops, dust and leaf scarring and postharvest handling period from mechanical injuries among others (Chege *et al.*, 1995). It is important to improve on the appearance of bananas hence the need to investigate the use of bunch covers. Blue polyethylene perforated bunch covers have been used elsewhere in the tropics and have been shown to improve fruit visual appeal among other postharvest qualities (Robinson,

1996). The covers may improve the appearance by minimizing dust, bird droppings, thus improving the marketability of the fruits while also reducing the water used for washing the dirty fruits thus conserving the environment. Bunch covers have been shown to reduce blemishes caused by leaf scarring on banana fruits which are inflicted during growing and harvesting periods. They also reduce blemishes caused by mechanical damage during transport stages. This is when the bunch is harvested while the cover is still on and hence reduces the level of damage caused by abrasion during harvest and transportation stages. The blemishes caused by leaf scarring and mechanical damage are not washable and hence reduce visual appeal of the fruits.

Banana fruits are highly perishable and they deteriorate fast at ambient conditions of temperature and humidity. In Kenya, the cool chain is lacking and there is the need to use non-sophisticated methods to prolong shelf-life at ambient conditions of temperature and humidity. It is also important to establish a cost-effective technology that will delay ripening, prolong postharvest storage life during retail/export distribution and maintain product quality thus enhance availability of this commodity not only for fresh sales but also for processing. Currently, the available technology is the cold chain but this is very expensive and out of reach of most farmers and traders. In this regard, the use of I-MCP is proposed to improve the postharvest green life of bananas. This chemical is not currently available in the Kenyan market but if its efficacy is tested and confirmed, then it can easily be imported and made available to the farmers.

1.3 Justification.

Bananas are the most popular and choicest commercial fruit produced in the tropics due to their extremely excellent flavour, attractive fragrance, beautiful yellow colour, delicious taste and health-giving properties. In Kenya, banana is the most important horticultural crop grown in terms of acreage and income generation (Nguthi, 1999) and is mainly grown by small scale farmers. It is estimated to cover about 2% of the arable land (MOA, 2006) or 7.4% of the cropped area (Acharya and Mackey, 2008) and thus of the area under fruit cultivation, it occupies 55% (Acharya and Mackey, 2008). The use of tissue culture technology for production of clean banana plantlets has led to large areas planted with tissue-cultured bananas. Production, storage and ripening of bananas continue to be a challenging problem due to low yields and high postharvest losses that need more attention. Indeed, poor postharvest handling has been identified as one of the constraints in the banana industry hence development of postharvest handling techniques and control of ripening processes are crucial for development of a sound banana industry in Kenya.

Most of banana cultivation systems in Kenya are low input sustainable farming methods involving organic fertilization, regular replanting, rotation cropping, hand weed control, mulching and intercropping combinations to increase cash flow. However, with banana commercialization, there is a need to establish nutrient needs especially in relation to achieving low cost/benefit and producing high yield and good quality fruits that can keep well. Establishing a tissue-culture banana orchard is expensive, hence the need to apply production methods such as inorganic fertilizer

application which has been found to increase banana yield and produce high quality fruits (Qaim, 1999).

Bunch covers have been shown to improve the quality of bananas in the tropics and sub-tropics by reducing blemishes caused by bird droppings, dust and even leaf scarring during growth, harvesting and transportation. Research on newly introduced banana cultivars has mainly been on agronomic practices and very limited work has been done on postharvest research (Nguthi, 1999). Establishing the maturity indices of the tissue-cultured bananas would allow for harvesting at the proper time for destined purpose and market and therefore reduce the postharvest losses. Harvesting fruits at the optimum maturity allows for enough green life to market the fruits while producing good quality ripened fruits acceptable by the consumers. Both bunch covers and maturity indices need to be studied to enhance quality of banana fruits.

Handling, transportation, ripening and marketing of banana fruits require sophisticated technology and facilities such as temperature control at 12°C to 13°C due to their highly perishable nature at ambient conditions. However, for developing countries like Kenya, there is need for alternative non-sophisticated technologies for extension of postharvest shelf life at ambient temperature. Such non-expensive technology is postharvest treatment with 1-MCP, a non-toxic gaseous product that has been used as a tool to extend postharvest life, delay softening and improve post-storage quality of different climacteric fruits. No reports on effects of 1-MCP on tissue-cultured banana fruits are available. This product has been approved in the US and its commercial application has been done on edible crops (Sisler and

Blankenship, 1996). The safety, toxicity and environmental profiles of 1-MCP in regard to humans, animals and environment are extremely favourable (Environmental Protection Agency, 2002). The compound is used at low rates, has non-toxic mode of action and is chemically similar to naturally occurring substances (Environmental Protection Agency, 2002). It is a simple and cheap method of extending the postharvest life of horticultural commodities, thus would enable the rural poor to access distant markets without unduly high postharvest losses. This technique is not available to banana farmers and traders in Kenya, since studies on its efficacy have not been carried out under local conditions despite its use being approved in other countries like the US (Sisler and Blankenship, 1996). However, testing it in this study will give sufficient evidence of its efficacy and hence recommend it to for use by the banana farmers and traders. The combined effect of bunch covers and postharvest treatments with 1-MCP on postharvest storage is necessary to study in order to take advantage of synergy between them if established. Reduction of postharvest losses increases food availability to the growing human population, decreases area needed for production and conserves natural resources (Kader, 2003).

1.4 Hypotheses

1.4.1 Inorganic fertilizer affects postharvest fruit quality.

1.4.2 Establishment of maturity indices for tissue-cultured bananas improves fruit quality.

1.4.3 Pre-harvest bagging of banana bunches of tissue-cultured plants improves fruit quality.

1.4.4 Use of 1-MCP in combination with bunch covers results in extension of the postharvest storage life of fruits of tissue-cultured plants than when used alone.

1.5 Research objectives

1.5.1 Main objective

The main objective of this study was to establish the effect of inorganic fertilizers and micronutrients on the postharvest quality, determine maturity indices and establish the postharvest responses of newly introduced tissue-cultured banana fruits to bunch covering alone and in combination with 1-MCP treatment.

1.5.2 Specific objectives

The specific objectives of this study were to determine:

(i) The influence of inorganic fertilizers and micronutrients on tissue-cultured banana physical and biochemical postharvest quality parameters.

(ii) The effect of harvest maturity on physical fruit quality, during growth, at harvest and fruit physiological, biochemical and qualitative changes during ripening.

(iii) The effect of pre-harvest bunch bagging on fruit quality at harvest and fruit physiological, biochemical and qualitative changes during ripening.

(iv) The effect of pre-harvest bunch bagging in combination with postharvest 1-MCP treatment on fruit quality at harvest and during storage.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Banana Nutrition

Crop yield can be improved by addition of fertilizers (Taiz and Zeiger, 2002). Bananas require high amounts of nutrients for growth and fruit production. These are supplied only in part by the soil and supplements are necessary as the banana crop extracts very large amounts of these nutrients from the soil (Lahav, 1995; Robinson, 1996). It is estimated that a crop harvest of 25 tonnes per hectare per year of fresh fruits removes 28 kg N, 7 kg P and 78 kg K from the soil (Purseglove, 1985).

Potassium is a key element in banana nutrition whose deficiency causes reduced total leaf area of the plant (Lahav, 1995). Potassium regulates the transfer of nutrients such as nitrogen, phosphorous, calcium, magnesium, sodium, manganese, copper and zinc to the xylem. Potassium deficiency causes reduced respiration and photosynthesis (Robinson, 1996). Its deficiency also impairs protein synthesis since free amino acids and soluble forms of nitrogen increase in low-potassium plants (Lahav, 1995). Fruit growth has also been found to be decreased by low potassium content in the plant by reducing the translocation of carbohydrates from leaves to fruit and restricting the conversion of the sugars to starch in the leaves resulting in production of ‘thin’ fruits. A potassium supply to the banana plant above that which influences growth and yield, has been observed to cause changes in reducing, non-reducing sugars and total sugars (Lahav, 1995; Robinson, 1996). Increased potassium supply causes an increased sugar/acid ratio due to increased sugars and

reduced acidity. Potassium supply has an effect on yield and fruit quality (Lahav, 1995).

Nitrogen is very important to bananas as growth is positively correlated with yield (Lahav, 1995; Robinson, 1996). It is considered second only to potassium in terms of the amount needed for growth and yield (Lahav, 1995; Robinson, 1996). High but balanced levels of N and K have been shown to considerably improve fruit size, TSS, sugars and ascorbic acid contents of fruits (Suresh and Hasan, 2002). Also, N application was shown to increase bunch weight, fingers per hand and finger weight but reduced TSS (Weeransinghe *et a*l., 2004). The banana plant cannot store nitrogen other than using it for growth and is, therefore, mostly in short supply even on very fertile soil (Lahav, 1995). Nitrogen also affects bunch maturation period and quality (Lahav, 1995). Nitrogen deficiency reduces plant growth while excess produces large plants with dark green leaves which produce small bunches that do not fill properly and have a short green life after harvest (Robinson, 1996). Besides, excess nitrogen increases green coloration, yet decrease soluble solids concentration, titratable acidity and fruit firmness (Lahav, 1995). Vitamin C has been shown to be decreased by increasing amount of nitrogen fertilizers possibly due to a relative dilution in the plant tissues as N generally enhances plant growth (Lee and Kader, 2000).

Phosphorous requirement of the banana is not large as they accumulate the amount they require over an extended period of time (Lahav, 1995; Robinson, 1996). However, a relatively small amount of phosphorous is exported with the fruit

(Lahav, 1995). Phosphorous is also easily distributed from old to young leaves, from leaves to bunch and from mother plant to followers. Low phosphorous supply results in very stunted growth and poor root development. Phosphorous uptake is enhanced by the association of roots with vesicular-arbuscular mycorrhizae (Lahav, 1995; Robinson, 1996). Low Mg supply to banana plants has been shown to reduce root uptake and distribution of P (Lahav, 1995; Robinson, 1996).

Calcium is very immobile in the banana plant and therefore deficiency symptoms appear on the youngest leaves (Lahav, 1995; Robinson, 1996). In Ca-deficient plants, fruit quality is inferior and the peel splits when the fruit is ripe (Robinson, 1996). Calcium uptake by the plant depends not only on the Ca concentration in the soil but also on the concentration of other elements especially K and Mg (Robinson, 1996). Calcium uptake during the course of plant growth is also influenced by cultivar and climate and follows dry matter accumulation (Lahav, 1995). During fruit growth, further uptake depends upon the site (Lahav, 1995).

Magnesium deficiency is the most common in the banana-growing countries after N and K. (Robinson, 1996). Magnesium uptake can be suppressed by high concentrations of Mn and K. Low Mg supply causes a reduced yield which is proportional to reduced growth in other plant parts. Sulfur deficiency occurs in the field which stunts growth and the bunch is small or choked (Lahav, 1995).

Other nutrients needed by the banana plant albeit in small quantities include zinc, copper, boron and molybdenum (Lahav, 1995; Robinson, 1996). To permit

continuous production of high yields and to maintain soil fertility, these nutrients need to be replaced. This is commonly achieved by applying organic matter and/or more efficiently by mineral fertilizers which supply the nutrients in a more concentrated and readily available form (Lahav, 1995). For example, Mg added to banana plants has been shown to increase number of fingers, hands and bunch weight, compared to the control (Mostafa *et al*., 2007). Parameters such as TSS, total sugars and ascorbic acid were also enhanced by addition of Mg to banana plants (Mostafa *et al*., 2007).

Fertilizer requirements and strategies can be determined by having knowledge of chemical composition of a plant (Turner and Barkus, 1983a). The overall requirement of nutrients can also be determined from analysis of the whole plant and estimated plant growth (Lahav, 1995; Memon *et al*., 2005). The grower must know the ability of the soil to meet the plant requirements and whether supplementary fertilizers are needed. This can be achieved through soil analysis and the establishment of field experiments on a range of soil types although the results of such trials have been shown to be dependent upon local conditions of climate, soil and cultivar and, therefore, their reliable extrapolation is limited (Lahav, 1995). In order to make results of field experiments more meaningful, a better method of plant and soil analysis is recommended with the aim of estimating the amount of fertilizer required to optimize the yields (Lahav, 1995).

In Kenya, blanket recommendations for bananas are in place (Anon, 2002). This is a combination of both inorganic and organic fertilizers where 2 *debes* (40 litres) of

manure are applied per stool every year mixed with 200g CAN. However, there is lack of knowledge on how much and what nutrients the bananas require and their impact on maturation and fruit quality. Also there is a complaint by some farmers that growing bananas using inorganic fertilizers cause them to have a hard inner core and do not sweeten on ripening. The physiological and biochemical basis of this has not been established. Further, investigation on effect of inorganic fertilizers on postharvest storage quality and life needs to be established.

2.2 Maturity indices

Maturity at harvest is an important factor affecting quality perception and the rate of change of quality during postharvest handling. It is, therefore, critical for measures of maturity to be obtained (Shewfelt, 2009). An ideal maturity index is one that can be measured non-destructively and is different at distinct levels of maturity and does not change with time of storage (Shewfelt, 2009). However, few such ideal maturity indices exist. Some of the ways of determining maturity indices include estimation of the duration of development, measurement of size, weight, or density. Physical attributes such as colour, firmness and moisture or solids content may also used. Chemical attributes such as starch, sugar or acid content and morphological evaluation can also be used (Shewfelt, 1993; Shewfelt 2009). Predictive modeling can then be permitted by development of such indices as they help separate maturity effects from storage and handling effects (Shewfelt, 1993). Maturity in banana can be defined as the stage of growth that enables maximum yield but allows the fruit to reach the market in a green condition while allowing it to ripen to acceptable eating quality (Turner, 1997). This implies that banana maturity will be a function of fruit

growth rate, bunch emergence to harvest interval, market needs for fruit size and environmental conditions during postharvest period (Turner, 1997). This makes it hard to determine the harvest maturity for bananas. However, for the banana crop, there are no universally recognized objective methods which can be used to determine when bananas should be harvested (Thompson and Burden, 1995; Dadzie and Orchard, 1997). Plate 2.1A and 2.1B show a banana bunch and hand.

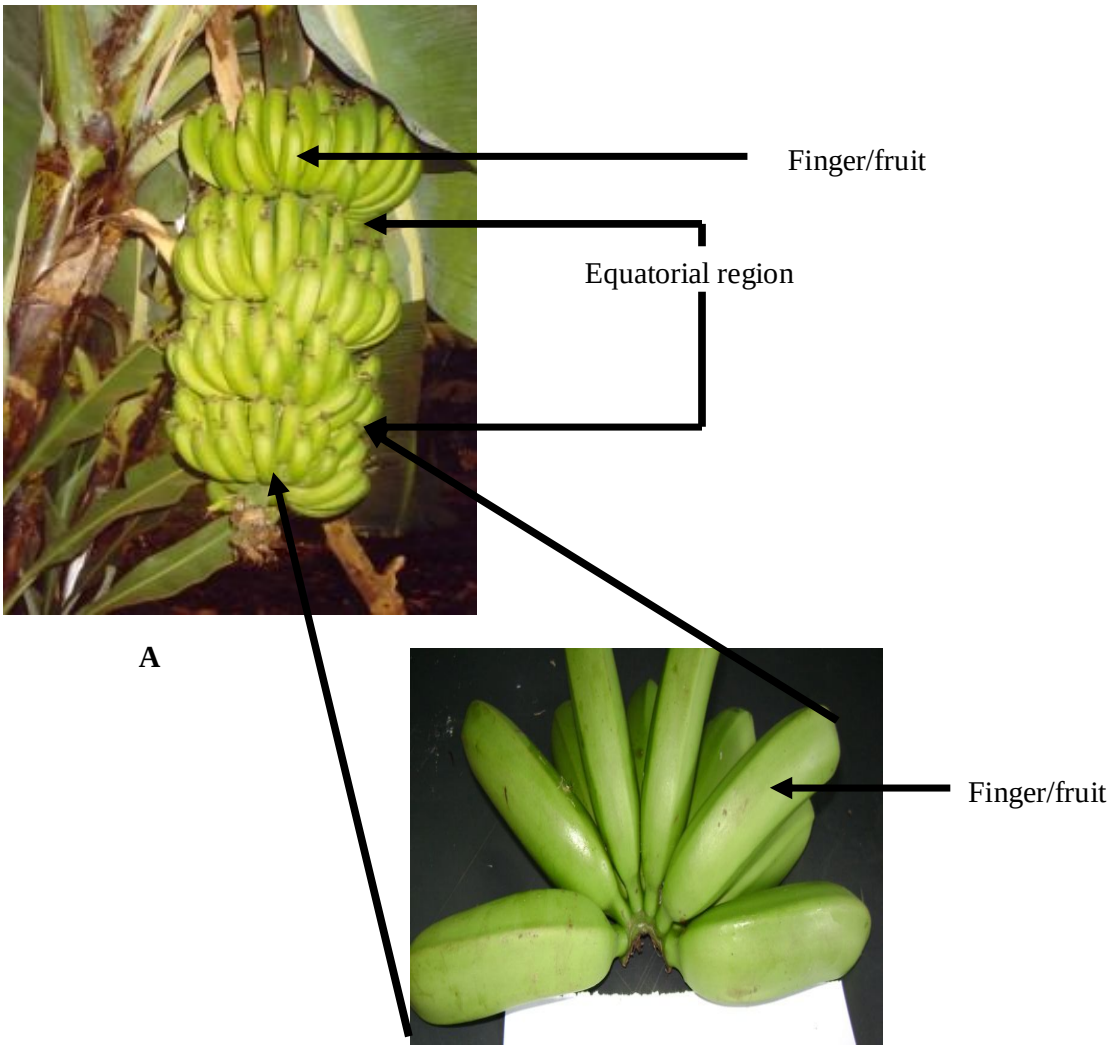

PLATE 2.1: Banana bunch (A) and banana hand (B)

For bananas, an optimum maturity index must consistently ensure a minimum acceptable quality and a long storage life for all producers, locations and years or seasons (Dadzie and Orchard, 1997). Some of the indices that have been used include, bunch emergence to harvest interval, angularity of the fruit fingers in the cross-section, pulp/peel ratio, finger length and diameter, thermal time, green life, fruit firmness and brittleness of the flower end of the bunch (Thompson and Burden, 1995; Turner, 1997). Simple and readily performed maturity measurements either in the field and/or laboratory or inspection point which require relatively inexpensive equipment are desirable. The index should preferably be objective than subjective (Dadzie and Orchard, 1997). However, the use of a single indicator of maturity may be applicable to one cultivar but may not be applicable to other cultivars hence the need to use a combination of several indicators to determine the time of harvest (Dadzie and Orchard, 1997). Maturity indices measure fruit characteristics and postharvest quality attributes which change consistently as the fruit develops so that harvesting the fruit at particular indices allows the final eating quality to be predicted (Dadzie and Orchard, 1997).

2.3 Bunch covers

The use of polyethylene bunch covers mainly in the subtropical and tropical areas has become universal (Robinson, 1996; Amarante *et al*., 2002). The covers are mainly used to improve fruit quality during winter experienced in subtropical climates (Stover and Simmonds 1987; Robinson, 1996). They are also used to reduce physical damage caused by wind-blown dust, leaf and prop chafing in strong winds in both tropical and subtropical climates (Robinson, 1993; 1996). Bunch

covers are also used to protect the bunches from insect attacks and fungicide sprays (Johns, 1996). In fact, preharvest bagging has been used in some fruits including banana (Daniell and Lindsay, 2005), mango (Hoffman *et a*l., 1997, 2000; Joyce *et a*l., 1997), apple (Fan *et al.,* 1999; DeEll *et al*., 2002) and pear (Amarante *et al*., 2002) to improve fruit appearance, reduce level of blemishes and protect them from pests and diseases during the attachment stage. Yield increase for winter growing bananas are attributed to increased bunch mass due to increased fruit size, reduced bunch emergence to harvest interval or both (Robinson, 1995).

The increase in size of fingers and of bunches is attributed to an increase in temperature under covers creating a "greenhouse" effect. An average temperature increase of 0.5ºC has been recorded under blue covers although the increase was much higher when direct sun reached the covers (Stover and Simmonds, 1987; Robinson, 1995; Robinson, 1996). The "greenhouse" effect can be increased by reducing the size of the perforations (Stover and Simmonds, 1987). The increased temperatures are conducive for enhanced metabolic reactions that enhance growth especially during cold winter climates.

Bunches developing over cool winters in South Africa have long development periods of about seven months (Robinson, 1996). Bunch covers have been shown to improve both yield and quality and also shorten maturation period in these areas. For example, a 16.7% increase in bunch mass of cultivar Williams growing over cool winters was recorded, due to a 10% increase in finger length under blue covers, compared with uncovered bunches (Robinson, 1996). The number of first grade

fruits increased by 10-15% under covers due to reduced mechanical damage and fewer undersized fruits on the distal hands while the flower emergence to bunch harvest interval was shortened by three days (Robinson, 1996). Banana bunches of cultivar Williams grown in Australia had their maturation period decreased by 5-11days by bunch covers open at the base, while the period was increased by 16 days by bunch covers sealed at the base (Daniells *et al*., 1992). Bunch weight was not increased by the covers open at the base, while a 9% increase was recorded for bunches grown under sealed covers (Daniells *et al*., 1992). Elsewhere in Australia, maturation period was significantly reduced by blue bunch covers by five days, while finger length was increased by five mm and bunch mass was also increased by 0.8 kg (Robinson, 1996). In Bangalore, India, banana cultivar Robusta had its finger weight and finger diameter significantly increased while time taken for bunch maturity was reduced by application of blue polyethylene bunch covers during growth and development (Reddy, 1989). Finger weight and diameter were increased by 35.1 g and 0.5 cm, respectively while bunch maturation period was decreased by 12.2 days (Reddy, 1989).

In America, the use of pigmented bags has not improved quality or yield of bananas. However, a white opaque bag has been shown to reduce sunburn incidence although these bags obscure Moko disease symptoms on fruit and harvesting time estimation is difficult (Stover and Simmonds, 1987). Elsewhere, polyethylene bunch covers applied on bananas grown under irrigation were shown to reduce maturation period by 12 days while having no influence on bunch weight (Vilela *et al.*, 2001). The

varying results are probably due to locality, cultivar, cultural practices and bunch cover characteristics such as colour, thickness and material among others.

In the tropics, bunch covers are mainly used for protecting the fruit from leaf scarring, dust, light, hail and handling damage during harvest and transport (Robinson, 1996). Perforated polyethylene bunch covers for aeration and cooling and pesticide-impregnated covers for pest control are commonly used in the tropics (Robinson, 1996). For bananas growing in the tropics, translucent blue covers are recommended as they allow heat transmission but reduce sunburn damage compared to other colours (Robinson, 1993; Robinson, 1996). Chemical companies have recently been importing bunch covers into Kenya and providing them to a few farmers for covering bananas. However, no research has been reported on the effect of these covers on the postharvest quality and behaviour of the tissue-cultured banana fruits.

2.4 Use of 1-Methylcyclopropene (1-MCP)

Most of the qualitative changes that take place in climacteric fruits are mediated by ethylene, the ripening hormone. Ethylene is the simplest organic compound with biological activity and plays a critical role in ripening of climacteric fruits (Abeles *et al.*, 1992). Therefore, regulating ethylene concentrations around climacteric fruits is an important commercial tool to achieve ripening at targeted times after harvest and to reduce the variability in ripening within fruit lots. Ethylene responses can be controlled by regulation of its production or action (Mathooko, 1996). The use of inhibitors of ethylene production is, however, limited because of the likelihood of

exposure of agricultural produce to exogenous ethylene (Feng *et al*., 2000). For this reason, inhibitors of ethylene action are considered favourable for use in agriculture since they provide protection against both exogenous and endogenous ethylene. Several inhibitors of ethylene action among them carbon dioxide, silver thiosulphate, diazocyclopentadiene, 2, 5-norbornadiene and 1-methylcyclopropene (1-MCP) have been identified. However, silver thiosulphate and 2, 5-norbornadiene have been tested but some are unstable for commercial agricultural purposes due to either toxicity such as the former, or strong odour or corrosive nature such as the latter (Feng *et al*., 2000). These inhibitors have other drawbacks such as need for continuous presence and need for sophisticated equipment. The one compound that seems to provide a new approach to manipulating ethylene action is 1-MCP, a synthetic cyclic olefin (Sisler and Blankenship, 1996; Sisler and Serek, 1997). 1-MCP is a non-toxic gas that has been approved by US Environmental Protection Agency for use in food crops in the US and many countries in Europe. Investigated at commercial scale, the efficacy of 1-MCP, an inhibitor of ethylene action and a new tool in the postharvest handling of horticultural commodities has been found to be effective. 1-Methylcyclopropene acts by binding permanently and irreversibly to ethylene receptor sites in plant tissue (Sisler and Serek, 1997). This causes fruits to ripen and soften slowly, thereby, facilitating distribution and maintaining their high edible quality conditions for longer periods of time and has commercial significance for distribution centers and supermarkets and even for export market. Eventual recovery of tissue to respond to ethylene is apparently via synthesis of new ethylene receptors (Blankenship and Dole, 2003; Adkins *et a*l., 2005) and unlike other inhibitors of ethylene action, the fruits do not need continuous exposure to 1-MCP.

It has been shown that in apple fruit, the response to 1-MCP is cultivar dependent (Blankenship and Dole, 2003) and such response has, however, not been tested in tissue-cultured banana fruits. Indeed, the use of 1-MCP for harvested fruits and vegetables represents a revolutionary advance in postharvest science and practices.

The discovery of 1-MCP, in the early 1990s (Sisler and Blankenship, 1996) was a major breakthrough in postharvest technology of horticultural commodities. In July 2002, 1-MCP (under trade name SmartFresh™) was approved by the US Environmental Protection Agency for use in various fruits and vegetables and its use has since expanded to Europe. Unfortunately few studies in developing countries have utilized this tool in extending the postharvest storage life of tropical commodities despite its simplicity and promising potential. Since ethylene is the plant growth regulator involved in fruit ripening (Abeles *et al*., 1992; Mathooko, 1996), preventing its action by the use of 1-MCP has the potential to extending the storage life of horticultural commodities. Not only does commercial use of 1-MCP promise to revolutionalize and advance commercial agriculture but also using 1-MCP in research programmes promises to advance our understanding and provide insights into plant ethylene responses. No death or clinical signs of systemic toxicity have been reported in tests for acute toxicity of 1-MCP (Blankenship and Dole, 2003), hence making it safe for use. Indeed, this tool could be the solution to postharvest handling problems in developing countries. The use of technologies that minimize the accumulation of ethylene in the storage environment or inhibit ethylene action would extend the time to ripen of climacteric fruits including bananas. A lot of research has been done using apple fruit as a model and it has been

shown that 1-MCP can enhance apple storability (Blankenship and Dole, 2003; Pre-Aymard *et al.*, 2003). Indeed, 1-MCP has been shown to inhibit the action of ethylene and extend the storage life of other fruits including peach (Mathooko *et al*., 2001), pear (Hiwasa *et al*, 2003; Mwaniki *et al*., 2005), tomato (Wills and Ku, 2002; Mostofi *et al*., 2003), banana (Jiang *et al*., 1999; Harris *et al*., 2000; Pelayo *et al*., 2003) and avocado (Jeong *et al*., 2003; Feng *et al*., 2000; Adkins *et al*., 2005). Therefore, the use of 1-MCP seems to have commercial potential for growers and traders to delay ripening and for retailers and consumers to delay senescence of fruits. It is against this background that the efficacy of this tool was tested in tissue-cultured banana fruits which has not hitherto been tested under tropical conditions. Moreover, although 1-MCP has been shown to delay the ripening of conventional banana, its effect has been shown to be variable and dependent on the fruit maturity (Harris *et al.*, 2000) and no such work has been reported using tissue-cultured bananas grown under bunch covers. Bunch covers have been shown to be beneficial as the fruits have fewer blemishes and are appealing. Combining both bunch covers and 1-MCP may have a high potential of increasing the postharvest storage life of tissue-cultured bananas.

2.5 Banana fruit ripening

Generally fruit ripening refers to the changes that make it ready for eating. Such changes typically include softening due to enzymatic breakdown of the cell walls, starch hydrolysis, sugar accumulation, and the disappearance or synthesis of organic acids and phenolic compounds, including tannins (Taiz and Zeiger, 2002). A harvested banana fruit passes through three physiological stages namely the pre-

climacteric or ‘green life’, the climacteric and the eat-ripe and senescence stage (Robinson, 1996). The green life stage refers to the period from harvest to the initiation of respiratory climacteric. Shelflife can be defined as the time over which the ripe fruit remains acceptable for consumption (Dadzie and Orchard, 1997; Turner, 1997). The commercial objective is to prolong the green life stage period as much as possible through various ways such as good pre-harvest management techniques, harvesting at an early maturity stage, low temperature management, hormonal treatment and modified atmosphere storage and ethylene scrubbing (Robinson, 1996). During the climacteric stage in the banana fruit, there is a rapid and massive increase in ethylene production which precedes the respiratory climacteric (Marriot, 1980; Seymour *et a*l., 1993; Burdon *et al.*, 1994; Robinson, 1996). Ethylene production is regulated through two enzymes namely, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase which generates ACC and ACC oxidase, formally known as the ethylene forming enzymes (EFE) which generates ethylene from ACC (Seymour *et al*., 1993; Burdon *et al.*, 1994; Robinson, 1996; Wills *et al.*, 1998). The enzyme systems become more efficient with maturity and are able to produce sufficient ethylene to initiate rapid respiration and ripening process (Robinson, 1996). The ripening process is accelerated when the respiratory maximum is attained which depends on temperature, humidity and ethylene concentration. During the respiratory climax, there is rapid oxygen uptake and carbon dioxide evolution to a maximum of 250 mg CO_2 $kg^{-1}h^{-1}$ from a pre-climacteric low of about 30 mg CO_2 $kg^{-1}h^{-1}$ (Robinson, 1996).

During the ripening process, there are several changes that take place simultaneously in the banana fruit. The major change in the pulp during ripening is conversion of starch to sugar (Stover and Simmonnds, 1987). Infact, banana peel colour has been found to be closely correlated with starch: sugar ratio (Stover and Simmonnds, 1987; Robinson, 1996). Tissue softening starts mainly due to starch degradation to sugars in both the pulp and the peel which causes the rupture strength of the cell to slowly decrease. Soluble pectic polysaccharides and uronic acid concentration and their related enzyme activity increases. Water-soluble pectins increase and the insoluble pectins decrease on ripening and this result in pulp softening, with the ripe pulp containing 0.5-0.7% pectin.

Most bananas change colour from green to yellow on ripening. This is mainly due to degradation of chlorophyll and unmasking of carotenoids (Seymour *et al.*, 1993; Ferguson and Boyd, 2002). The peel then turns to spotted yellow as senescence sets in (Stover and Simmonds, 1987; Robinson, 1996). During this colour change process, the pulp becomes softer and sweet as the sugars are formed from starch and a characteristic banana aroma is produced making the fruit palatable. There are various enzymes involved in these changes whose action is well coordinated. During ripening, organic acids change with malic and citric acids increasing while oxalic acid decreases while there are other acids present in trace amounts. Oxo acids play a role in chilling injury (Stover and Simmonds, 1987). Aromatic compounds such as esters, acetates, alcohols and carbonyl compounds are produced during ripening. These are responsible for the characteristic aroma of a ripening banana. (Stover and Simmonds, 1987; Robinson, 1996). Phenolics cause astringency in unripe bananas

and also are responsible for some browning reactions of which latex staining is commercially important (Seymour *et al*., 1993; Robinson, 1996). These compounds are localized in the latex vessels of the peel and pulp (Robinson, 1996). Loss of astringency during ripening is mainly due to polymerization of the phenolics. Dopamine which forms about 80% of the water soluble phenolics referred to as tannins decreases during ripening. Browning is linked to enzymatic oxidation of phenolics with dopamine being the main one and polyphenoloxidase is the enzyme (Robinson, 1996). The browning is accelerated by temperatures below 13ºC which results in underpeel discolouration due to chilling injury which is of commercial importance as it reduces the visual appeal of the fruits (Marriot, 1980; Robinson, 1996) although it does not affect the flavour unless it is severe (Muchui, 2000; Klieber and Muchui, 2002).

Water is the most abundant constituent in the pulp and peel and it increases in the pulp but decreases in the peel during ripening (Marriot, 1980; Robinson, 1996). The increase in the pulp is due to respiratory breakdown of pulp and osmotic movement of water from peel to pulp while the loss from the peel is also due to transpiration until the ripening processes degrade the peel tissue preventing further water loss (Burdon *et al*., 1994; Robinson, 1996). Water occupies about 75% of pulp mass in a ripe banana fruit. Ripe banana contains large amounts of K and lesser amounts of Ca, P, Mg and S while it is also a good source of vitamin C, A, B and B_6 compared to other fruits (Robinson, 1996). Loss of water from the banana peel during ripening explains why it has high contents of K, Ca and Mg. A proportion of these elements

migrate with the water towards the pulp making the contents of K and Ca to rise considerably (John and Marchal, 1995).

Protein content though low in bananas increases during ripening to about 1.1% of its total composition and contains some important amino acids such as lysine. Histidine, asparagines and glutamine are predominant amino acids at the full ripe stage of the banana fruit (Robinson, 1996). Lipids content is fairly low in banana fruits comprising about 1% of the fresh mass of peel and between 0.2 and 0.5% of the pulp. The content remains fairly constant during ripening and linoleic acid forms a major part of the lipids (Seymour *et al.*, 1993; Robinson, 1996). After the climacteric stage, the senescence stage sets in where the peel becomes spotted brown and then completely brown and the pulp loses its firm white texture to become brown and gelatinous (Marriot, 1980; Seymour *et al.*, 1993; Robinson, 1996). Banana has low fibre content of about 0.8% in the pulp and a bit more in the peel with the non-cellulosic polysaccharides being more abundant. (Stover and Simmonds, 1987; Robinson, 1996). Commercially, banana fruits are ripened artificially by use of exogenous ethylene in order to produce a firm pulp texture, good flavour and bright yellow peel colour. This is done under controlled temperatures and humidity of about 16º-18ºC and 95%, respectively. Varying the temperature is used to control time taken for the fruits to ripen. However, if the fruits are ripened at very high temperatures (>25ºC), the pulp becomes soft while the peel colour is still greenish yellow, a condition referred to as 'cooked-ripe'. Banana fruits ripened at temperatures below 13ºC have a greyish yellow peel colour due to discolouration of latex vessels (underpeel discolouration) due to chilling injury.

Uneven pulp ripening has been found to be caused by low temperatures and insufficient ethylene (Robinson, 1996).

2.6 Sensory quality and evaluation

Sensory quality of fresh fruits includes such attributes as appearance, colour, shape, size, hand-evaluated firmness, flavour (taste and aroma) and oral texture. The external sensory attributes of fresh fruits such as appearance, color, shape, size and hand-evaluated firmness are intrinsic quality cues that the consumers evaluate before consumption (Wismer, 2009). Other sensory attributes such as flavour and oral texture are experience-quality attributes that are evaluated during consumption (Wismer, 2009). Sensory quality is evaluated using human senses as these cannot be reproduced exactly by laboratory instruments (O'Mohany, 1988). Sensory evaluation involves the measurement, quantification and interpretation of sensory characteristics of foods and consumer products through the use of human subjects acting as judges (Heintz and Kader, 1983). It is used as a tool to study chemical and physical characteristics of food products, and is carried out using various sensory methods depending on the objectives of the evaluation. Methods used include, affective, difference, attribute difference and descriptive tests (Lawless and Heyman, 1998; Meilgaard *et al*., 1999). Panelists are selected and may be trained or not trained depending on the objectives of the sensory evaluation. Panelists are also given instructions on how to conduct the evaluation verbally and in questionnaires (Meilgaard *et al*., 1999). Sensory evaluation is used for general screening analysis to be supplemented by further instrumental analysis, or where it is inconvenient or sufficiently insensitive (O'Mohany, 1988). The major role of sensory evaluation is

therefore to provide valid and reliable information to allow the sensory analyst to make sound decisions about the perceived sensory properties of the product (Meilgaard, 1999). For horticultural crops, testing variables may include, for example, a comparison of cultivars, production areas, cultural practices, harvesting procedures, maturity stages at harvest or postharvest handling procedures (Hentz and Kader, 1983).

CHAPTER THREE

3.0 INFLUENCE OF INORGANIC FERTILIZERS AND MICRONUTRIENTS ON YIELD AND POSTHARVEST QUALITY PARAMETERS OF THE FRUITS OF TISSUE-CULTURED BANANA (*Musa* spp.)

3.1 Introduction

Increasing banana yield and profit is the main target of commercial banana producers (Mostafa *et al*., 2007). Good banana orchard management, especially fertilization is paramount in achieving this goal (Kumar and Kumar, 2007). The wide gap between production potential and realized productivity may be reduced by efficient crop management techniques among them, balanced banana nutrition. Fertilizers provide useful nutrients to banana plants and these plants have been shown to require large amounts of nutrients for proper growth and production, with a nutritional requirement of 320 kg N, 32 kg P_2O_5 and 925 kg K_2O/ha per year (Kumar and Kumar, 2007). Banana fruits are a rich source of minerals and carbohydrates. The nutrient content of the fruits is mainly determined by the mineral content of the growing medium among other factors (Suresh and Hasan, 2002). Fertilizers applied affect both the physical and biochemical quality of banana fruits. Physical and biochemical parameters of fingers were positively affected by fertilizers containing N, P and K in combination (Suresh and Hasan, 2002; Sanjui *et al.*, 2003; Hongwei *et al.*, 2004). Other elements required by banana plants include Ca, S, Mn, Zn, Fe, Cu, B, Mo, Na and Cl albeit in smaller quantities (Lahav, 1995).

Potassium is a major plant nutrient, taken up in large amounts by plants. It is involved in enzyme activation in cells, charge balance and osmoregulation (Wakeel,

et al., 2009). It has been observed that about 90% of K taken up by plants is accumulated in vacuoles for osmotic functions and only a small fraction is involved in other cytoplasmic functions (Subbarao *et al.*, 2000; Wakeel *et al*., 2009). Potassium applied as a foliar spray prior to the shooting stage occurring 7 months after planting, was found to enhance preharvest quality parameters such as hand and finger number, bunch weight, finger length, girth and weight (Kumar and Kumar, 2007). Other postharvest parameters such as green life, shelflife, TSS, reducing, non-reducing sugars, total sugars and acidity were also enhanced by foliar application of K compared to the control (Kumar and Kumar, 2007). The increased green life and shelflife through nutrient use is very important because most bananas are sold to far-away markets.

Nitrogen promotes growth and is second only to K in terms of amount needed for growth and production (Lahav, 1995). Adequate N has been shown to increase fruit growth and hence hasten maturation rate in bananas (Srikul and Turner, 1995). Excess N delays maturation by encouraging more vegetative growth thus delaying synthesis of carotenoid pigments in the banana peel which influences the colour of bananas (Sanjui *et al*., 2003). Excess N has also been shown to reduce green life in bananas (Srikul and Turner, 1995). Nitrogen has a direct effect on fruit size and weight due to its association with nucleic acids as it has a vital role in the control of cell elongation and replication. Lee and Kader (2000) reported that excess N reduces levels of vitamin C in many plants including, cauliflower, oranges, grapefruits and mandarins while high levels of K increase the vitamin C content. Nitrogen

deficiency has been shown to decrease the number and size of banana fruits (Sanjui *et al*., 2003).

Phosphorous is one of the most unavailable and inaccessible macronutrients by the plants and it plays a key role in an array of plant processes (Yaryura *et al*., 2009). It is a major essential element and deficiency of this element primarily reduces CO_2 assimilation in leaf photosynthesis (Fujita *et al*., 2003) since it is a primary substrate of photosynthesis and has structural functions in membranes (Yaryura *et al*., 2009). Phosphorous deficiency was found to affect fruit expansion by lowering the water potential of the plant (Fujita *et al*., 2003). Magnesium is important for banana nutrition. Large amounts of K and N depress Mg uptake by banana plants (Mostafa *et al*., 2007). Magnesium is essential for chlorophyll molecule structure, and it is a co-factor with most enzymes related to active phosphorylation process. It also acts as a bridge between pyrophosphate structures of ATP or ADP, the enzyme molecule and stabilizes the ribosome particles in the configuration for protein synthesis (Mostafa *et al*., 2007. Magnesium has been found to impact positively on productivity and fruit quality of banana plants. Indeed, Mg added to banana plants was found to increase number of fingers, hands and bunch weight, compared to the control (Mostafa *et al*., 2007). Other parameters such as TSS, total sugars and ascorbic acid were enhanced by addition of Mg to banana plants (Mostafa *et al*., 2007). Foliar application of Mg, B and Zn were shown to improve days to shooting, number of fingers per hand, number of hands per bunch, number of fingers per bunch, hand weight, bunch length and bunch weight (Mandal *et al*., 2002).

Low soil fertility has been shown to adversely affect banana production in Kenya (Nguthi, 1999; MoA, 2006). Further, Kenyan farmers have complained that the use of inorganic fertilizers on bananas reduces the quality of the fruits. They complained that tissue-cultured bananas grown using inorganic fertilizers develop a hard core and do not soften and become sweet on ripening which renders them unpalatable. The physiological and biochemical basis of this had not been investigated. It was, therefore, important to study the effect of inorganic fertilizers and micronutrients on tissue-cultured bananas with a view of establishing the nutrients that influence the fruit quality and storage favourably and also establish the limiting nutrients. In this study, the pre-harvest and postharvest quality of tissue-cultured banana fruits grown under different nutrient regimes was established. The objective was to determine the influence of inorganic fertilizers and micronutrients on physical and biochemical fruit quality and storage of tissue-cultured bananas.

3.2. Materials and methods

3.2.1 Study area and plant materials

Experiments were undertaken at an established banana farm in 2006 in Maragua ridge, Maragua District, Agro-Ecological Zone, upper midland 3 (AEZ UM3) (Jaetzold and Schmidt, 1983). The farm is located at latitude 00º 49’ 56”S and longitude 037º 09’ 52” E as marked by a Global Positioning Satellite (GPS) instrument (Magellan,Triton, China) and the bananas had been grown using the recommended agronomic practices (Anon, 2002). The prevailing mean temperatures and rainfall climatic conditions at the time were 20.2ºC and 102.1 mm, respectively. Tissue-cultured banana (*Musa* spp., AAA group, Cavendish subgroup, cultivar

'Giant Cavendish') was grown in this experiment as the test variety. The plantlets had been obtained from Jomo Kenyatta University of Agriculture and Technology (JKUAT). Soil analyses were carried out before establishing the banana crop at the study site.

3.2.2 Experimental design

Three banana bunches were randomly selected and tagged with ribbons in an already existing nutrient omission trial in Maragua region. The nutrient omission trial is a long term trial that is addressing the nutrients limiting banana production in Maragua Region. The experimental design was randomized complete block design (RCBD) with four replications. Fertilizer rates under investigation were nitrogen (N) in form of urea at 400kg/ha, phosphorous (P) in the form of tri superphosphate (TSP) at 50kg/ha and potassium (K) in the form of Muriate of potash (MOP) at 600kg/ha. Micronutrients were applied as one combination in the following rates; 60 kg/ha, 6kg/ha, 0.5 kg/ha and 1kg/ha for magnesium (Mg), zinc (Zn), molybdenum (Mo) and boron (B), respectively. The treatments were combined such that a particular nutrient was omitted in each treatment and one treatment had all the nutrients applied. A control treatment was with no nutrients applied. The treatments were denoted as follows:

1. All nutrients=400kg/ha N, 50kg/ha P, 600kg/ha K, and 60 kg/ha Mg, 6kg/ha Zn, 0.5 kg/ha Mo and 1kg/ha Bo
2. Omitting K=400kg/ha N, 50kg/ha P, and 60 kg/ha Mg, 6kg/ha Zn, 0.5 kg/ha Mo and 1kg/ha Bo

3. Omitting N=50kg/ha P, 600kg/ha K, and 60 kg/ha Mg, 6kg/ha Zn, 0.5 kg/ha Mo and 1kg/ha Bo

4. Omitting P=400kg/ha N, 600kg/ha K, and 60 kg/ha Mg, 6kg/ha Zn, 0.5 kg/ha Mo and 1kg/ha Bo

5. Omitting micronutrients=400kg/ha N, 50kg/ha P, 600kg/ha K

6. Control=No nutrients applied.

The nutrients were applied in three splits, once at planting and twice during the growing season for the plant crop. Bunches were harvested at angularity of full three quarters (¾) as determined using the banana maturity stages chart (Plate 3.1). The angularity looks at banana fruit size and edges since as the fruit matures, it enlarges and the edges become rounded.

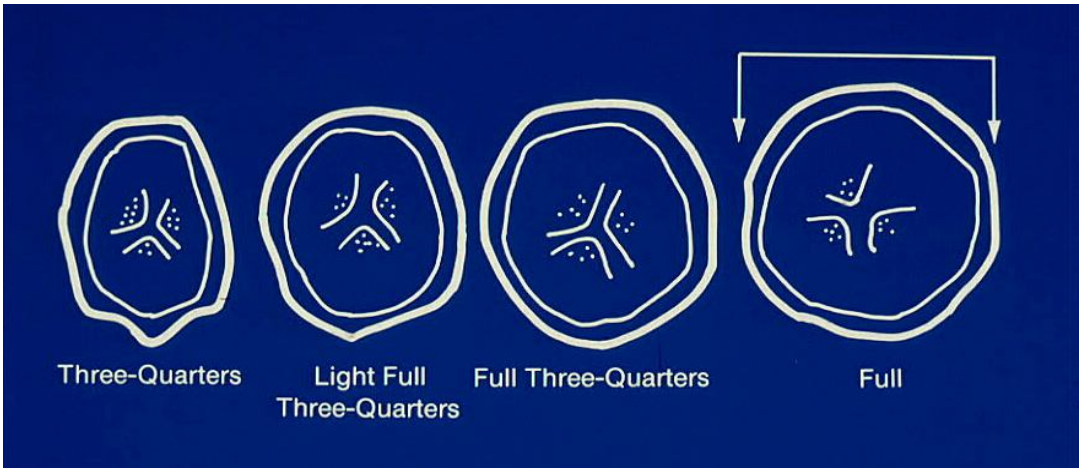

PLATE 3.1: Banana maturity stages chart

Source: Postharvest Horticulture Series No. 23-CD, Second Edition; March, 2010.POSTHARVEST TECHNOLOGY Research and Information Center, UC Davis http://postharvest.ucdavis.edu

Data were collected on: bunch weight, fruit weight, fruit diameter, fruit curvature, finger weights and numbers, hand weights and numbers and finger grade. The harvested bunches were dehanded and then packed in plastic crates and transported to the JKUAT laboratory on the same day. During transportation all precautions were taken to avoid damage to the fruit. Equatorial region hands of the bunches were selected for ripening and storage experiments in order to maintain uniformity of the samples. The position of the hand has an effect on the size of the fruit (Stover and Simmonds, 1987). The fruits were then washed with tap water to remove latex and dirt and were then subjected to fungicidal treatments by dipping for 1 min in 100 ppm sodium hypochlorite (Jik, Reckitt Benckiser-East Africa Limited, Kenya) in order to control spoilage during postharvest storage due to common fungal diseases such as anthracnose (*Colletotrichum musae*) and crown rot. The fruits were allowed to air-dry before being placed in the ripening chamber.

Green fruits were analysed for moisture content, flesh firmness, starch index, pulp to peel ratio, chlorophyll content of the peel and green life. A preliminary study to investigate optimal ripening conditions of temperature, ethylene levels and relative humidity for the fruits had been carried out earlier (Muchui, unpublished). The study found that fruits ripened optimally at 18-25˚C and 75-85% relative humidity with passion fruits for ethylene production at 2kg passion fruit for every four banana bunches. Fruits in the current study were then ripened in a ripening chamber at 18-25˚C and using passion fruit as the ethylene source (2 kg passion fruit/ 4 bunches) at a relative humidity of 75-85% and then left until fully ripe, stage 6 (Appendix I) (CSIRO, 1972; Marin *et al.*, 1996; Paull, 1996; Jiang *et al.*, 1999). Ripe fruits at

stage 6 were analysed for the following quality parameters: total soluble solids (TSS), total titratable acidity (TTA), vitamin C content, crude fibre, mineral content, pulp to peel ratio and flesh firmness. Shelflife and green life were also evaluated.

3.2.3 Analyses and Determinations

3.2.3.1 Fruit weight, diameter (grade) and length

Three bunches selected randomly and tagged in the banana orchard in Maragua Ridge were harvested and transported carefully on the same day to the postharvest laboratory in Jomo Kenyatta University of Agriculture and Technology (JKUAT). They were dehanded and the hands weighed to give the bunch weights. Also three fruits from the second hand in the three bunches per plot were weighed with an electronic scientific balance (Model Libro AEG-220, Shimadzu Corp. Kyoto, Japan) to give finger weights. Finger grade was measured with a caliper (Model CD-20C, Mitutoyo, Japan) as diameter of the middle finger of the outer whorl of the second hand (Stover and Simmonds, 1987). Finger length was measured using a standard tape measure on the outer curve of the finger.

3.2.3.2 Pulp/peel ratio

Pulp: peel ratio was calculated after measuring the pulp and the peel weights with a scientific balance (Type 1240, Shimadzu, Japan) for both green and ripe fruits. Three fingers selected randomly from the equatorial region hands per bunch were peeled and the pulp and peel weighed separately.

3.2.3.3 Starch content

This was done by cutting the three randomly selected fully green banana fruits across at the equatorial region, dipping in I/KI (2g/10g) solution and waiting for at least one minute for starch patterns to develop and rating using the Cornell Starch Chart (Watkins, 1981) for comparison (Appendix II). This chart has been used to assess maturity of apples and pears and was adopted for bananas in the current study. This chart has a scale of 3-8 with 3, all starch and 8, no starch.

3.2.3.4 Chlorophyll content

This was determined using the method of Arnon (1949). Four grams of the green tissue of the peel of 3 randomly selected fingers per treatment per block from the equatorial region hands was weighed. The peel sample was taken from the middle portion of the fruit and ground in 16 ml of cold 100% acetone in a mortar with a pestle in the presence of some acid washed sand. The homogenate was filtered and the residue rewashed with 80% acetone until the filtrate was colourless. The volume was brought to 40 ml with 80% cold acetone. An aliquot of the crude extract was taken and absorbances measured at 645 and 663 nm with a uv-visible spectrophotometer (Model UV mini 1240, Shimadzu Corp. Kyoto, Japan). Total chlorophyll in the crude extract was calculated using MacKinney's coefficients (MacKinney, 1941) after measuring absorbances at 645 and 663 nm and calculated as follows:

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

3.2.3.5 Vitamin C content

This was determined by visual titration using 2, 6-dichlorophenol indophenol according to AOAC methods (1996). Three ripe fruits sampled randomly were used for analysis. Five grams of ripe fruit pulp was weighed and ground with mortar and pestle with acid washed sand and some 10% trichloroacetic acid (TCA) solution. The ground sample was transferred quantitatively into a 100ml volumetric flask. Rinsing was done with the TCA solution, which was also used to make the mark to 100 ml. The homogenate was mixed well and filtered. Ten milliliters of the solution was put into conical flasks and titrated with indophenol solution until pink colour appeared. For blank, 10 ml of TCA solution was pipetted and distilled water equivalent to the indophenol solution used in the previous step added. Titration with indophenol solution was carried out until pink colour appeared. The following equation was used for calculating vitamin C content

Vitamin C content (mg/100g) = (A-B) xCx100/10x1/Sx100

Where:

A= Volume in ml of the indophenol solution used for sample.

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

C= Mass in mg of ascorbic acid equivalent to 1.0 ml of standard indophenol solution

S = Weight of sample taken (g).

100/10= Total extraction volume/volume of titrated sample.

3.2.3.6 Fruit firmness

This was determined at ripeness stages 1 and 6. Both subjective and objective methods were used. Hand firmness assessment was carried out using the scale 1 =

hard, 2 = firm, 3 = slightly soft, 4 = moderately soft, 5 = soft and 6 = very soft (Joyce *et al*., 1993; Jiang *et al*., 1999) for fully yellow ripe fruits. Objective fruit firmness measurement was determined along three equatorial regions of the fruit: at the base, middle and apical sections of the fruit using a rheometer (Fudoh, Model NRM-2010J-CW, Japan) with a 3 mm sharp probe for the green fruit and 6 mm round probe for the ripe fruit**.** The average of these three measurements was considered as one replicate. Firmness was expressed as Newton (N) (Joyce *et al*., 1993; Jiang *et al.*, 1999).

3.2.3.7 Total soluble solids content

Total soluble solids (TSS) content was measured after ripening to colour stage 6. The TSS content was measured as ºBrix using Atago hand refractometer (Type 500, Atago, Tokyo, Japan). Three randomly selected fingers from the equatorial region hands were used for TSS determination.

3.2.3.8 Total titratable acidity

Total titratable acidity (TTA) was determined by titration with 0.1N NaOH in the presence of phenolphthalein indicator. It was expressed as percent malic acid. Ripe pulp (10 g) of fruit at stage 6 from three fingers from the equatorial region hands was blended separately with distilled water (50 ml). 0.3 ml of phenolphthalein indicator was added to 10 ml of the solution and titrated with 0.1 N sodium hydroxide to a permanent pink colour. Total titratable acidity was then calculated using the following formula:

$$\text{TTA (\%)} = \frac{\text{Vol. of 0.1\% NaOH used X Conversion factor}}{\text{Volume of sample used}} \text{ X } 100$$

The conversion factor used was 0.067 for malic acid.

3.2.3.9 Moisture content

The pulp and the peel were analysed for moisture content at green stage 1 using the oven drying method as described by AOAC (1996).

3.2.3.10 Crude fibre content

The pulp and the peel were analysed for crude fibre content at ripeness stage 6 as described by AOAC (1996).

3.2.3.11 Finger curvature

Three fingers from the outer whorl of the second hand in three bunches per plot were measured for outer and inner length using a tape measure. The curvature was calculated as the ratio between the outer and inner length.

3.2.3.12 Green life

Fifteen fingers from the equatorial region hands were placed on a bench at ambient conditions of temperature and humidity of about 24±1ºC and 60±5%, respectively. Five fingers served as a replicate. Green life was determined as the number of days taken by half of the fruits to progress from green stage to show a yellow tinge (Peacock and Blake, 1970; Dadzie and Orchard, 1997).

3.2.3.13 Shelflife

Fifteen fingers from the equatorial region hands were placed on a bench at ambient conditions of temperature (24±1˚C) and humidity (60±5%). Five fingers served as a replicate. Shelflife was then determined as the number of days taken by the fruit to progress from ripeness stage 6 to 8 (CSIRO, 1972, Marin *et al*., 1996; Paull, 1996; Turner, 1997; Jiang *et al*., 1999).

3.2.3.14 Sensory analysis

The bananas were evaluated for their taste, aroma, sweetness and texture at ripeness stage 6 using a hedonic scale (Larmond, 1977; Meilgaard *et al*., 1999) by untrained panelists from the Department of Food Science and Technology (JKUAT) to determine consumer preference. The scores used for taste were: 9= like extremely and 1= dislike extremely, aroma: 1= no banana aroma and 5= extremely good banana aroma, sweetness: 1= not sweet and 6= extremely sweet and texture: 1= very smooth and 6= very rough. Samples were peeled freshly and cut into pieces of about 10 cm and placed randomly on white plastic plates. The form used is attached (Appendix III)

3.2.3.15 Sucrose, fructose and glucose contents

Sugars were analysed using the AOAC method (1996). Ten grams of the ripe fruit pulp was refluxed in ethanol for one hour. The sample was then concentrated with rotary evaporator and diluted with 75% acetonitrile. The individual sugars were analyzed using a high performance liquid chromatograph (HPLC) (Model LC-10AS, Shimadzu Corp., Kyoto, Japan) using a refractive index (RI) detector. Conditions included: oven temperature, 35^{o}C, recorder speed: 3, attenuation: 2, range: 4 and flow rate: 0.5ml/min.

3.2.3.16 Mineral contents

Five grams of the pulp and peel sample of fully ripe fruits was weighed in a crucible and was then incinerated in a muffle furnace to ash. The residue was then mixed with 1N HCl. The solution was filtered and topped up to 100ml in a volumetric flask. Mineral analysis was done by atomic absorption spectrophotometry (AOAC,

1996) method using an atomic absorption spectrophotometer (Model AA-6200, Shimadzu Corp., Kyoto, Japan). The minerals determined were Mg, K and Ca. Phosphorus content was determined using ascorbic acid method (AOAC, 1996) with the UV-Vis spectrophotometer (Model UV mini 1240, Shimandzu Corp., Kyoto, Japan).

3.2.3.17 Colour assessment

Colour of both the pulp and peel at ripeness stage 1 and 6 were measured using a Minolta colour difference meter (Model CR-200, Osaka, Japan) that was calibrated with a white and black standard tile. Measurements were made on three spots along the equatorial region and the average of these was considered as a replicate. The L*, a* and b* coordinates were recorded and, a* and b* values converted to hue angle ($H°$), where $H°$ = (arc tan(b/a), for first quadrant +a and +b, 180+arc tan (b/a) for second quadrant (-a, +b) and third quadrant (-a,-b) and hue=360+arc tan (b/a) for fourth quadrant (Mclellan *et al*., 1995).

3.2.4. Statistical analysis

Data were examined for normality using R software and outliers by scatter plot using the Ms Excel software. Outliers were mainly from improper data entry and once confirmed they were rectified. Data were then subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS statistical programme (SAS, 2001). Differences among means were compared using Student Newman Keul's (SNK) test ($\alpha = 0.05$).

3.3 Results and Discussion

3.3.1 Soil characteristics of the study site

Results of soil analyses of Maragua region study site and optimum banana requirements are presented in Table 3.1. Soils in the study site had adequate levels of K, Mg and N while P content was inadequate. Clay content was adequate in most areas of the site while pH range was adequate for absorption of most nutrients.

Table 3.1: Study site soil characteristics and optimum banana crop requirements

Nutrient/parameter	Study site soil characteristics	Banana plant requirements	Remarks
K(cmol(+)/L]	0.18-1.26	0.2-1.5	adequate
P (mg/kg)	0.05-3.91	10-40	inadequate
N (%)	>0.1%	>0.1%	adequate
Mg (me/100g)	1.03-8.21	1-10	adequate
pH range	5-5.5	5.5-6.5	acidic
Clay content (%)	22-44	30-55	Adequate in most areas of the site

3.3.2 Bunch weight, number of hands and number of fingers

There was a significant difference ($p \leq 0.05$) in bunch weights, number of hands and fingers due to the different nutrients applied. The treatment omitting K and having all other nutrients such as P, N, and micronutrients applied had the highest mean bunch weight of 11.23 kg and was significantly different ($p \leq 0.05$) from the treatment omitting P which had the least mean bunch weight of 2.86 kg (Fig. 3.1). The effect of treatments on the number of hands and fingers is shown in Fig. 3.2. The treatment omitting K had the highest number of hands followed by the treatment omitting micronutrients, N, followed by the treatment where all nutrients were applied and then by control and was significantly different ($p \leq 0.05$) from the treatment omitting P. The treatment omitting K had a significantly ($p \leq 0.05$) large

number of fingers, approximately 85 compared to control and treatment omitting P which had 48 and 36 fingers, respectively.

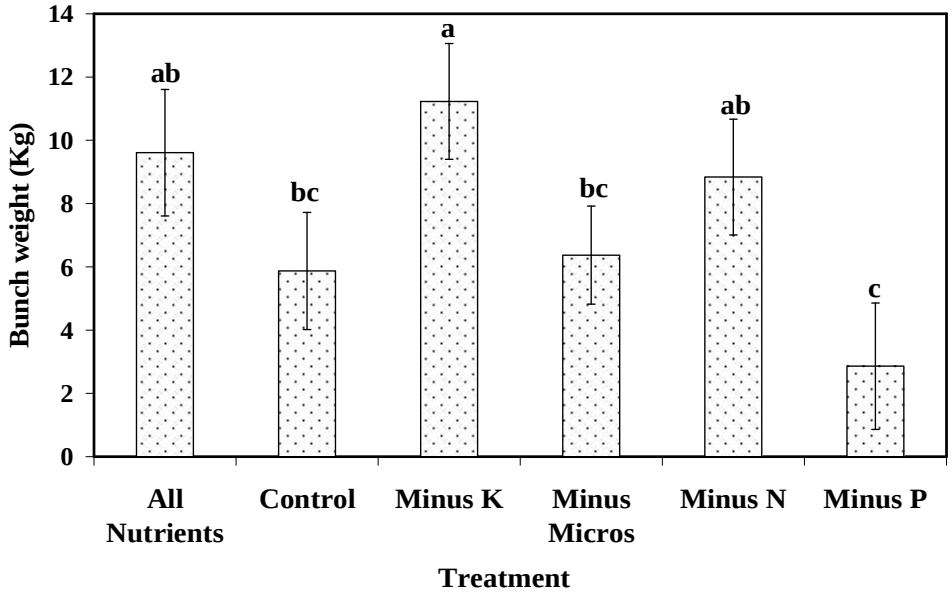

Figure 3.1: Effect of nutrients on bunch weight of tissue cultured banana cultivar Giant Cavendish. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

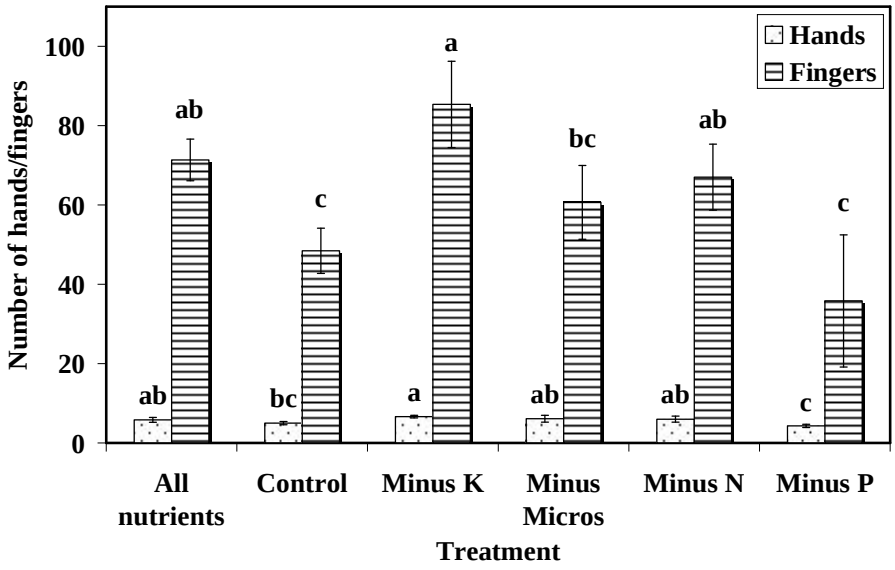

Figure 3.2: Effect of nutrients on number of hands and fingers of tissue cultured banana cultivar Giant Cavendish. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P

Addition of K seemed to negatively affect physical fruit quality (bunch weight, number of hands and fingers) since the fruits from the treatment omitting K had good physical quality. Therefore, the K amount in the soil was already adequate and hence more K applied did not influence the quality positively. Soil analysis of the plot showed that K concentration was between 0.18 and 1.26 [cmol (+)/L] in the upper layer of the soil (Table 3.1). Bananas have been shown to grow optimally in soils with K concentration of between 0.2-1.5 [coml (+)/L] (Lopez and Espinosa, 2000). Excess K added as KCl could have reached levels which may have negative

interactions with the other nutrients like Mg which affects chlorophyll formation (Bennett, 1993). Also, the high rate of KCl could have displaced the Ca and Mg off cation exchange surfaces into the soil solution from where they could be lost through leaching (Johns and Vimpay, 1999). Clayey soils have been shown to contain more exchangeable K than sandy soils (Defoer *et al*., 2000). Soil analysis of the plot indicated a high clay content of between 22-44%. Potassium has been shown to boost fruit size and quality (Jones, 2002). However, the uptake of K from the soil depends on the soil K concentration and development stage of the plant (Turner and Barkus, 1983b) which may explain the negative effect of K application on fruit size and some quality attributes in the current study. The maximum level above which K cannot be absorbed by the soil from the soil phase is determined by several factors such as climate, growth rate, root vigour, soil water status, disease and over or under supply of other cations (Robinson, 1996; Sathiamoorthy and Jeyabaskaran (undated). Imbalanced nutrition with K is well known and becoming an important constraint to crop production. Also, impaired nutrient management and related nutrient input-output imbalances, especially with K may represent a serious issue with crop nutrition (Cakmak, 2010).

Results from the treatment omitting N followed a similar trend with those of the treatment omitting K, although with slightly lower values. The plot where the bananas were grown had been bush and it is possible that organic matter in the soil provided some amounts of N. Indeed, soil analysis of the plot showed that an N content of >0.1% which is considered to be a good level of the element in the soil (Defoer *et al*., 2000). Mineralization of organic residues on the soil surface is an

important source of N for banana (Lopez and Espinosa, 2000). It has been shown that nitrogen fertilizer does not affect the bunch physical quality in the first growing cycle (Fontes *et al.*, 2003).

Phosphorous had a positive effect especially on physical fruit quality hence the fruits from the treatment omitting P had very poor qualities. For instance, banana plants from the treatment omitting P had the smallest bunch of 2.86 kg while the treatment omitting K had the highest bunch weight of 11.23 kg. Bunches from the treatment omitting P also had the lowest number of hands and fingers. Phosphorous applications have a beneficial effect on the number of hands and hence the number of fingers per bunch (Smith, 1993) and would, therefore, have an effect on bunch size and other finger characteristics. Bananas require a P concentration of about 10-40 mg/kg (Lopez and Espinosa, 2000). The P concentration of the plot was much lower as it ranged from 0.05 and 3.91 mg/kg which was below 4 mg/kg which is regarded as the lower limit requiring fertilization (Robinson, 1996) hence the negative response of bananas to P omission. Most of the soil in the plot had a P concentration of below 2 mg/kg. Phosphorous deficiency has been shown to reduce growth in mother plants (Lopez and Espinosa, 2000) resulting in stunted plants and poor root development (Robinson, 1996). This could explain why plants from the treatment omitting P had very small bunches with few hands and fingers. P has been shown to be fixed in soils where pH is less than 5.5 (Karugaba and Kimaru, 1999). Soil analysis report of the plot showed that a large proportion of the area had a pH of between 5 and 5.5. It promotes root formation, early growth and early maturity (Jones, 2002) hence stunted plants with P deficiency. The plot had previously been

under monocrop sisal. Sisal has been shown to deplete P at a very high rate. In a study on nutrient balance in a monocrop sisal farm, P was depleted most (85%) compared to other nutrients such as N (45%), K (78%), Ca (73%) and Mg (73%) (Hartemink, 1997).This shows that sisal depletes P from the soil and hence the need to replace it for optimal yields of subsequent crops.

Fruits from the treatment omitting micronutrients generally had lower values for bunch weight, hand numbers and finger numbers (Figs. 3.1 and 3.2) than the treatment where all the nutrients were applied indicating that the micronutrients, just like P were limiting fruit quality. As mentioned earlier, most of the soils in this region had a pH value of between 5 and 5.5. Lahav and Turner (1983) warned that trace element availability may be restricted at high pH and survey results of Turner *et al*. (1989) indicate decreasing banana yields associated with pH values >5.

Micronutrients are very important in leaf functions (Smith, 1993), hence the negative effect caused by their omission. Micronutrients have been shown to improve the size of the bunch by increasing the number of fingers per hand and number of hands per bunch (Mandal *et al*., 2002). Where all nutrients were applied, the physical quality of the fruit was not severely affected although it was generally lower than that of fruits from the treatment omitting K. The banana requires balanced nutrition (Smith, 1993).

3.3.3 Finger grade, weight and length

Finger grades were significantly ($p \leq 0.05$) influenced by the fertilizer treatments (Fig. 3.3A). Fruits from the treatment where all nutrients were applied and where K was omitted had significantly ($p \leq 0.05$) better grades of about 36 mm compared to those from the treatment omitting P. However, fruits from all treatments except where P and micronutrients were omitted attained an export grade of between 33 and 38 mm (Stover and Simmonds, 1987). Grade is one of the most important pomological characteristics of the banana. It is measured as a diameter with calipers and for determining harvest grade, the middle finger in the outer whorl of the second hand is measured at its thickest part (Stover and Simmonds, 1987). It is one of the parameters used for quality evaluation especially with the conversion of packing bananas as hands or portions of hands (clusters).

There was significant difference ($p \leq 0.05$) in finger weights as influenced by the nutrients. Fruits from the treatment omitting P had significantly ($p \leq 0.05$) lower weights than those from all the other treatments (Fig. 3.3B). Fruits from all treatments except those from the treatment where P was omitted had fingers that weighed between 110-144 g. Export carton sizes vary from 12 to 20 kg (Stover and Simmonds, 1987). This means that few heavy fingers would be used in the cartons which would mean fewer fingers for similar weight compared to the light fingers would be sold earning more money.

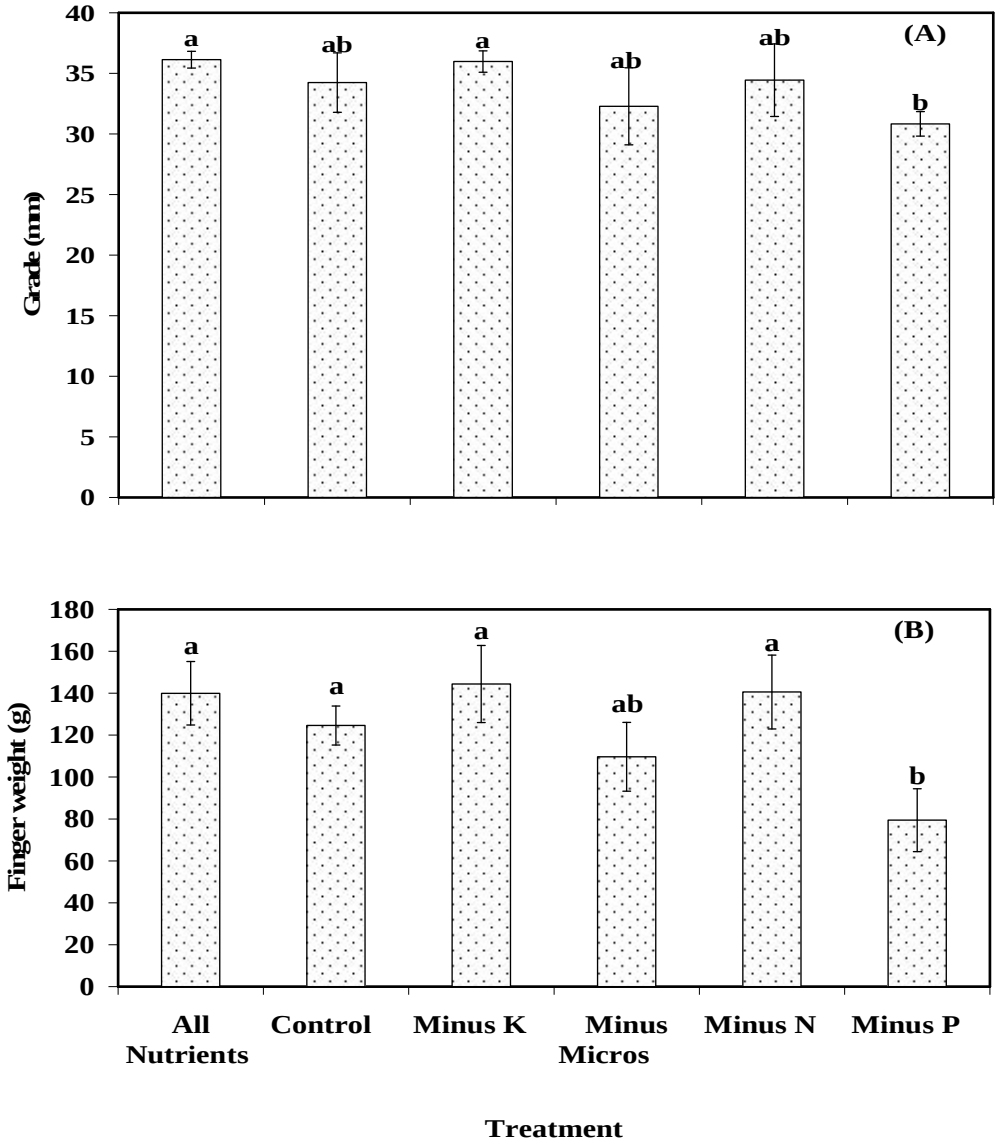

Figure 3.3: Effect of nutrients on the grade of middle finger of second hand of outer whorl (A) and on finger weight (B) of tissue cultured banana cultivar Giant Cavendish. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

Finger length was significantly ($p \leq 0.05$) influenced by the nutrients. Fingers from the treatment omitting P were significantly ($p \leq 0.05$) shorter than those from all the other treatments (Fig.3.4A). Fruits from all treatments except where P was omitted fell within the acceptable minimum finger length of about 15 cm for various export categories (Stover and Simmonds, 1987). The most preferred finger length by consumers according to Stover and Simmonds (1987) is in the range 15-20 cm. There was no significant difference ($p > 0.05$) in finger curvature between the different nutrients applied (Fig. 3.4B). Generally, banana fingers have an arc shape during early stages of maturation but straighten as they mature (Dadzie, 1998).

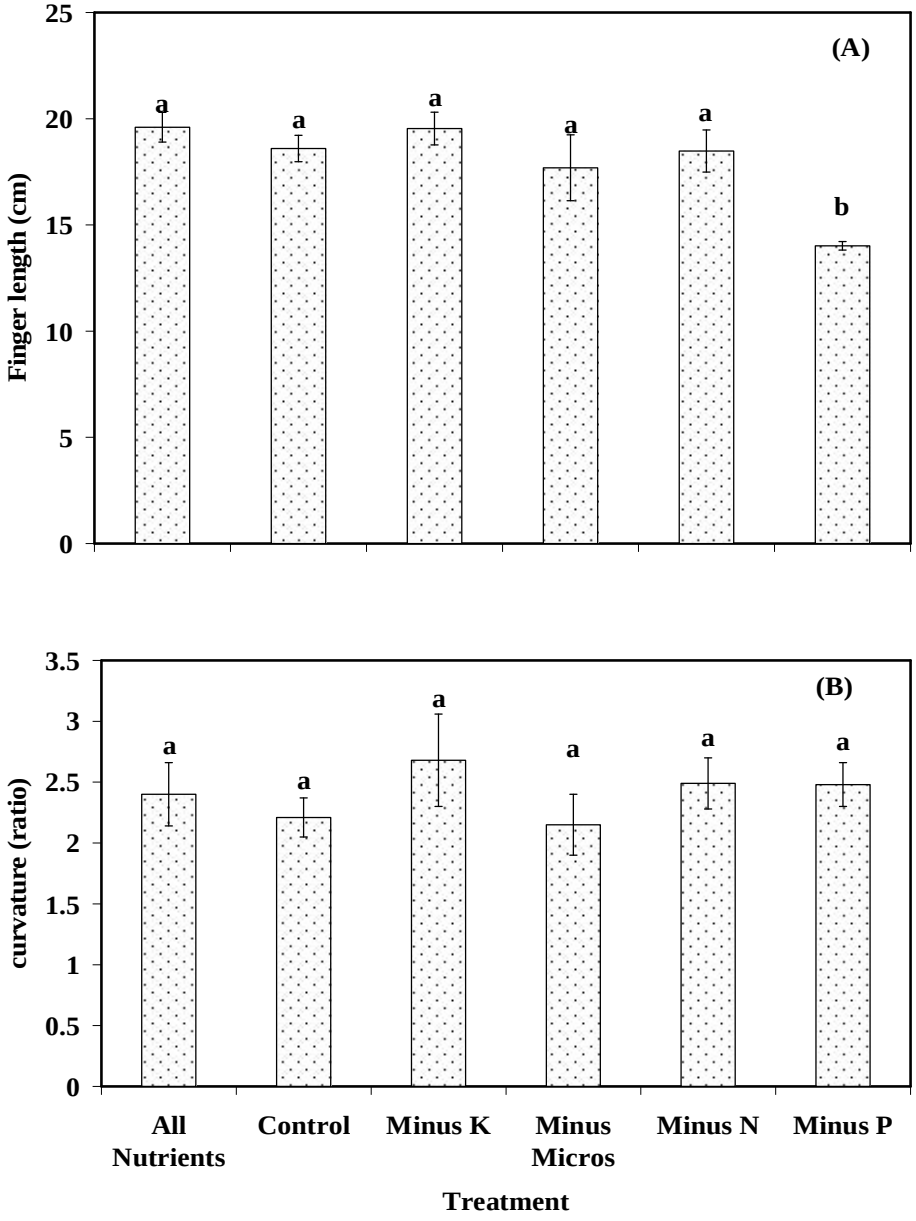

Figure 3.4: Effect of nutrients on finger length (A) and finger curvature (B) of tissue cultured banana cultivar Giant Cavendish. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

3.3.4 Pulp/peel ratio

The nutrients applied significantly ($p \leq 05$) influenced the pulp to peel ratio of green bananas with the fruits from the treatment omitting K having significantly larger ratios compared to all other treatments (Fig. 3.5). However, the pulp-peel ratio of ripe fruits was not significantly ($p > 0.05$) affected by the nutrients applied (Fig 3.5).

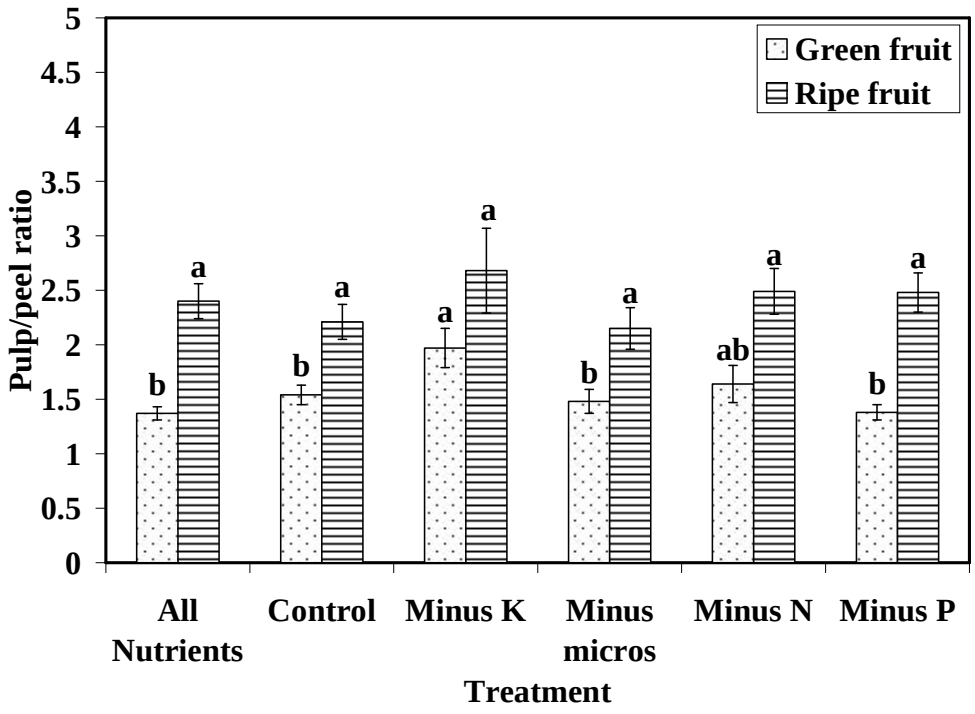

Figure 3.5: Effect of nutrients on pulp to peel ratios of green and ripe fruit of tissue cultured banana cultivar Giant Cavendish. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

Pulp and peel are the two obvious plant tissues of the banana fruit where the peel is the ovary wall and commences growth soon after the flower initials appear on each hand when the inflorescence is deep within the pseudostem (Turner, 1997). The pulp

initials begin to grow one to two weeks after bunch emergence. The rate of growth of the fruit peel begins to slow down as the fruit matures but the pulp continues to grow. The pulp to peel ratio gives the proportion of the banana fruit that is edible. A pulp to peel ratio of about 1 at 70 days and 1.82 at three quarter maturity for AAA (Cavendish subgroup) 'poyo' bananas has been achieved in the tropics (Robinson, 1996). Fruits from all the treatments gave a green pulp to peel ratio of above 1.34 although those from the treatment omitting K gave the highest ratio of 1.97 which was significantly ($p \leq 0.05$) higher than all other treatments except the one omitting N which had a ratio of 1.64.

In India, "Giant Cavendish" bananas have been shown to acquire a pulp to peel ratio of 1.34 while green and 2.17 while ripe (Marriot, 1980). In our experiment, the pulp to peel ratio of the green fruits ranged from 1.37 to 1.97 and 2.15 to 2.68 for the ripe fruits which is consistent with the findings of Marriot (1980). The peel of the green bananas has high water content estimated at about 90% (Turner, 1997). Ripening has been shown to increase water content in the pulp of "Giant Cavendish" bananas as reported by Marchal *et al.* (1988). As the fruit ripens water is lost from the peel to the pulp in response to change of osmotic potential of each (Turner, 1997). This explains the low and high ratios of pulp: peel of green and ripe banana, respectively.

3.3.5 Fruit firmness

Results of the effect of inorganic fertilizers on ripe fruit firmness are shown in Fig. 3.6. The nutrients applied did not influence ripe finger firmness significantly ($p > 0.05$) measured subjectively.

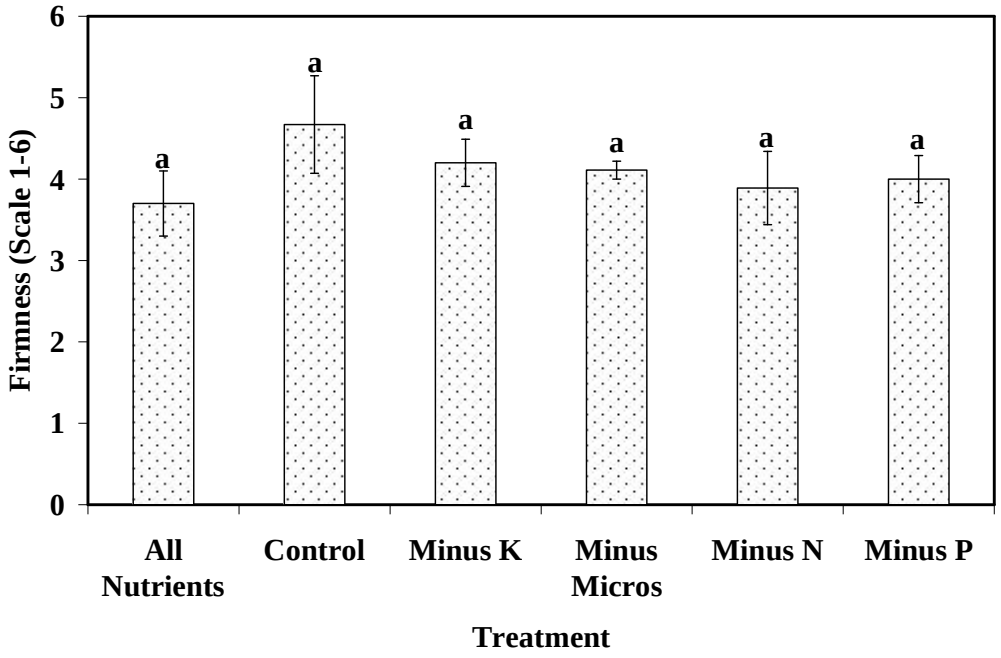

Figure 3.6: Effect of nutrients on ripe finger firmness of tissue cultured banana cultivar Giant Cavendish. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

However, some fruits from the treatment omitting N, P and K and where all nutrients were applied developed a hard inner core on ripening. In our case only some fruits from plots 1 and 12, where all nutrients were applied, plot 5, where P was omitted, plots 14 and 32, where N was omitted and plot 28, where K was omitted, developed a hard inner core. However, not all the fruits had this hard core in these treatments hence the non-significance in the overall rating of firmness. This may indicate that indeed an interaction between macronutrients and micronutrients could play a role in hardening of fruit pulp. Potassium which

is a cofactor of enzymes including those responsible for softening has been shown to be negatively affected by Mg (Jones, 2002).The peel firmness measured objectively of the green and ripe fruits was affected significantly ($p \leq 0.05$) (Fig. 3.7A) while pulp firmness of the raw and the ripe fruit were not significantly ($p > 0.05$) affected by nutrient applied (Fig. 3.7 B).

The fruits from the treatment where all nutrients were applied had the highest green peel firmness compared to those from the treatment omitting K. High K content has been shown to increase fruit firmness (Ferguson and Boyd, 2002). Fruits from all treatments softened on ripening as expected (Seymour *et al*, 1993). For the ripe peel, fruits from the treatment omitting micronutrients had significantly ($p \leq 0.05$) higher firmness than the fruits from the control and those from the treatment omitting P. Raw and ripe pulp firmness were not significantly ($p > 0.05$) influenced by the nutrients applied. This agrees with data for finger touch of subjective ripe finger firmness as reported above, which was not influenced by plant nutrition.

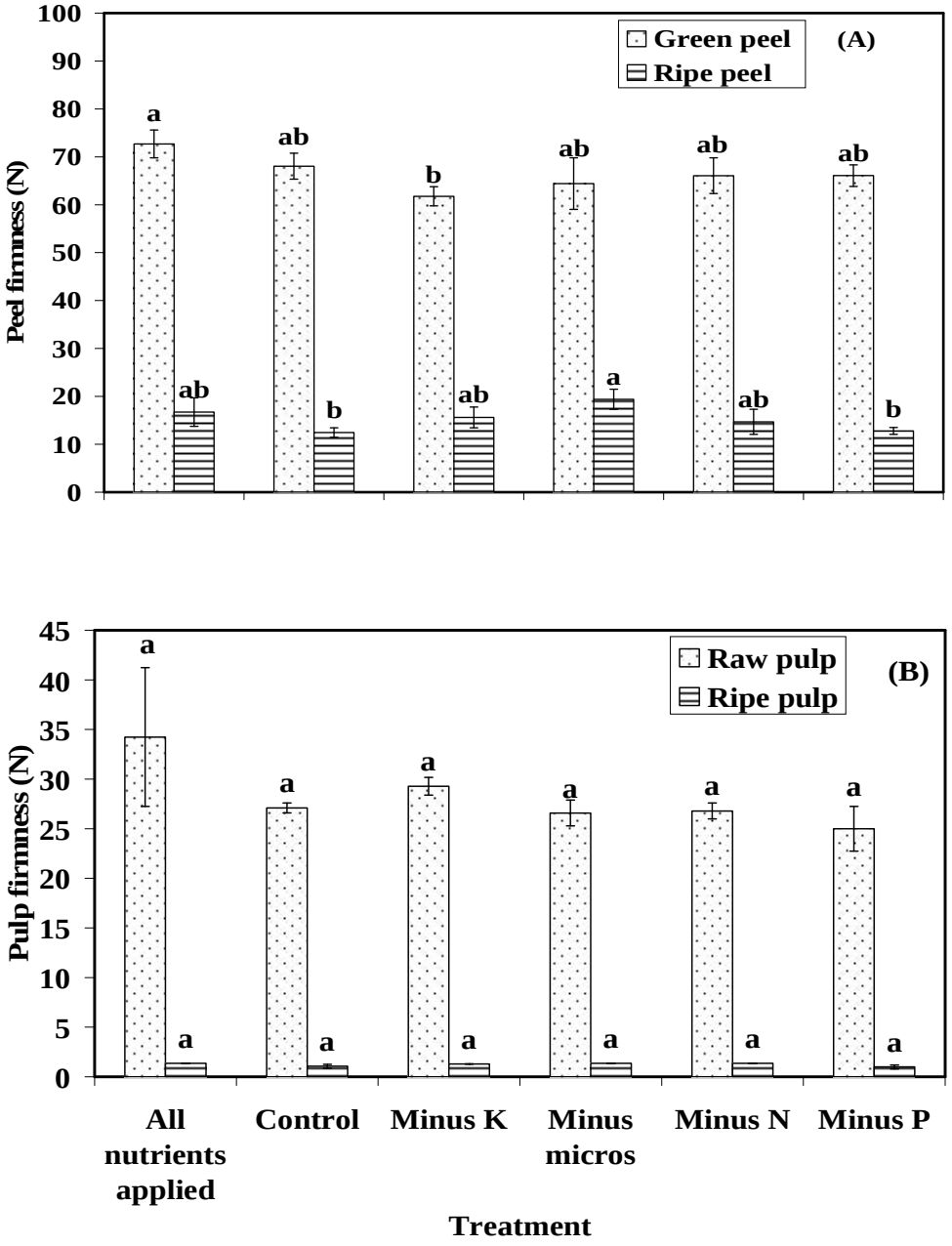

Figure 3.7: Effect of inorganic fertilizers on the peel (A) and pulp (B) firmness of green and ripe banana fruit. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

Firmness is an important quality criterion that can be measured by both sensory and instrumental methods. Firmness is defined as specific force required to deform a product. The fruit that is more firmed shows less deformation from an applied force. For the bananas a moderate to soft firmness would be appropriate depending on the consumer. However, the presence of a hard core highly reduces the textural quality of the banana fruit. During ripening in fruits, protopectin which is a large polymer is broken down to lower molecular weight fractions which are more soluble in water and the rate of degradation of this substance is directly correlated to the rate of softening of the fruit. This breakdown is facilitated by cell wall degrading enzymes (Wills *et al*., 1998; Ali *et al*., 2004).

Banana fruit softening is also attributed to breakdown of starch to sugar and loss of turgor (Ratule *et al*., 2007). The formation of the hard core in the banana fruit could come from interference in protopectin breakdown and or lack of starch degradation to sugar. Further research should be carried out in this area. Hard core presence in ripe bananas has been associated with a CO_2 concentration of above 10% in the ripening environment. This has been shown to produce fruits with uneven ripening, with the peripheral zone of the pulp becoming soft and watery, while the central zone stays hard (John and Marchal, 1995). Also, low temperatures and insufficient ethylene have been found to cause uneven ripening in bananas (Robinson, 1996). None of the fruits from the control and from the treatment omitting micronutrients developed a hard core on ripening which may indicate an interaction of the nutrients applied, and the above mentioned conditions of more than 10% CO_2 concentration in the ripening atmosphere, insufficient ethylene and low temperatures.

3.3.6 Starch content and total soluble solids (TSS)

The nutrients applied had a significant ($p \leq 0.05$) effect on the starch content of green fruit (Fig 3.8). The fruits from the treatment omitting P, micronutrients and the control had the highest content of about 97%, 96% and 96%, respectively. The treatment omitting K had the least starch content of about 90% at the green mature stage.

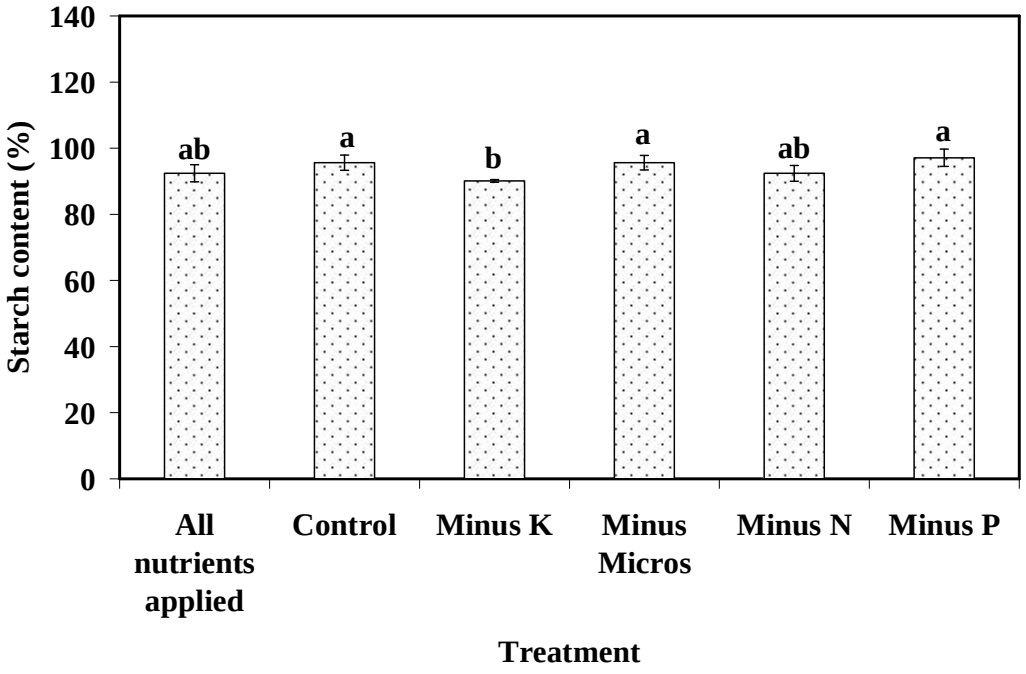

Figure 3.8: Effect of nutrients on starch content of green fruit pulp. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

Total soluble solids content was significantly ($p \leq 0.05$) affected by nutrients applied (Fig. 3.9). The fruits from the control and from the treatment omitting micros had

the highest mean TSS content of 28.1 ˚Brix while the fruit from the treatment omitting P had the lowest mean TSS content of 25.6 ˚Brix.

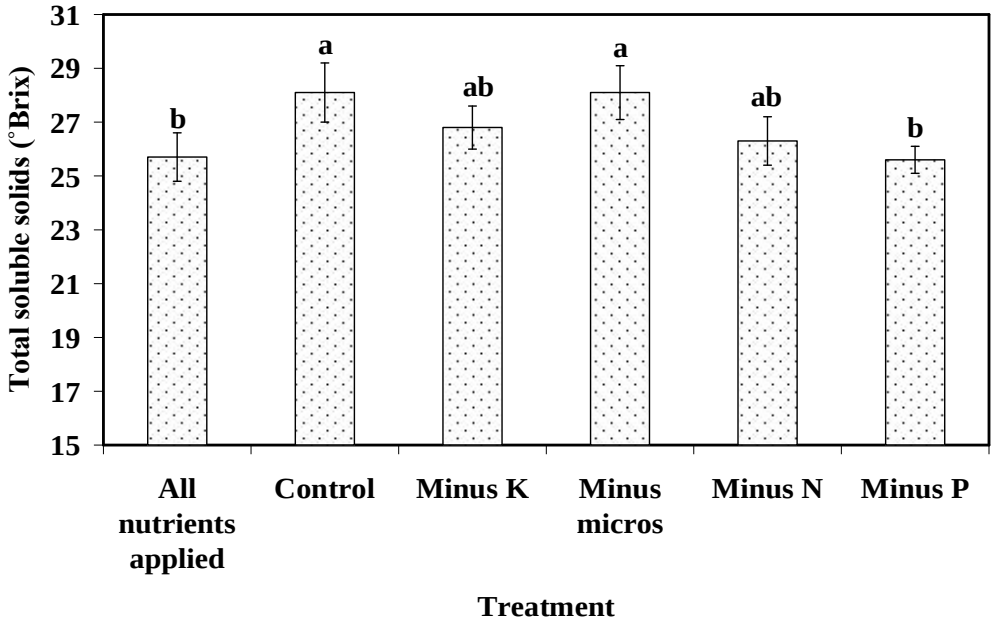

Figure 3.9: Effect of nutrients on total soluble solids content of fruit pulp. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

In this study, the starch content ranged from 90% to 97% on fresh weight basis. Starch comprises 85 to 95% of the dry matter of unripe pulp (Marriot, 1980). Phosphorus deficiency promotes starch synthesis. This could be due to the role P plays in starch formation as a low concentration of orthophospahate in the cytosol limits the export of triose phosphate from the chloroplast through the translocator, thereby promoting the synthesis of starch (Taiz and Zeiger, 2002). Phosphorous deficiency has been shown to reduce growth by reducing leaf number and size (Lynch *et al.*, 1991). This could be

the result of reduced assimilates including the sugars in the banana fruit. Infact, P has also been shown to be involved in formation of sugars (Brian, 2006). Indeed, the fruits from the minus P treatment had low sugar, as evidenced by the low TSS and high starch contents. Marriot (1980) reported that in bananas, there is a high positive correlation between sweetness and TSS.

The treatment omitting micronutrients also had high TSS content. Omitting Mg did not affect the process of carbohydrate manufacture as the banana requires a Mg soil content of between 1-10 me/100g (Lopez and Espinosa, 2000) and most soils in this plot had a content of about 1 me/100g. Magnesium is part of chlorophyll and is essential for photosynthesis, the process by which plants manufacture carbohydrates (Muriuki and Qureshi, 2001) which are eventually turned into sugars in the banana fruit during ripening. The soil in the experimental plot had adequate K which allowed for more cation-balance and uptake of the mineral (Jones, 2002). Other micronutrients like Boron have been implicated in production of sugars and starches while Zn has been shown to be essential for carbohydrate translocation. Molybdenum is essential for N utilisation, which in turn is part of chlorophyll necessary for carbohydrate manufacture (Brian, 2006). It is possible that these micronutrients were adequate for carbohydrate synthesis and therefore did not affect TSS content.

3.3.7 Total titratable acidity (TTA)

In the current experiment, the TTA of the fruits was not affected significantly ($p>0.05$) by the nutrients applied (Fig. 3.10). Total titratable acidity content ranged from 0.4%-0.6%. Bananas have been shown to have acidity of about 0.4% (Robinson, 1996). The

higher acid content of the fruits in this study may be attributed to the varying nutrients applied to the soils.

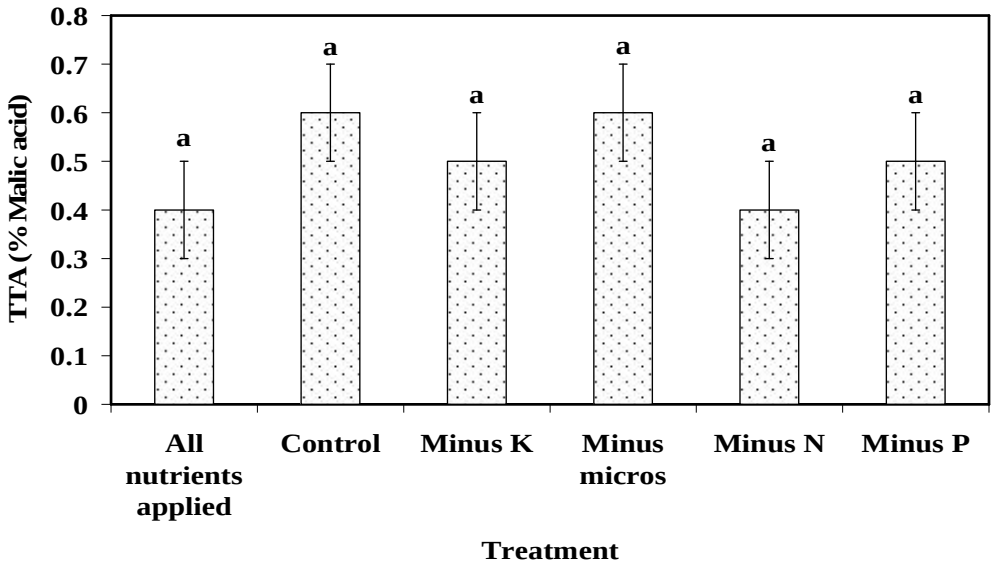

Figure 3.10: Effect of nutrients on total titratable acidity of fruit pulp. Data are means ± SE of twelve fingers. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

3.3.8 Fruit pulp and peel vitamin C, moisture and of crude fibre contents

The effect of the nutrients applied on vitamin C content of the ripe fruit, the moisture content and crude fibre content of pulp and the peel is summarised in Figs. 3.11, 3.12 and 3.13 respectively. Vitamin C content of ripe banana pulp was significantly ($p \leq 0.05$) influenced by the nutrients applied. The moisture content of both banana pulp and peel was not significantly ($p > 0.05$) affected by the nutrients applied. Also, the crude fibre content of the peel was not influenced significantly

($p>0.05$) while that of the pulp was influenced significantly ($p\leq0.05$) by the nutrients applied. Fruits from the treatments where micros were omitted and from the control had the highest content of 21.3 mg/100g, while the fruits from the treatment omitting K had the lowest of 14 mg/100g. The contents were within the expected levels of 10 to about 20 mg/100g for banana fruits (Stover and Simmonds, 1987; Robinson, 1996). The low level of vitamin C in the treatment omitting K was possibly due to reduced K uptake due to increased Mg (Jones, 2002).

Potassium fertilization has been found to increase vitamin C content of fruit (Ferguson and Boyd, 2000). Treatments where all nutrients were applied, and N and P were omitted also had low vitamin C content possibly due to negative interactions of the nutrients applied. Application of K in the treatment omitting N may have lead to reduced N content in the plant hence reduced vitamin c content in the fruits (Jones, 2002). In the treatment where all nutrients were applied it is possible that there were negative interactions between K and Mg and N hence the low levels of vitamin C content in the fruits. Fruits from the treatment omitting P also had low levels of vitamin C possibly due to reduced uptake of P by Zn (Jones, 2002) and also due to the fact that P in the study site soil was below optimal. Phosphorous deficiency reduces CO_2 assimilation in leaf photosynthesis (Fujita *et al.*, 2003) which leads to low carbohydrate production from which vitamin C is made. Phosphorous also regulates activities of enzymes involved in controlling starch synthesis and climacteric respiration during fruit ripening (Jones, 2002) hence reduced vitamin C due to low P content.

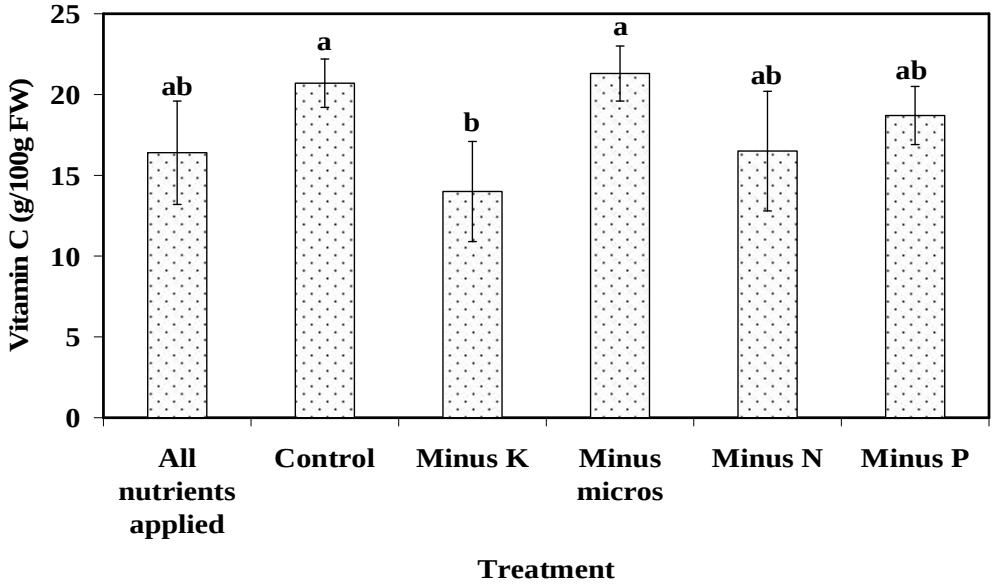

Figure 3.11: Effect of inorganic fertilizers on vitamin C of fruit pulp. Data are means of 12 fingers. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

The moisture content had a narrow range of 78% to 87% in the peel and 68 to 71% in the pulp and compares well with moisture levels for banana fruits of about 89% for the peel and 71-72.3% for the pulp (John and Marchal, 1995; Yousaf *et al*., 2006). This also agrees with what other workers have reported (Marriot, 1980; Turner, 1997). The nutrients applied had no significant ($p>0.05$) influence on the crude fibre content of the peel while that of the pulp was significantly ($p\leq0.05$) affected. Fruits from the treatment omitting K had the highest crude fibre content in the pulp of 0.8%, while fruits from the treatment where all nutrients were applied had the lowest in the pulp of 0.4%.

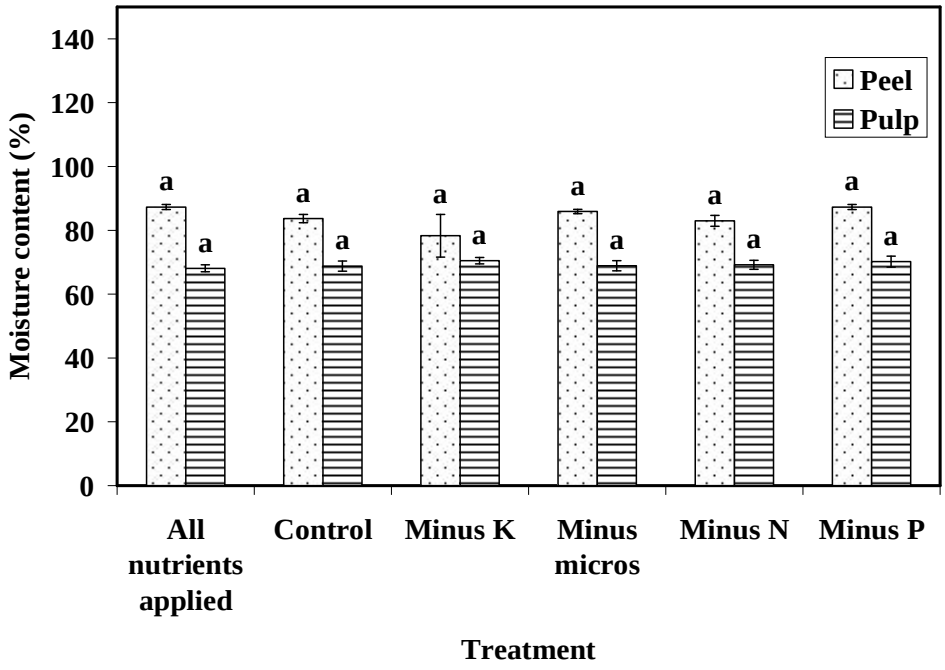

Figure 3.12: The effect of nutrients on moisture content of pulp and peel. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

Generally, the crude fibre of the pulp was slightly lower than what has been recorded earlier of 0.84% (Stover and Simmonds, 1987; Robinson, 1996) except for the treatment omitting K which could be due to the effect of the nutrients applied, cultivar and growing conditions. In all the treatments, moisture and crude fibre contents were higher in the peels than in the pulp. Similar findings have been reported by Izonfuo and Omuaru (1988).

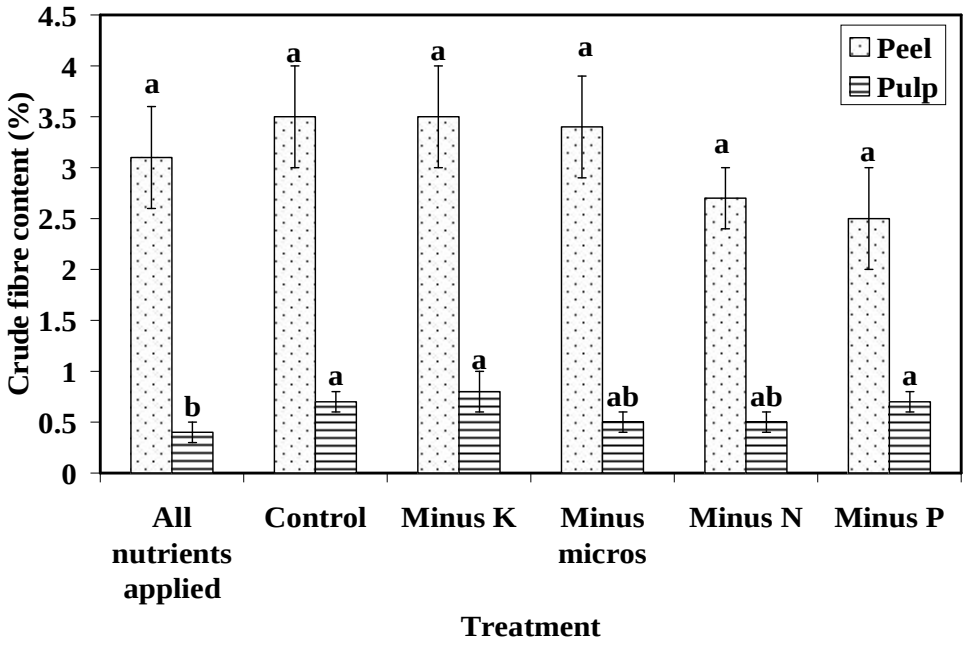

Figure 3.13: The effect of nutrients on crude fibre content of pulp (A) and the peel (B). Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

3.3.9 Chlorophyll content of the green fruit

The nutrients applied had no significant ($p>0.05$) effect on total chlorophyll content of the peel of the green fruit (Fig. 3.14). This shows that the nutrient combinations had no significant effect on chlorophyll synthesis. The chlorophyll levels achieved ranged from 85.1 to 93.4 to µg/g and were consistent with those reported earlier of about 50 to 100µg/g fresh weight (Stover and Simmonds, 1987) and 93µg/g as recorded by Marriot (1980).

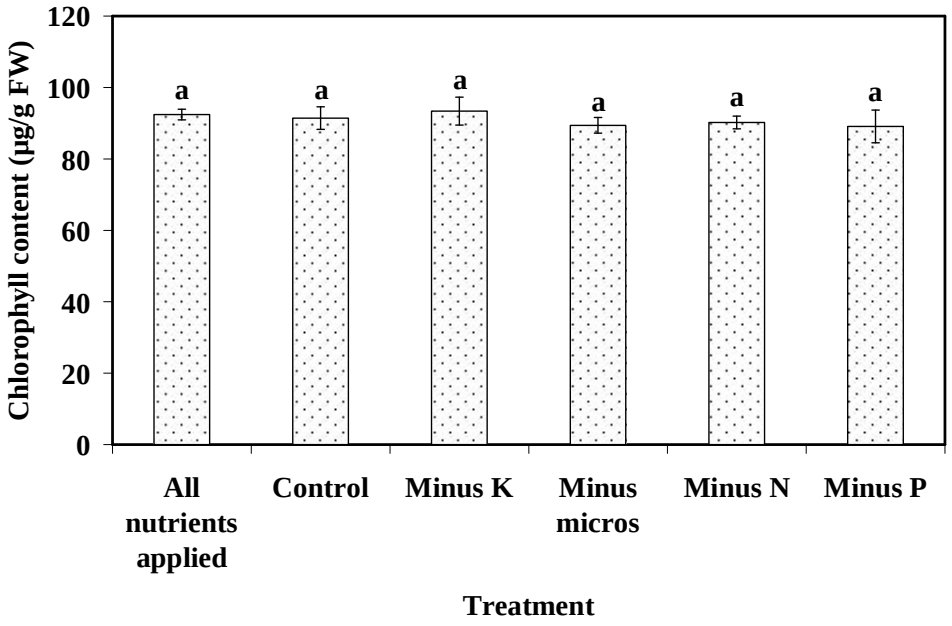

Figure 3.14: Effect of inorganic fertilizers on total chlorophyll content at harvest of banana fruits. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

The low chlorophyll content of the fruits from the treatment omitting P could have been due to reduced chlorophyll synthesis due to P deficiency. Chlorophyll synthesis requires ATP and P plays a key role in reactions that involve ATP (Taiz and Zeiger, 2002). Magnesium is a constituent of the chlorophyll molecule. Although the soils were low in Mg (<1 me/100g), the fruits from the treatment omitting micros had a high content of chlorophyll of 89.4 µg/g. This could be due to the added the P that allowed for the chlorophyll synthesis using the available Mg in the soil.

3.3.10 Colour

Colour is a very important quality as it is used by consumers to make decisions on purchase of a product (Kays, 1999). The hue angle is the actual colour perceived as it 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. Lightness (L*) is a measure of lightness on a scale from zero to 100. Zero represents black and 100 equals white (McGuire, 1992). At the green stage, the peel of the fruits from the treatment where all nutrients were applied were significantly ($p \leq 0.05$) darker, had a dark green colour (lower L* value) compared to those from all other treatments while, yellow peels of fruits from all treatments had similar lightness (L* value) (Fig. 3.15).

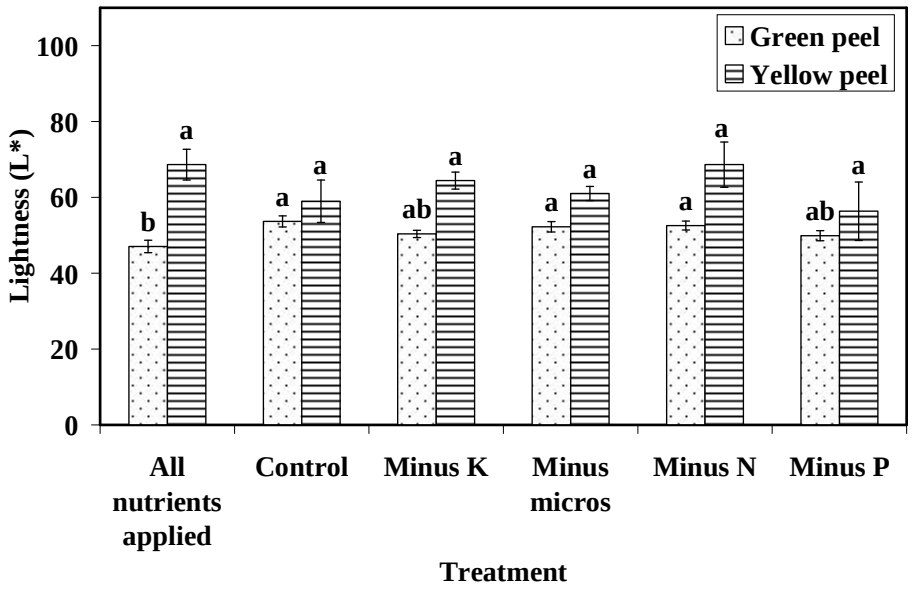

Figure 3.15: Effect of inorganic fertilizers on the lightness of green (A) and yellow (B) peels of banana. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

The effect of nutrients on hue angle of the green and yellow peel is shown in Fig. 3.16. Nutrition had a significant ($p \leq 0.05$) effect on hue angle of green peel with fruits from the treatment where all nutrients were applied having significantly ($p \leq 0.05$) higher values compared to fruits from where micronutrients were omitted. The hue angle of the yellow peel was similar for all nutrient combinations. As expected, fruit peels for all treatments changed from green to yellow which corresponded to decreases in the hue angle values. Lightness (L* value) of the peel increased as the bananas turned from green to yellow for all treatments. This is mainly due to degradation of chlorophyll during ripening and/or unmasking of carotenoids (Seymour *et al*., 1993; Ferguson and Boyd, 2002). Nitrogen, potassium and manganese nutrition has been shown to affect colour changes (Ferguson and Boyd, 2002). In fact, nitrogen and manganese have been found to impart green colour of lemons and 'jonagold' apples, respectively (Ferguson and Boyd, 2002). Potassium and N nutrition has been shown to affect colour changes (Ferguson and Boyd, 2002).

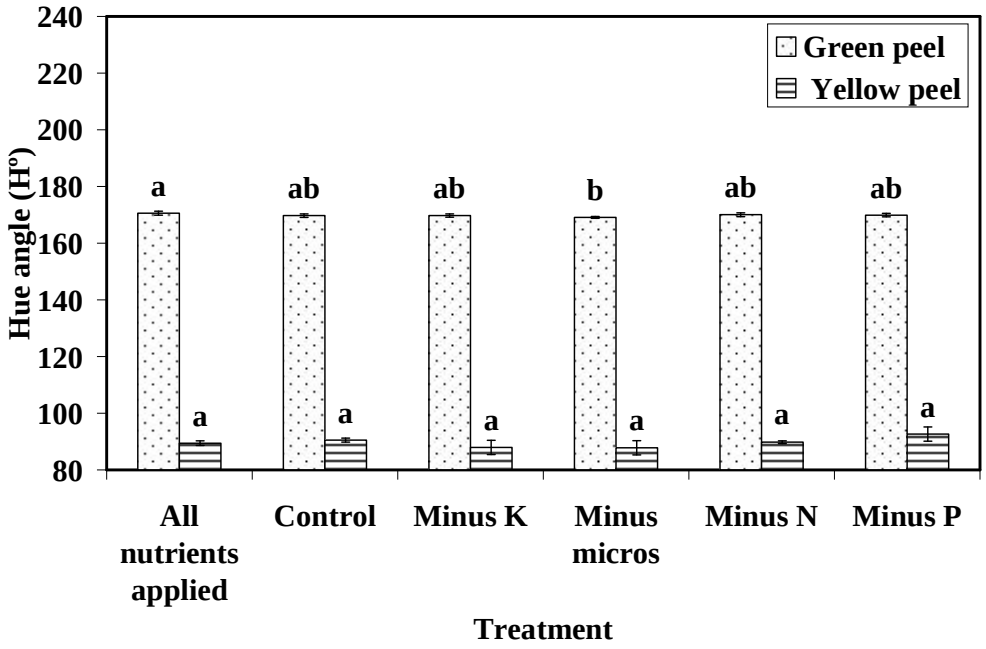

Figure 3.16: Effect of inorganic fertilizers on the hue angle of green and yellow peels of banana. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

Mineral nutrition significantly ($p \leq 0.05$) affected the L* value of the unripe pulp with pulp of the fruits from where all nutrients were applied significantly ($p \leq 0.05$) lighter white colour compared to fruits from the treatments omitting micros (Fig. 3.17). The L* value of ripe pulp was not influenced significantly ($p > 0.05$) by the fertilizer regime (Fig. 3.17).

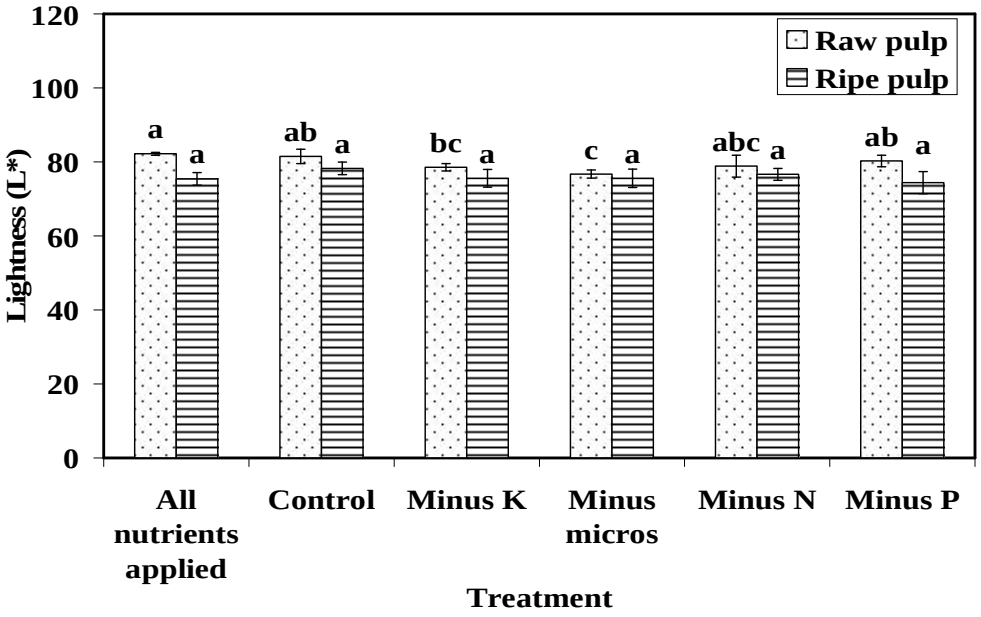

Figure 3.17: Effect of inorganic fertilizers on the lightness of raw and ripe pulp of banana. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

The significantly low L* value of fruits from the treatment omitting micros may have been caused by a high K/Mg ratio that has been shown to cause a 'yellow pulp' condition in the banana fruit (Lahav, 1995). 'Yellow pulp' condition is also caused by high soil calcium and magnesium deficiency (Lahav, 1995). Hue angle of the unripe pulp was significantly ($p \leq 0.05$) affected, with ripe pulp of fruits from treatment omitting micros having lower values compared to that of fruits from the treatment where all nutrients were applied (Fig. 3.18). Ripe pulp Hue angle was also affected significantly ($p \leq 0.05$) by plant nutrition with fruits from the treatments omitting K, control and all nutrients applied having higher values compared to those from the treatment omitting micros, N and P (Fig. 3.18). Generally the hue angle decreased on

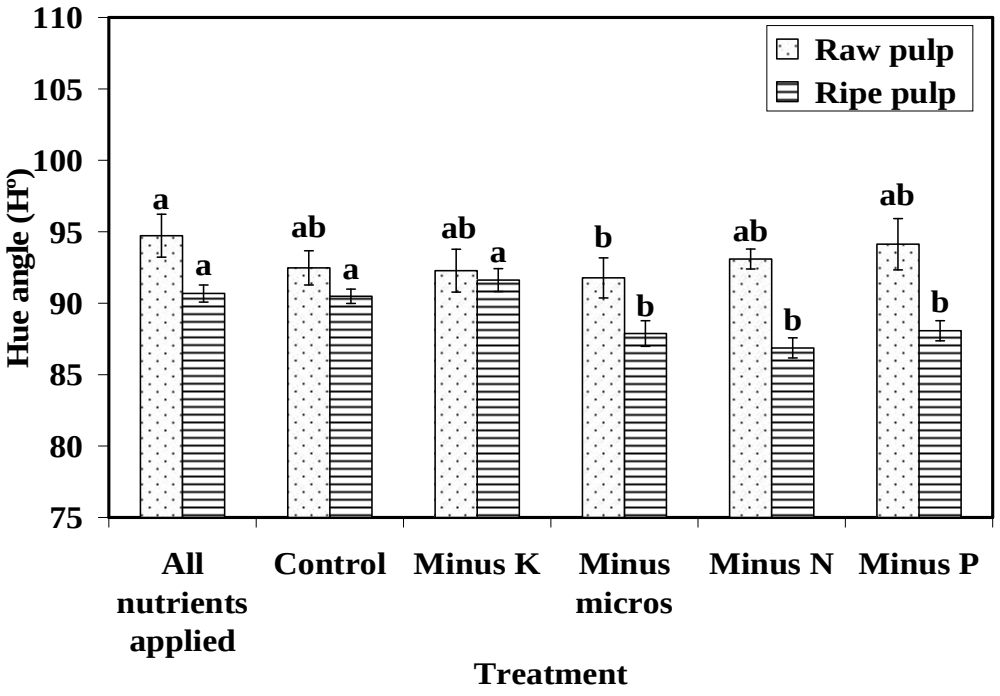

Figure 3.18: Effect of inorganic fertilizers on the hue angle of raw and ripe pulp of banana. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

ripening for all treatments as the pulp colour changed from whitish to creamish probably due to appearance of yellow pigments such as carotenoids (Osman *et al.*, 1998; Harnandez *et al.*, 2006).

3.3.11 Sugar content

Nutrients applied did not significantly ($p>0.05$) influence the glucose, sucrose and fructose levels of the fruits (Fig. 3.19).

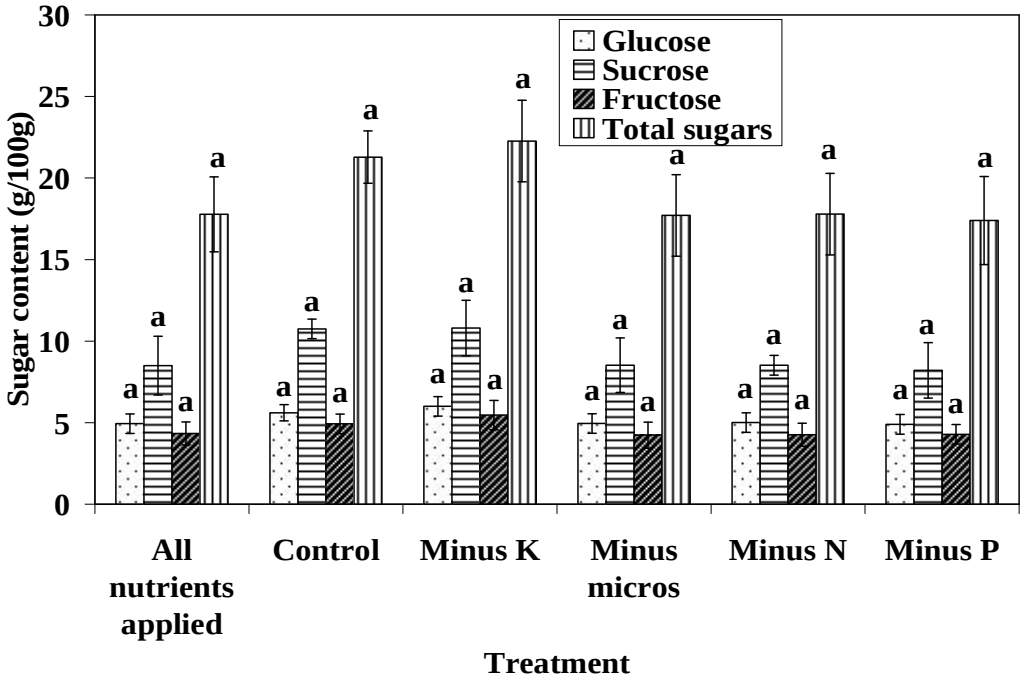

Figure 3.19: Effect of inorganic fertilizers on the sugar content of ripe pulp of banana fruits. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test ($\alpha=0.05$). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

3.3.12 Pulp and peel mineral content

Results of the effect of nutrients on pulp and peel mineral content are summarized in Fig. 3.20 A and Fig. 3.20 B, respectively. In the pulp, Ca, Mg and P were influenced significantly ($p\leq0.05$) while the K content was not affected significantly ($p>0.05$) by the nutrients applied possibly because the K content in the field had been adequate as

discussed earlier in section 3.31 and 3.3.2. However, for the peel, only P content was influenced significantly ($p \leq 0.05$) by the nutrients applied. Fruits from the treatment omitting the micronutrients and where K was omitted had significantly ($p \leq 0.05$) lower P values compared to fruits where all nutrients had been applied for the pulp. Low magnesium supply has been shown to reduce uptake of P by the root and also to restrict transfer of P to the tops (Lahav, 1995). Potassium has been found to regulate the transfer of nutrients such as P to the xylem (Lahav, 1995; Robinson, 1996).

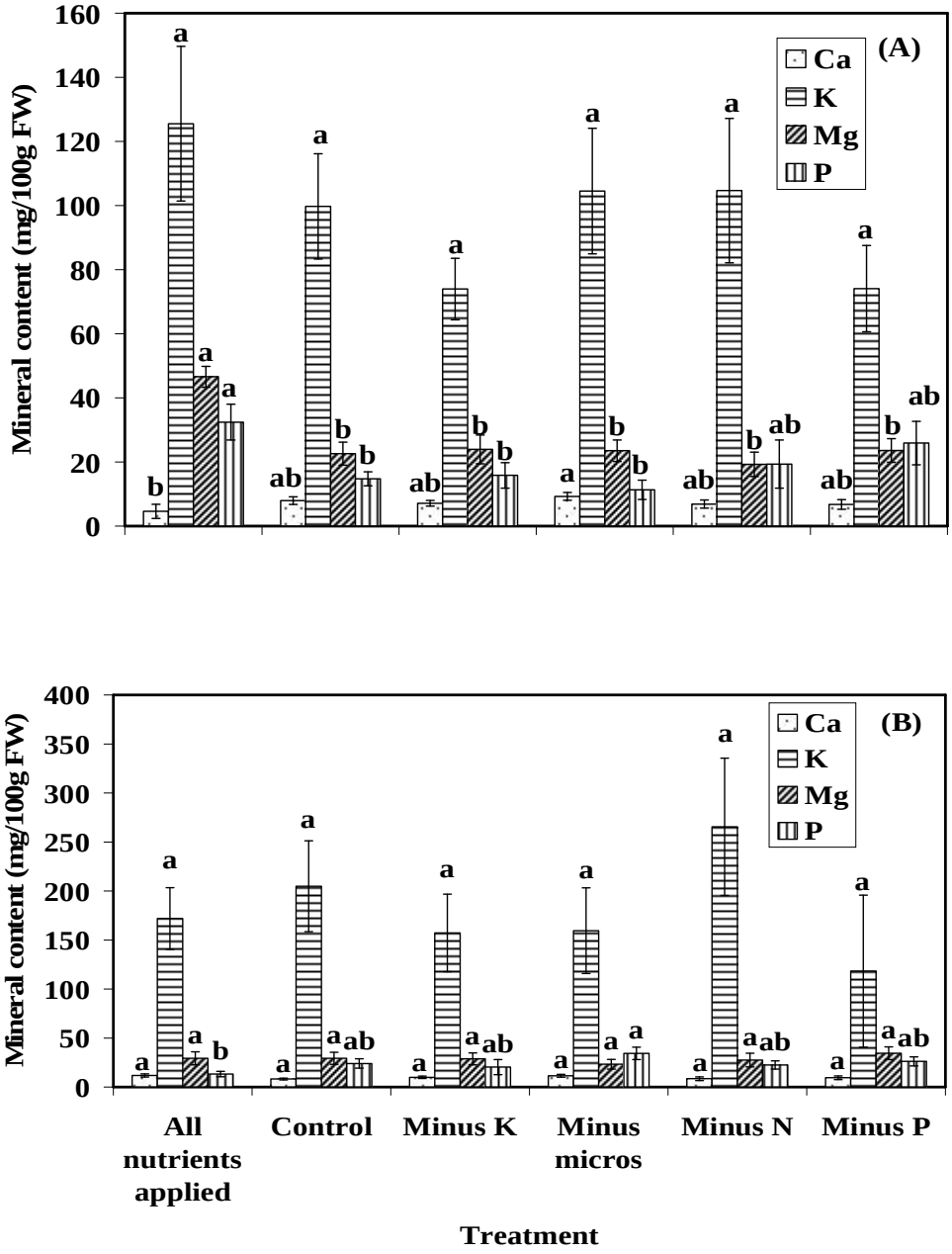

Figure 3.20: Effects of inorganic fertilizers on mineral content of banana pulp (A) and peel (B). Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

Magnesium content was significantly ($p \leq 0.05$) high in the pulp of the fruits where all nutrients were applied compared to other treatments. Large uptake of K has been shown to promote the uptake of Mg by the fruits (Lahav, 1995). Calcium content was significantly ($p \leq 0.05$) higher in the pulp of fruits from the treatment omitting micros compared to fruits from the treatment where all nutrients were applied. Increased K and N have been shown to reduce Ca uptake (Robinson, 1996), while Mn, B and Mg has been shown to have an antagonistic influence on Ca uptake (Lahav, 1995; Ferguson and Boyd, 2002). Preharvest nutrition has been found to affect fruit mineral composition (Ferguson and Boyd, 2002). Generally, the mineral contents were higher in the peel than the pulp for all the nutrient combinations. Several studies have shown that mineral content in the peel is normally higher than in the pulp (Robinson, 1996; Ferguson and Boyd, 2002). This is due to some proportion of the mineral elements migrating with water towards the pulp in the course of ripening and those remaining behind increasing in percentage due to less water in the peel (John and Marchal, 1995). Mineral content is rarely affected by one season one nutrient application (Ferguson and Boyd, 2002). However, in our case the bananas had the nutrients applied three times before harvest hence the effect on some of the fruit mineral contents.

3.3.13 Sensory attributes

Results of the sensory evaluation are presented in Figs. 3.21 and 3.22. The fruits from the control treatment were liked best while those from the treatment where micronutrients were omitted were least preferred (Fig. 3.21 A). The nutrients did not influence banana sweetness significantly ($p > 0.05$) (Fig. 3.21 B). This differs from the

TSS values discussed earlier (Section 3.3.6, Fig. 3.9) where fruits from the control treatment had significantly ($p \leq 0.05$) higher values than fruits from the treatment where P was omitted. In banana, TSS has been found to contribute significantly to sweetness (Marriot, 1980). However, sensory analysis has been shown to differ with instrumental analysis since even if there are significant differences according to an objective measurement, this difference may not be perceptible due to the fact that human senses and laboratory equipments do not have the same sensitivity (O'Mahony, 1988).

Fruits from the control treatment had the best banana aroma with a rating of good banana aroma while the rest had moderate banana aroma (Fig. 3.22 A). Texture was perceptibly influenced by nutrients applied, with fruits from the treatment omitting P, N and control being smoother compared to those from the treatment omitting K (Fig. 3.22 B).

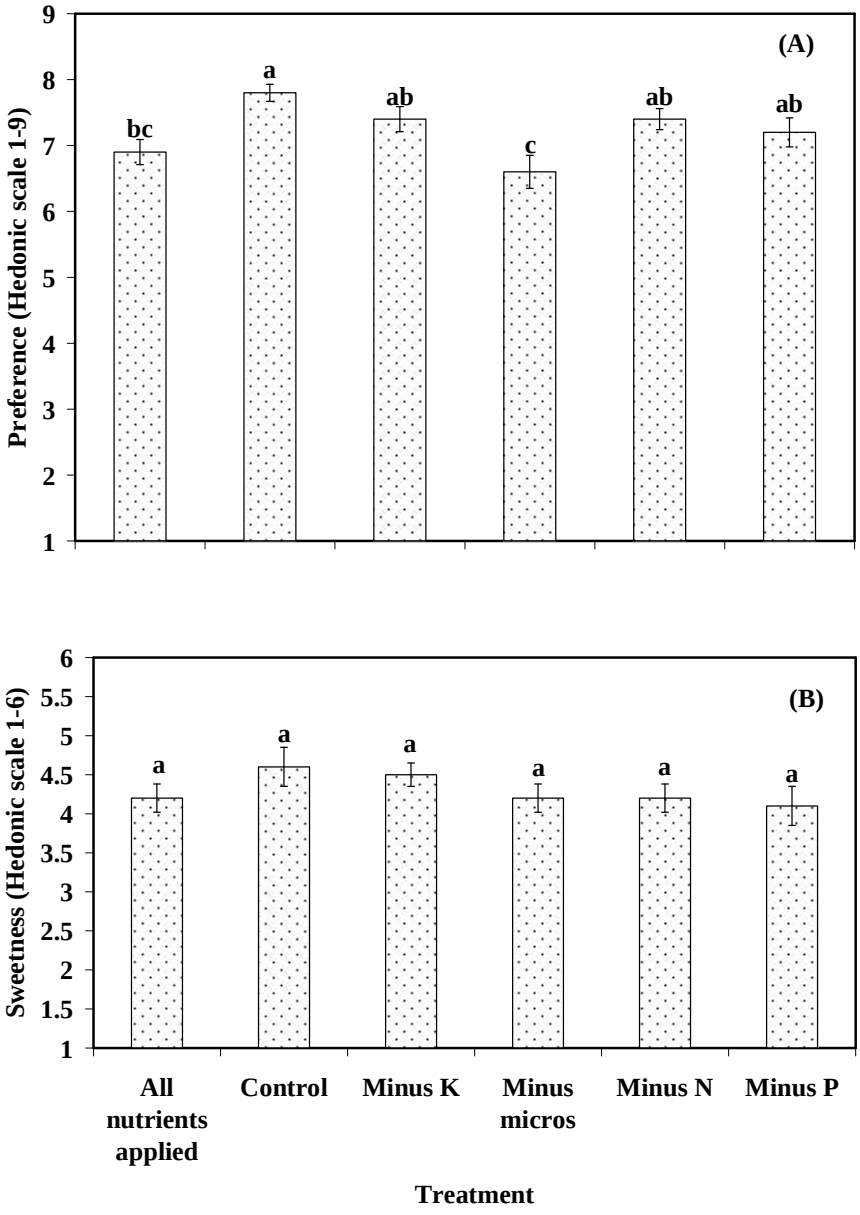

Figure 3.21: Effect of inorganic fertilizers on the taste (A), sweetness (B). Vertical bars represent SE of the means of 25 panelists. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

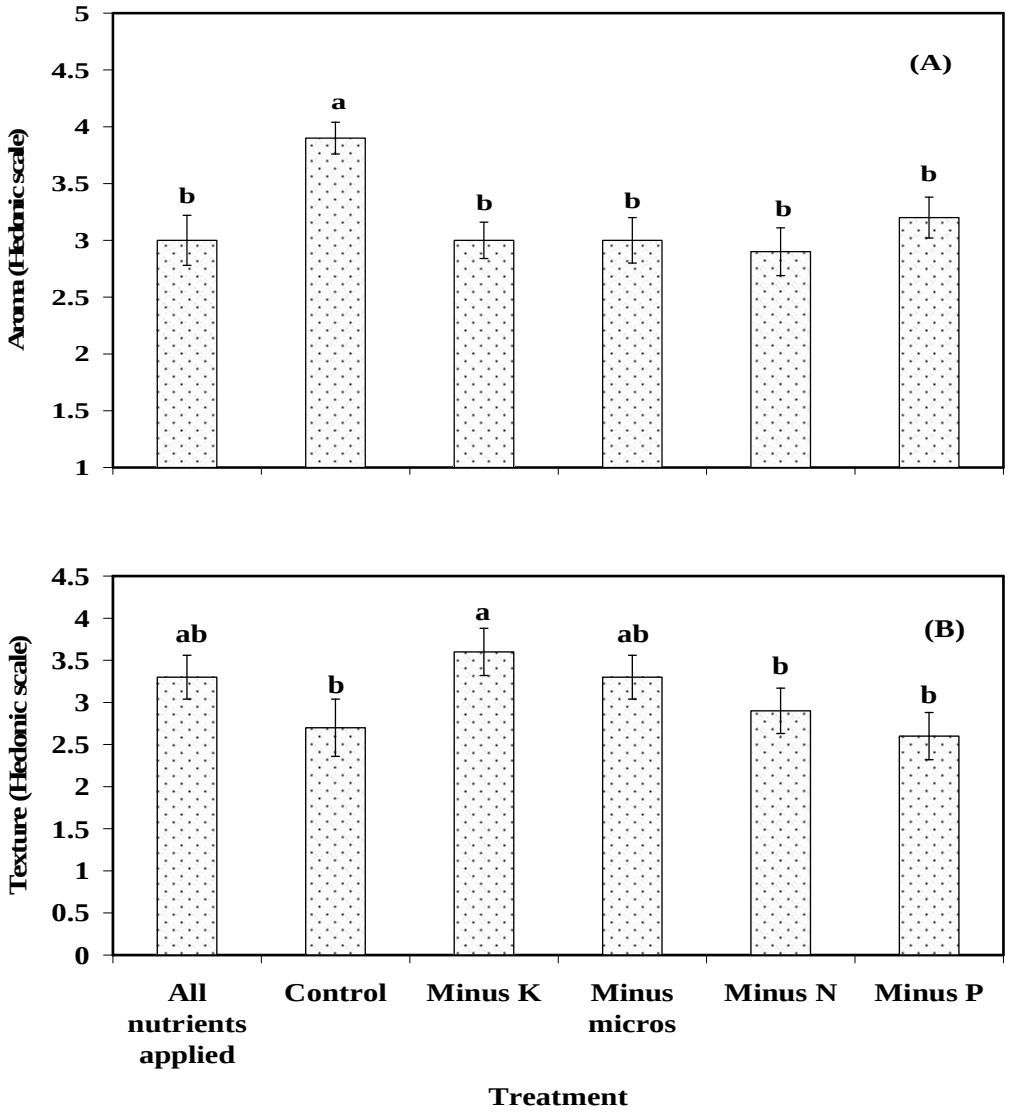

Figure 3.22: Effect of inorganic fertilizers on the aroma (A) and texture (B) at eating ripe stage 6 of plant crop. Vertical bars represent SE of the means of 25 panelists. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

These findings contrast the results of subjective ripe finger firmness which was not influenced by nutrition in the current study. Consumer acceptability tends to be based on appearance and sensory properties associated with texture and flavour rather than on laboratory measurements such as TTA, dry matter and firmness (Ferguson and Boyd, 2002). Fruits from the control treatment were liked best probably due to the fact that they had the highest scores for sweetness and aroma and the fruits ripened to a soft texture. Fruits from the treatment omitting micros and where all nutrients were applied were preferred least compared to the control. Fruits from the treatment omitting P despite having low sweetness scores had relatively high aroma scores and softened well on ripening hence the higher preference ratings. During ripening, starch is degraded into sugars which are eventually broken down in the presence of oxygen via glycolysis, the TCA cycle and the mitochondrial electron transport chain (Turner, 1997). The oxidative pentose phosphate pathway achieves the initial steps of the glycolytic pathway and provides precursors for aromatic compounds thus eventually influencing the fruit flavour (Turner, 1997). Earlier studies have indicated that banana flavour and aroma is a result of a wide variety of volatile compounds that emanate from ripe bananas with the 'banana like' flavour being due to amyl esters, while the 'fruity' note is mainly attributable to butyl esters (Seymour, 1993). Fruit flavour which highly influences consumer preference is a combination of taste such as sweetness, aroma and tartness (Ferguson and Boyd, 2002).

The low aroma ratings in fruits from the treatment omitting N could be attributed to low production of aroma volatiles as N fertilization is associated positively with volatile production of some fruits (Ferguson and Boyd, 2002). Fruits from the

treatment omitting K had highest texture scores suggesting rough textured fruits, while those from the treatment omitting P, N and control had low scores associated with smooth mouthfeel. The changes in texture of pulp during banana ripening probably results from alterations in both cell wall structure and starch degradation (Seymour, 1993). This influences the texture of the fruit and ultimately consumer perceptions and fruit acceptability (Redgewell and Fischer, 2002). The changes in the cell wall occur due to depolymerization and solubilization of cellulose microfibrils, hemicellulose and pectins (Asha, *et al*., 2007). This process requires enzymes such as hydrolyses (Nascimento *et al.*, 2006). Although soil analyses showed that the K content of the plot was within the optimum range for banana growing, the content in the treatment omitting K may have been negatively affected by the applied Mg. Magnesium has been shown to have a depressing effect on K uptake from the soil (Jones, 2002) and this may have made K not adequate for the processes of softening as it is a co-factor of many enzymes (Taiz and Zeiger, 2002) which may explain the rough texture of fruits from the treatment omitting K. Fruits with low K content have been shown to have uneven ripening (Ferguson and Boyd, 2002). Mineral nutrition has been shown to affect sensory attributes of fruits (Ferguson and Boyd, 2002).

3.3.14 Green life and shelflife

Both green life and shelflife were not significantly ($p>0.05$) influenced by the nutrients applied (Fig. 3.23). Green life and shelflife are a function of respiration and ethylene production (Wills *et al*., 1998). . The inorganic fertilizers in this case possibly did not affect the two processes significantly. Fruits from inorganic fertilizer

treatments with higher respiration and ethylene production rates were found to have a shorter shelflife than those from treatments with lower rates (Miriti, 2009).

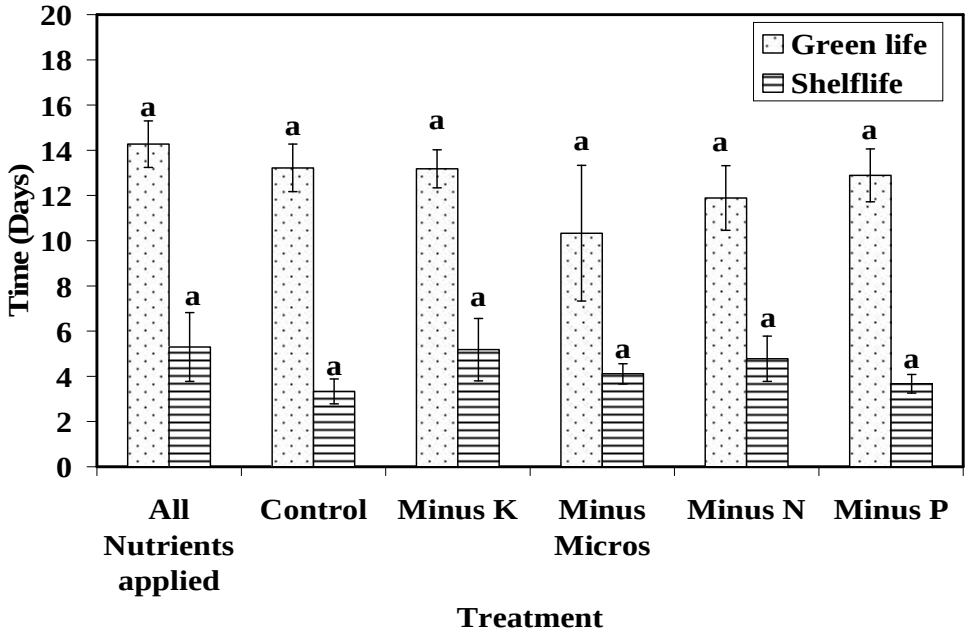

Figure 3.23: Effect of inorganic fertilizers on green life and shelflife. Vertical bars represent SE of the means of 4 replications. Columns denoted by the same letter represent values that are not significantly different according to Student Newman Keul's (SNK) test (α=0.05). All nutrients=all nutrients applied, control=no nutrients applied, Minus K=All nutrients applied except K, Minus Micros=All nutrients applied except micronutrients, Minus N=All nutrients applied except N and Minus P= All nutrients applied except P.

However, green life in the current study varied with a range of about 4 days with the treatment where all nutrients were applied and minus micros treatment having the longest green life of 14 days and the shortest green life 10 days, respectively.

In this study, the fruits from the treatment where all nutrients were applied had the longest shelflife of about 5 days while the fruits from the minus P treatment had the shortest shelflife of 4 days although it was not significantly influenced by the

nutrients applied. The shelflife was higher than what has been found in Australia of about 3 days (Australian Banana Grower's Council, 1999). However, the results in the current study are lower than those of Peacock (1980), where bananas of cultivar Giant Cavendish ripened at 21˚C and 24˚C had a shelflife of about 7 and 6 days, respectively. This may be attributed to different ripening temperatures and growing conditions.

3.4 Conclusion and Recommendations

The nutrients that seem to be limiting postharvest fruit quality are P and micronutrients. This could change with time because K is removed judiciously by the banana fruit and may become deficient in ratoon crops. Nitrogen may follow the same trend as K. Nitrogen is of major importance in banana nutrition management as it is a component of various plant proteins. Hence, the role of N is paramount since most of the enzymes that control all metabolic processes are involved in plant proteins (Lopez and Espinosa, 2000). From the findings of this study indicate that an interaction of the N, P, K and micronutrients could be the cause of the hard core development since no fruits from the treatment omitting micronutrients and from the control developed the hard core. However, further research should be carried out to verify this. Most of the postharvest parameters were however not significantly influenced by the nutrients applied probably because this was the plant crop. The harvested banana bunch has been shown to take away a lot of nutrients (Purseglove, 1985) and this will probably have a negative effect on soil fertility during the growth of ratoon banana crops. In the long term then, the ratoon plants will show more reactions to the omitted nutrients.

Soils planted with bananas will deteriorate fast unless the mineral elements used by the banana plants are replenished regularly.

The outcome of the study indicates the need for use of inorganic fertilizers and micronutrients in order to improve both physical and nutritional content of the banana fruits. Effect of nutrients should be evaluated for the ratoon crops in order to come up with comprehensive fertiliser requirements. This research should also be carried out in other agro-ecological zones and also under controlled water regimes and different soil types. The research should also be carried out in different seasons in order to establish weather effects on yield and postharvest quality under different fertilizer regimes.

The results of this study indicate that fertilizers providing NP and micronutrients could be recommended for use in this area for the plant crop as they generally gave superior quality fruits compared to other fertilizer combinations. However, it is known that K is removed judiciously by the banana during growth and harvest compared to other nutrients and hence the need to replenish it for the ratoon crop even though it may have been adequate in the plant crop, therefore leaving the fertilizer combination providing all nutrients as the best option for this area. The study has also shown the importance of soil analysis before planting orchards with commercial banana crop so that only the nutrients needed are added and this may cut on cost and any detrimental effect of excess nutrients in the soil due to interactions. The detrimental interactions are caused by an excess of one element in the soil causing unavailability of another element to the plant. There is also the need to establish the cost/benefit ratio of using the fertilizers for a commercial banana enterprise.

CHAPTER FOUR

4.0 DETERMINATION OF MATURITY INDICES FOR NEWLY INTRODUCED TISSUE-CULTURED BANANA (*Musa* spp.)

4.1 Introduction

In Kenya, banana farming has recently shifted from backyard farming to commercial production (Acharya and Mackey, 2008). New cultivars of bananas among them Williams and Grand Nain have been introduced into Kenya. Most of these cultivars are established using tissue-cultured plantlets produced by both institutional and private laboratories in Kenya (Acharya and Mackey, 2008). Banana, being a delicate and highly perishable fruit encounters very high postharvest losses mainly due to several factors, among them, harvesting at improper maturity stage (Chege *et al*., 1995; Ramma *et al*., 1999). The stage at which the fruit is harvested greatly influences the green life, shelflife and its final eating quality (Dadzie and Orchard, 1997; Kader, 2002).

Fruits develop their full characteristic flavour, taste and colour during storage if picked during an optimum period. Fruits harvested at an early stage of maturity are susceptible to shriveling, mechanical damage and develop poor flavour and taste despite having long storage life (Mattheis and Fellman, 1999; Kader, 2002). However, harvesting at an advanced stage of maturity produces fruits that have good taste and flavour, but have a short storage life and are not suitable for transporting for long distances (Dadzie and Orchard, 1997). Also, overmature fruits are likely to become soft and mealy with insipid flavour soon after harvest (Kader, 2002). It is, therefore, important to harvest fruits at the right maturity stage to suit the purpose.

Maturity at harvest affects quality perception and the rate of change of quality during postharvest handling (Dadzie and Orchard, 1997; Shewfelt, 2009). Establishing a maturity index for a fruit makes it possible to schedule harvesting, handling and marketing operations efficiently (Robinson, 1996).

Maturity measurements are normally carried out by producers, handlers and quality control personnel and, therefore, need to be simple, readily performed in the field, or laboratory, or inspection point, and should require relatively cheap equipment (Dadzie and Orchard, 1997). The maturity indices should be objective rather than subjective and should preferably be non-destructive (Dadzie and Orchard, 1997). Many features have been used to estimate maturity, among them caliper grade in combination with bunch age (Ramma *et al.*, 1999). Conversely, the maturity indices for bananas must consistently meet two requirements for all producers, localities and seasons, namely, that they should ensure minimum eating quality whereby, they have a sweet taste, are soft and have a characteristic banana aroma (Dadzie and Orchard, 1997). There are no universally recognized objective criteria for determining harvest time for bananas. Some commonly used indices are angularity of fingers, bunch age and grade (Robinson, 1996). Both field and laboratory methods and procedures should be used in identification of key indicators of fruit maturation. Field methods and procedures include tagging of plants at flower emergence and visual observation of bunch and fruit development. Laboratory methods and procedures include measurements of diameter (grade), length, volume, density, pulp to peel ratio, fruit cross-sectional area, locular architecture, peel and pulp colour, pulp firmness, pulp pH and total titratable acidity, peel and pulp

moisture and dry matter content (Dadzie and Orchard, 1997). However, the use of a single indicator of maturity may be applicable to one cultivar but not to other cultivars. This makes it necessary to use a combination of several indicators to determine time of harvest. The indices must measure fruit characteristics and postharvest quality attributes which change consistently as the fruit develops and correlate well to fruit development so that harvesting the fruit at particular indices enable final eating quality to be predicted (Dadzie and Orchard, 1997).

In most international markets such as America, Europe and Asia, finger grade is the maturity index commonly used for bananas (Stover and Simmonds, 1987: Robinson, 1996). Grade is determined as the diameter of the thickest part of the middle finger of the outer whorl of the second hand measured using a caliper (Stover and Simmonds, 1987). Grade of the fruit is very important as it dictates the price of banana bunches especially in the export market where grade standards are already in place. For the European and United States of America (USA) markets, fruits with grades within 31-41 mm are acceptable (Robinson, 1996). Some multinational company plantations in Central America which produce high quality fruit for export use phenology (flowering duration to harvest), coloured ribbons and grade for estimation of harvest maturity (Robinson, 1996). Other methods have been tried to determine the correct stage of harvest but these have either been destructive, impractical or subjective. These include, pulp to peel ratio and a firmness index of fruit skin (Robinson, 1996). In subtropical countries, the three-quarter round index (full ¾) is used which is only satisfactory for local markets but it is difficult to maintain accuracy and consistency since this is a highly subjective method.

There are no standards existing for the Kenyan local market although they could be more useful when the fruits are destined for the export market where standards are already in place. A long green life is very important for it allows fruits to be transported for long distances before ripening. A long shelflife is preferred as it allows for flexibility in marketing and also storage by the retailers and the final consumers without spoilage. Lack of proper maturity indices of bananas continue to be a challenge to the banana industry in Kenya with some fruits reaching the market in mixed ripe state due to harvesting too late while others have poor flavour on ripening as they were harvested too early. Determination of proper maturity indices of the newly introduced tissue-cultured bananas would improve banana farming as the farmers and traders can predict time of production and storage and hence improve marketing and distribution which would impact positively on the economy. This would reduce postharvest losses and help all stakeholders in the banana industry get better returns. The objective of this study was to establish the optimum maturity indices for tissue-cultured banana cultivars Grand Nain and Williams.

4.2 Materials and methods

Trials were laid out in an already existing banana orchard in 2007-2008 in Maragua Ridge, Maragua District, agro-ecological zone UM3 (Jaetzold and Schmidt, 1983). The farm is located at latitude 00º 49’ 14”S and longitude 037º 08’ 34” E as marked by a Global Positioning Satellite (GPS) instrument (Magellan, Triton, China) and the bananas had been grown using the recommended agronomic practices (Anon, 2002). Three banana plants were randomly selected from the field for each treatment and the experimental design was completely randomized design. The treatments

were four and these were harvesting the bananas at different maturity stages namely, ¾, light full ¾, full ¾ and full maturity stages (Plate 3.1). The twelve plants of a first ratoon crop of banana cultivar Grand Nain and Williams were then tagged and the date of flower emergence of each plant was noted. During growth to harvest, the outer and inner finger length and diameter of three fruits selected from the top-third, middle and lower third of the bunch were measured in order to come up with a growth curve. The three randomly selected bunches were harvested at ¾, light full ¾, full ¾ and full maturity using angularity of the fruit to determine time of harvest. The bunches were harvested carefully, dehanded and placed in plastic crates and then transported to the postharvest laboratory of Jomo Kenyatta University of Agriculture and Technology (JKUAT). The fruits were then washed with tap water to remove latex and dirt and were then subjected to fungicidal treatments by dipping for 1 min in 100 ppm sodium hypochlorite (Jik, Reckitt Benckiser-East Africa Limited, Kenya) in order to control spoilage during postharvest storage due to common fungal diseases such as anthracnose (*Colletotrichum musae*) and crown rot. The fruits were then evaluated for postharvest quality.

4.2.1 Analyses and determinations

4.2.1.1 Fruit weight, diameter (grade) and length

Fruit weight, diameter and length measurements were carried out as previously described in section 3.2.3.1.

4.2.1.2 Finger curvature

Finger curvature was determined as earlier indicated in section 3.2.3.11.

4.2.1.3 Pulp/peel ratio

Pulp: peel ratio was determined as previously described in section 3.2.3.2 with a few modifications. The measurements were done at harvest (ripeness stage 1) through the ripening stages, until the fruits were fully ripe (ripeness stage 6) (CSIRO, 1972, Marin *et al*., 1996) unlike in 3.2.3.2 where the measurements were done only at stage 1 and 6

4.2.1.4 Starch content

Starch content was determined as earlier indicated in section 3.2.3.3. Starch content was determined from stage 1 through to ripeness stage 6 (CSIRO, 1972, Marin *et al*., 1996).

4.2.1.5 Ripening

Four hands per bunch from the equatorial region were ripened in a humidity chamber at 18˚C and 95% RH using passion fruit as ethylene source until ripeness stage 6 (CSIRO, 1972, Marin *et al*., 1996; Paull, 1996; Jiang *et al*., 1999)

4.2.1.6 Firmness

Hand fruit firmness assessment was determined as described earlier in section 3.2.3.6 although the measurements were carried out at ripeness stage one to six (CSIRO, 1972; Joyce *et al*., 1993; Jiang *et al*., 1999). Objective firmness was determined at the apical, middle and basal regions of the fruit using a rheometer (Model CR-1000, SUN SCIENTIFIC Co. Ltd, Japan) with an 8 mm probe.

4.2.1.7 Colour

Both subjective and objective colour was determined at harvest and during ripening. The assessment methods are as described earlier in section 3.2.3.17.

4.2.1.8 Total soluble solids content

Total soluble solids content was measured at harvest and during ripening until colour stage 6 as described earlier in section 3.2.3.7.

4.2.1.9 Green life

Green life was determined as indicated earlier in section 3.2.3.12.

4.2.1.10 Shelflife

Shelflife was determined as described earlier in section 3.2.3.13.

4.2.1.11 Chlorophyll content

Chlorophyll content was determined using the method of Arnon, (1949) as indicated earlier in section 3.2.3.4 with a few modifications. Three fingers per treatment were used and chlorophyll determination was carried out at harvest and during ripening until ripeness stage 6 (CSIRO, 1972).

4.2.1.12 Ethylene and carbon dioxide production (respiration) rates

Banana fruits were placed in 3L plastic jars whose covers were fitted with a self-sealing rubber septum for gas sampling. The fruits were incubated for one hour at room temperature. Gas samples from the headspace gas were removed using an airtight syringe and injected into a gas chromatograph (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 Q column and that of ethylene determination fitted with an activated alumina column and a flame ionization detector. Rates of carbon dioxide production were calculated as ml per kg per hr at standard atmospheric pressure while, the rates of ethylene production were calculated as nl per kg per hr.

4.2.1.13 Sucrose, fructose and glucose contents

Sucrose, fructose and glucose contents were determined as earlier indicated in section 3.2.3.15 for fruits at harvest through ripening until ripeness stage 6 (CSIRO, 1972).

4.2.1.14 Vitamin C content

Vitamin C content was determined by visual titration using 2, 6-dichlophenolindophenol according to AOAC methods (1996) as described earlier in section 3.2.3.5. The analysis was done on fruits at harvest and during ripening until ripeness stage 6 (CSIRO, 1972).

4.2.1.15 Total titratable acidity (TTA)

The method of TTA determination was as earlier described in section 3.2.3.8 with slight modifications. Total titratable acidity was measured on fruits from colour stage 1 to 6.

4.2.1.16 Mineral contents

The minerals determined included Zn, Fe, Mg and K. Analysis was done by atomic absorption spectrophotometry (AOAC, 1996) method as described in section 3.2.3.16. However, the analysis was carried out on fruits at harvest and during ripening to ripeness stage 6 (CSIRO, 1972).

4.2.1.17 Total polyphenol content

A standard curve was prepared using gallic acid (0, 50, 100, 200, 300, 400 and 500 mg/l). Ten grams of pulp was blended in 50 ml distilled water and filtered using cotton wool. 0.1 aliquot of solution was mixed with 5.0 ml 0f 0.2 N Folin-Ciolcateau reagent and 4.0 ml of saturated sodium carbonate. After 2 hrs, the absorbance was

measured at 765 nm in a UV-Vis spectrophotometer (Model UV mini 1240, Kyoto, Shimadzu, Japan). The concentration was read from the standard curve and expressed as mg gallic acid per 100g sample.

4.2.2 Statistical analysis

Data were examined for normality using R software and outliers by scatter plot using the Ms Excel software. Data were then subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS statistical programme (SAS, 2001). The means were compared according to Student Newman Keul's (SNK) test ($\alpha = 0.05$) and Least Significant Difference (LSD) ($\alpha = 0.05$) to test for significant effects. Correlations among maturity indices were tested using MS Excel software.

4.3 Results and discussion

4.3.1 Banana fruit growth curves

Finger development measured as finger length, diameter and curvature over the growing period after flower emergence for cultivars Williams and Grand Nain is shown in Fig. 4.1 A and 4.1B. Fruit growth curves for both banana cultivars followed the pattern of bananas grown in the tropics (Robinson, 1996). The length and diameter of the fruit growth measured displayed a single sigmoid curve typical of bananas and other fruits like the mango (Subhadra and Subramanyan, 1970; Worrell *et al*, 1998). Generally, there was a fast growth for both varieties in the first phase of finger growth followed by a slower but longer growth period which evened out towards the end.

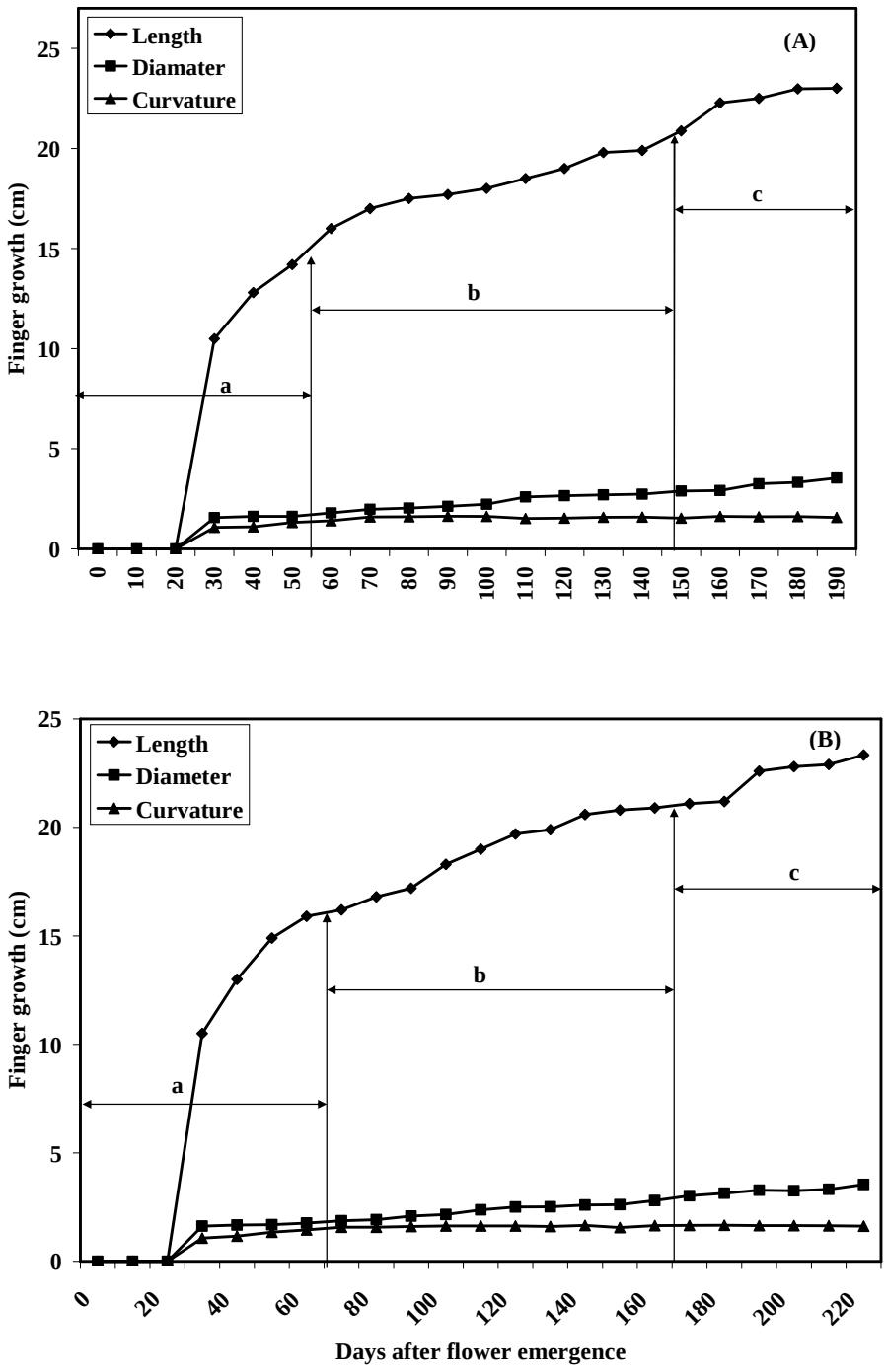

Figure 4.1: Fruit development of cultivar Grand Nain (A) and Williams (B) grown in 2007-2008 in Maragua. a represents cell division, b represents cell enlargement, c represents fruit maturation period according to Robinson (1996).

Finger length increased rapidly in the first phase followed by a slower increase which eventually evened out towards fruit maturation for both cultivars. Diameter, on the other hand, increased at a lower rate until harvest. Curvature also increased at a low rate and then reduced towards harvest time. The rapid growth in the first phase of fruit development represented cell division while rapid cell expansion occurred after this phase followed by fruit maturation (Robinson, 1996). Curvature of both cultivars Grande Nain and Williams followed the trend that is typical of most bananas (Robinson, 1996; Dadzie and Orchard, 1997). The fruits are highly curved as they emerge from the inflorescence and straighten out during growth and development to a characteristic curve of the banana cultivar.

4.3.2 Harvest age, grade, green life and shelflife

The effect of harvest maturity on grade, green life and shelflife of tissue-cultured banana cultivars Grand Nain and Williams is shown in Table 4.1 A and 4.1B. The age of the bunch corresponding to three quarter, light full three quarter, full three quarter and full maturity stages for banana cultivar Grand Nain was 22, 24, 26 and 28 weeks, respectively while for cultivar Williams it was 24, 26, 28 and 30 weeks, respectively. The grade for both banana cultivars was significantly ($p \leq 0.05$) affected by the bunch age with earlier harvested fruits having lower grades than those left to grow for longer periods. Currently, banana fruits for export market need to have a diameter of 31-41 mm (Robinson, 1996) although some competitive markets require a grade of 33-38 mm (Stover and Simmonds, 1987). For both banana cultivars Williams and Grand Nain, fruits harvested at ¾ mature through to full mature had acceptable grade for export. In banana finger diameter (grade) has been shown to

increase with age (Nakasone and Paull, 1998). This is due to rapid cell division that occur about 6 weeks before emergence to 4 weeks after emergence followed by rapid cell expansion at 4-12 weeks after emergence and fruit maturation at 12-15 weeks after emergence. The period may however vary according to growing conditions (Robinson, 1996).

Green life was also affected significantly ($p \leq 0.05$) by the maturity period for both banana cultivars. Full mature fruits having significantly lower green life of about 11 and 6 days for cultivar Williams and Grand Nain, respectively while all the other stages of both banana cultivars had statistically similar green life.

Table 4.1A: Effect of ripening time on grade, green life and shelflife for banana cultivar Grand Nain harvested at different maturity stages.

Maturity stage	Harvest age (weeks)	Grade (mm)	Green life (days)	Shelflife (days)
¾ mature	22	34.50^{c}	14.00^{a}	7.00^{a}
Light full ¾	24	35.53^{c}	11.67^{ab}	6.00^{a}
Full ¾	26	36.83^{b}	9.33^{b}	5.67^{a}
Full mature	28	38.33^{a}	5.67^{c}	5.33^{a}
LSD		1.28	3.52	1.80

Data are means of three replicates. Means followed by the same letter in a column are not significantly different according to LSD test (α=0.05).

Table 4.1B: Effect of ripening time on grade, green life and shelflife for banana cultivar Williams harvested at different maturity stages.

Maturity stage	Harvest age (weeks)	Grade (mm)	Green life (days)	Shelflife (days)
¾ mature	24	32.50^{b}	16.67^{a}	6.33^{a}
Light full ¾	26	34.80^{ab}	15.67^{a}	6.00^{a}
Full ¾	28	34.93^{ab}	15.00^{a}	5.67^{a}
Full mature	30	37.50^{a}	11.00^{b}	5.33^{a}
LSD		4.02	3.07	2.11

Data are means of three replicates. Means followed by the same letter in a column are not significantly different according to LSD test (α=0.05).

Green life represents the period from harvest until the initiation of the respiratory climacteric (Robinson, 1996; Turner, 1997) and it is also defined as the period the fruit remains green and firm (Dadzie, 1998). In order to maintain a firm pulp texture, good colour and flavour, and a bruise-free product, bananas are harvested at a mature green stage and transported to markets (Li *et al*., 1997) hence long green life is very important. A longer green life is preferred for far-off markets or for holding for long durations for orderly marketing. Green life has been found to be negatively correlated to bunch age (Turner, 1997). Harvest time has been found to represent a compromise between leaving the fruit on the plant long enough to maximize yield, but harvesting it soon enough so that sufficient green life remains to market the fruit in an acceptable manner (Turner, 1997). This is confirmed by the results of this study since the optimum maturity stages were found to be ¾ mature, light ¾ and full ¾, corresponding to 22, 24, and 26 weeks for cv. Grand Nain and 24-28 weeks for cv. Williams, which were harvested earlier than full mature stage but which had significantly longer green life compared to the full mature bunches (Table 4.1A and B). Short green life allows the fruit to be sold locally or even consumed in the households. Shelflife was not affected significantly ($p>0.05$) by maturity stage for both cultivars. Similar findings were reported for bunches harvested at different ages where the shelflife was not affected which indicated that the bunches could be harvested together (Ramma *et al*., 1999).

4.3.3 Physical parameters of fingers

The effect of harvest age and stage on finger weight, finger length and peel diameter for cultivars Grand Nain (A) and Williams (B) are shown in Figs. 4.2, 4.3 and 4.4.

The finger weights were significantly ($p \leq 0.05$) influenced by maturity stages for both banana cultivars Grand Nain and Williams. Fingers from full mature bunches were heavier followed by those from full ¾ mature bunches, light full ¾ bunches and lastly by those from the ¾ mature bunches. This agrees with the findings of Ramma *et al*. (1999) that finger weight increases with banana maturation. This could be attributed to cell division followed by cell elongation, maturation and starch deposition as the fruit matures (Nakasone and Paull, 1998). The finger weight for cultivar Grand Nain ranged from 159.88-201.27 g while that for cultivar Williams ranged from 165.22-207.95g.

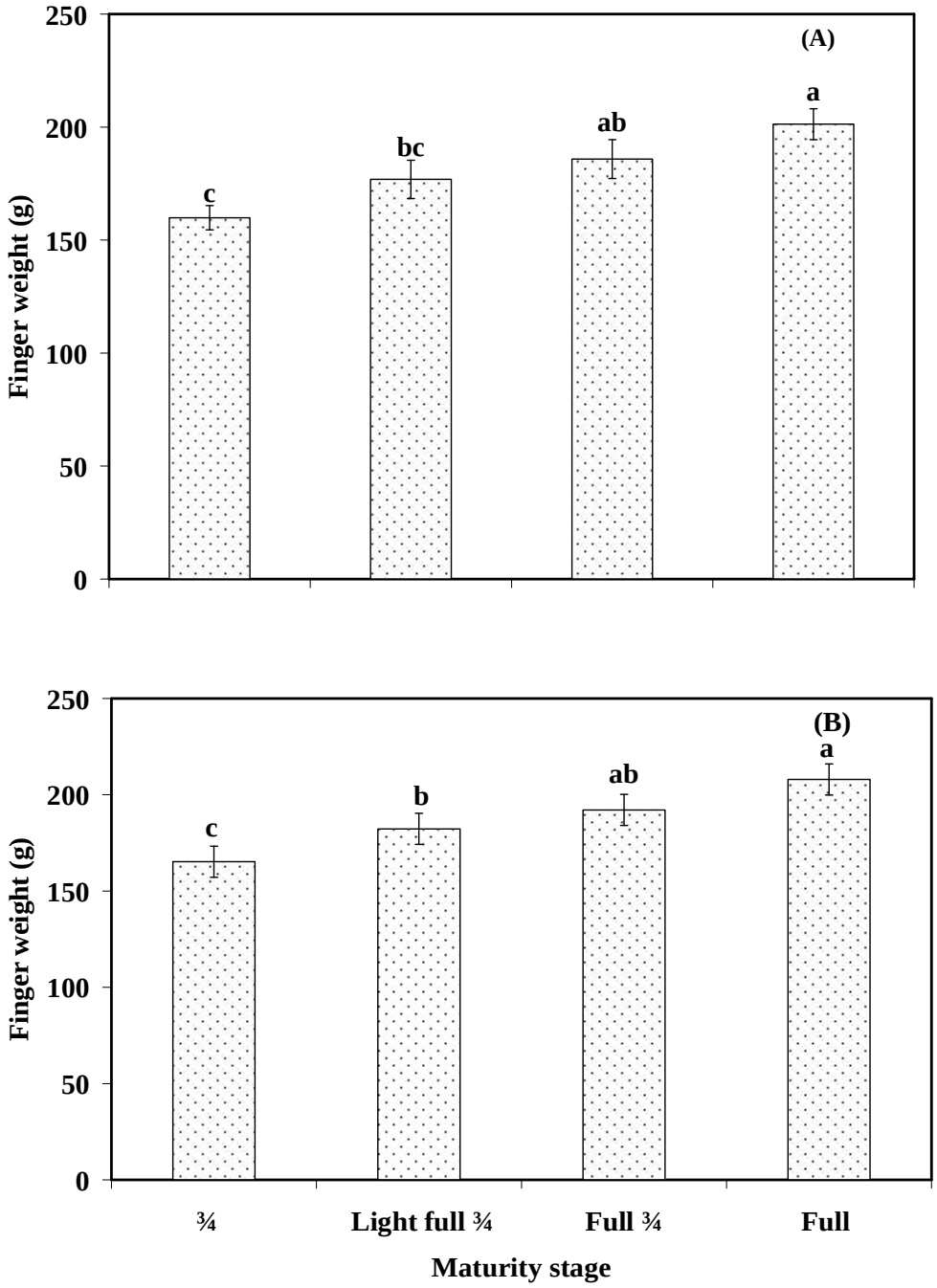

Figure 4.2: Effect of ripening time on finger weight of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Means followed by the same letter in a column are not significantly different according to Student Newman Keul's (SNK) test (α=0.05).

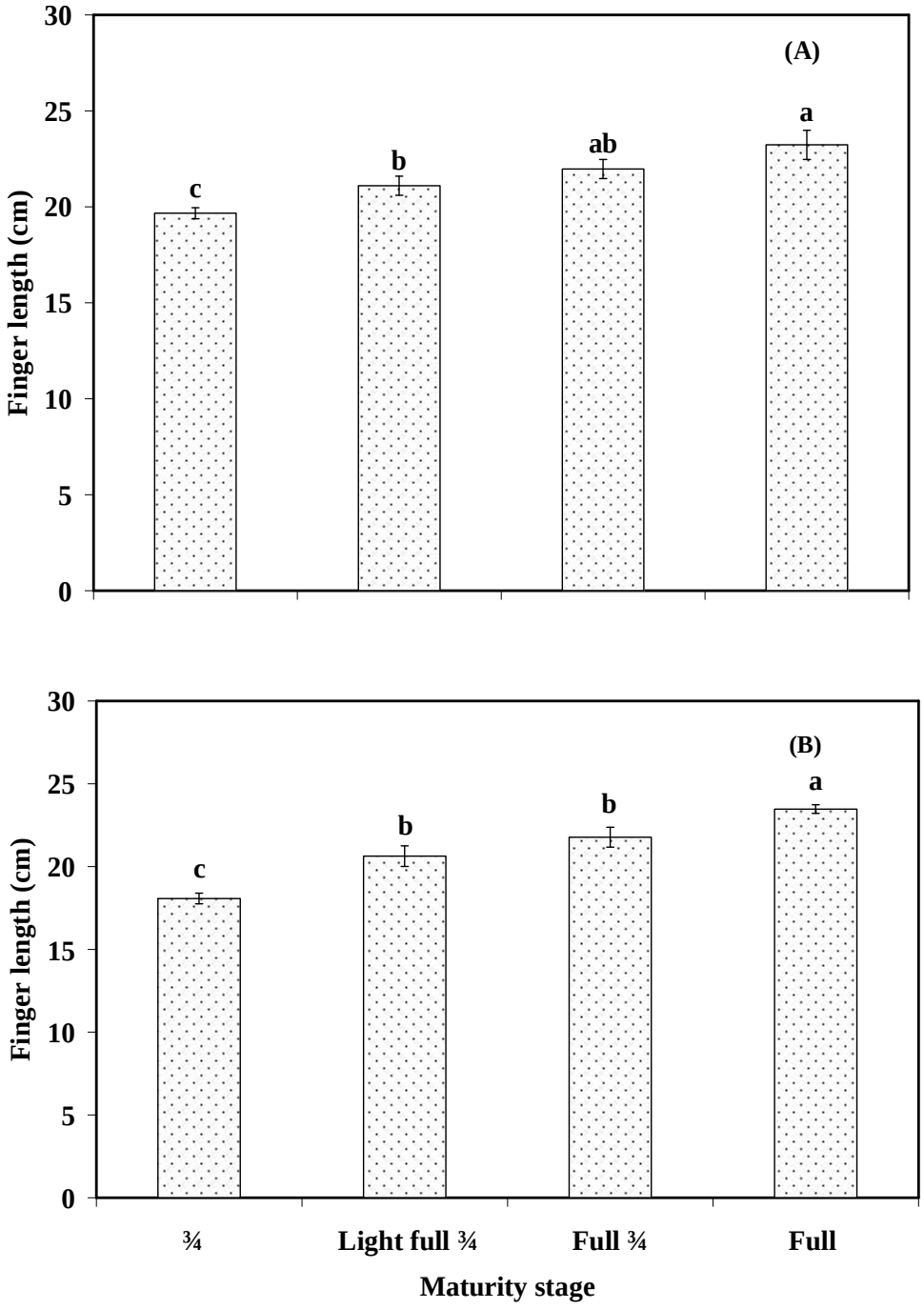

Figure 4.3: Effect of ripening time on finger length of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Means followed by the same letter in a column are not significantly different according to Student Newman Keul's (SNK) test (α=0.05).

Finger length was also affected by maturity stages with fingers from full mature bunches having significantly ($p \leq 0.05$) longer fingers compared to fingers from bunches harvested at earlier maturity stages. Finger length ranged from 19.67-23.23 cm and 18.07-23.47 for cultivars Grand Nain and Williams, respectively. Finger length has been shown to increase with banana maturity with the increase being rapid early in fruit development and reducing during fruit maturation (Stover and Simmonds, 1987; Robinson, 1996). Ramma *et al.* (1999) observed a similar trend in finger length of bunches harvested at different maturities. Acceptable finger length depends on the market. For some international markets, the acceptable finger length should be above 20.3 cm (Robinson, 1996) while others prefer medium size fingers (15-20 cm) (Stover and Simmonds, 1987).

Peel diameter generally decreased with maturity with fingers from the ¾ mature bunches having larger diameters than fingers from other bunch maturities. Banana peel has been shown to increase rapidly during the first month after inflorescence emergence but the growth is reduced with maturation (Robinson, 1996; Turner, 1997).

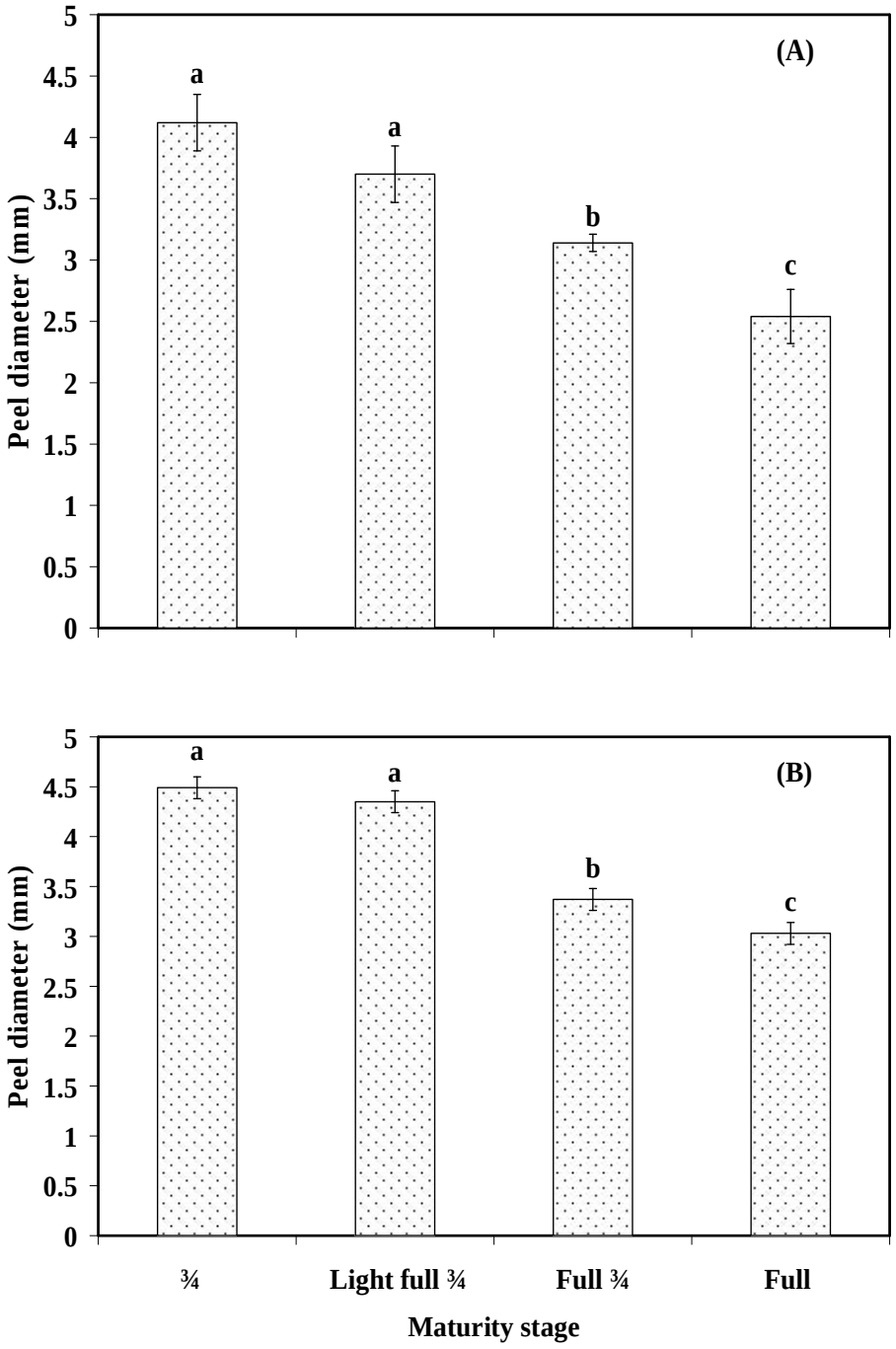

Figure 4.4: Effect of ripening time on peel diameter of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Means followed by the same letter in a column are not significantly different according to Student Newman Keul's (SNK) test (α=0.05).

4.3.4 Pulp/peel ratio

Effect of maturity period on pulp/peel ratio at harvest and during ripening of banana cv. Grand Nain and Williams is shown in Figs. 4.5A and 4.5B, respectively.

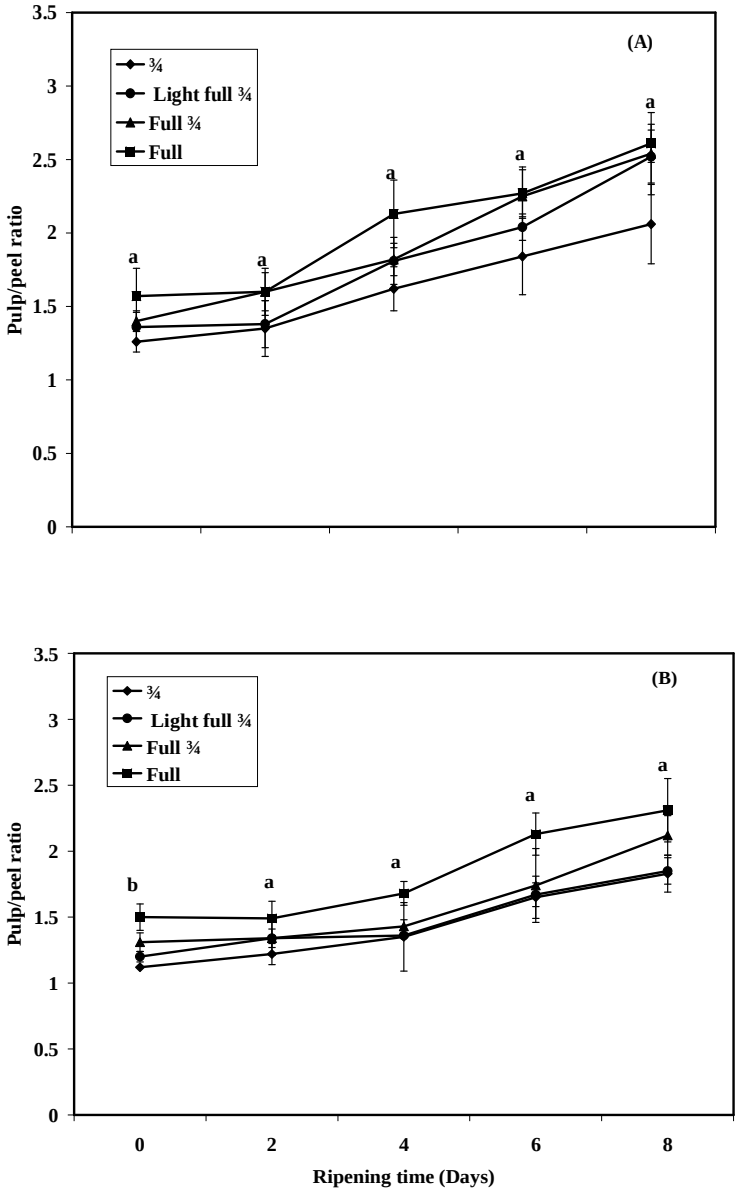

Figure 4.5: Effect of ripening time on pulp/peel ratio of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

For cultivar Grand Nain, there was no significant ($p>0.05$) effect of harvest stage on pulp/peel ratio at harvest and also during the ripening period (Fig. 4.5A). However, more mature fruits had generally higher ratios at harvest and during ripening compared to the less mature fruits. Pulp/peel ratios at harvest ranged from 1.26 – 1.57 for cultivar Grand Nain. Fruits harvested at fully mature stage had significantly ($p\leq0.05$) higher ratios at harvest compared to the other stages of maturity for cultivar Williams (Fig. 4.5B). The ratios ranged from 1.12 to 1.5 at harvest. However, during the ripening period there was no significant ($p>0.05$) effect of maturity stage on pulp/peel ratios.

As the banana fruit develops, the peel develops earlier compared to the pulp hence an initial low pulp/peel ratio (Robinson, 1996). However, as the fruit matures, the pulp increases rapidly and the pulp/peel ratio increases and hence the generally higher pulp/peel ratios of more mature fruits at harvest (Turner, 1997). The pulp/peel ratio represents the edible portion of the banana and hence more mature fruits have more edible portion compared to the less mature ones due to more filling at harvest (Bananuka *et al.*, 1996). This could be used to predict maturity although it is a destructive method but correlates well with diameter at +0.98 and +0.86 for Grand Nain and Williams, respectively (Table 4.2). Indeed, pulp/peel ratio has been used as a maturity index where a ratio of more than 1 has been found to be a good indicator of banana harvest maturity for optimum postharvest quality which ensures an acceptable eating quality of the fruits on ripening (Turner, 1997; Ramma *et al.*, 1999). Pulp/peel ratio increased throughout the ripening period for all maturity stages for both banana cultivars but not significantly ($p>0.05$). Moisture has been

shown to move from the peel to the pulp of banana peel during ripening in response to the osmotic potential (Turner, 1997) as starch is converted to sugar in the pulp, hence increased pulp/peel ratio on ripening.

4.3.5 Peel and pulp firmness

The effect of maturity stage on changes during ripening in peel firmness measured objectively and subjectively for cultivars Grand Nain and Williams is presented in Figs. 4.6A and 4.6B and 4.7 A and 4.7B, respectively. There was no significant difference in peel firmness measured objectively (Figs. 4.6 A and 4.6B) and subjectively (Figs. 4.7A and 4.7B) for the different maturity stages at harvest and during ripening. Similar findings were noted for pulp firmness (Figs. 4.8 A and 4.8B) for both banana cultivars.

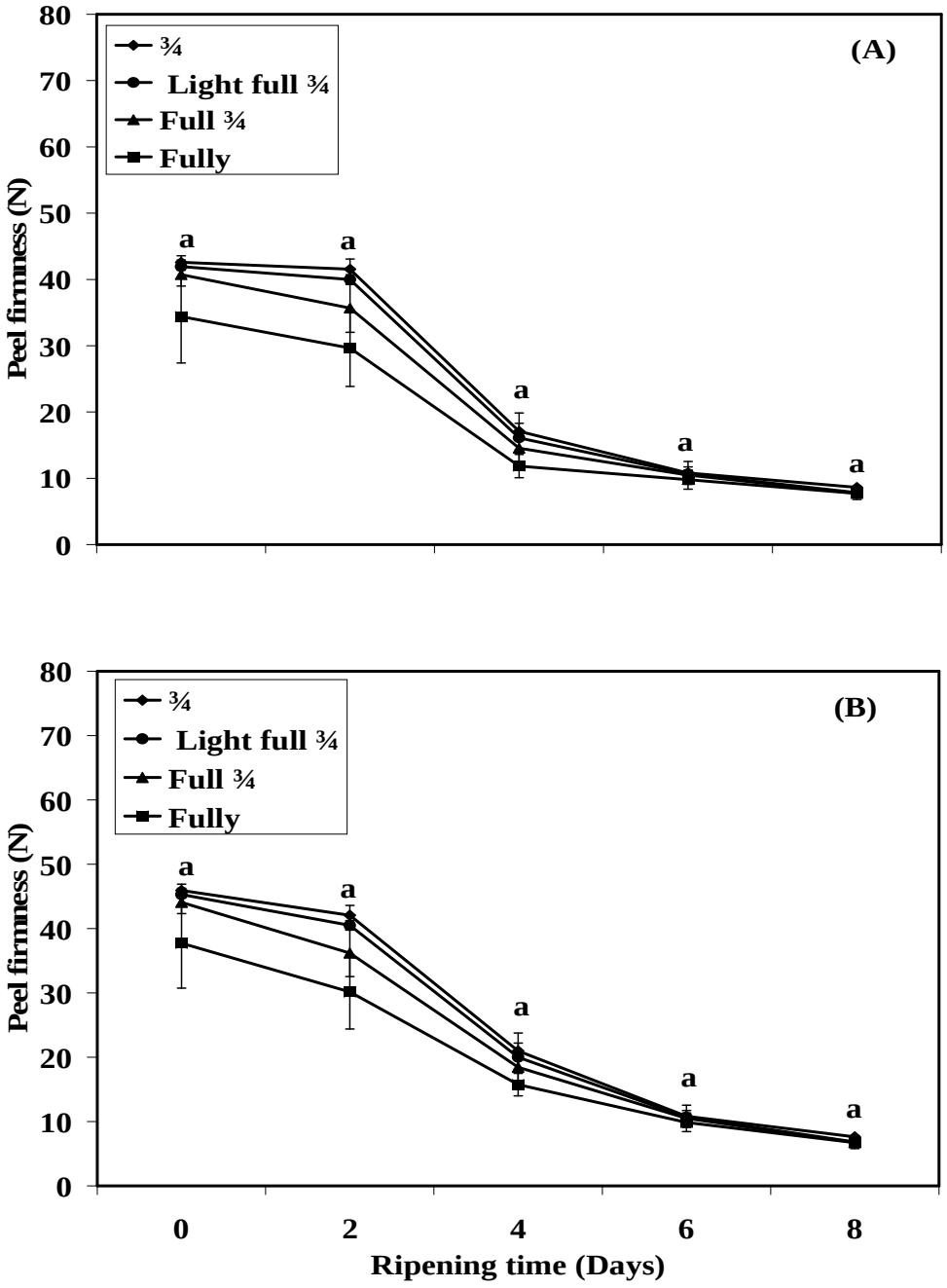

Figure 4.6: Effect of ripening time on peel firmness (N) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

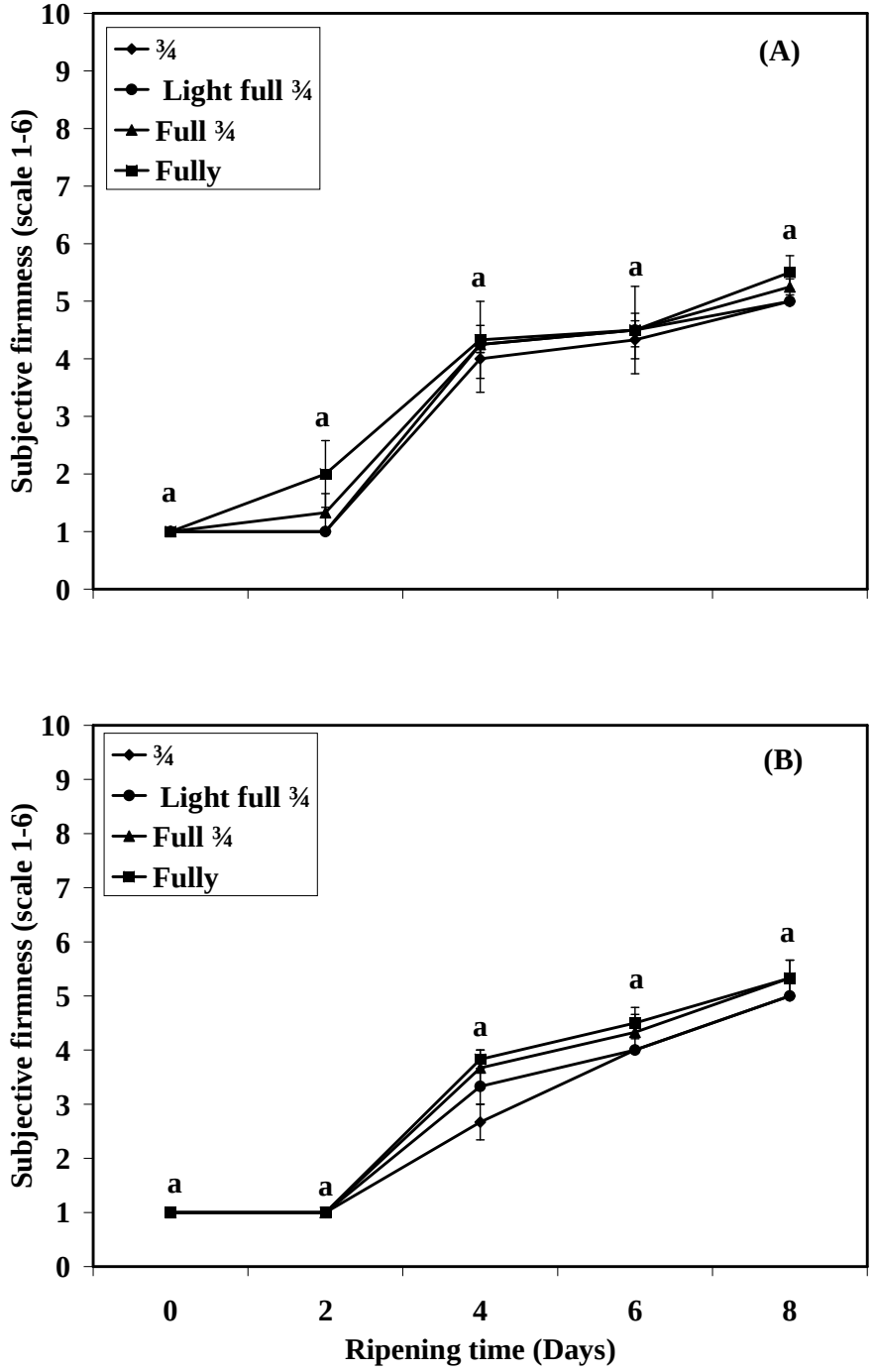

Figure 4.7: Effect of ripening time on subjective firmness (1=hard to 6=very soft) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

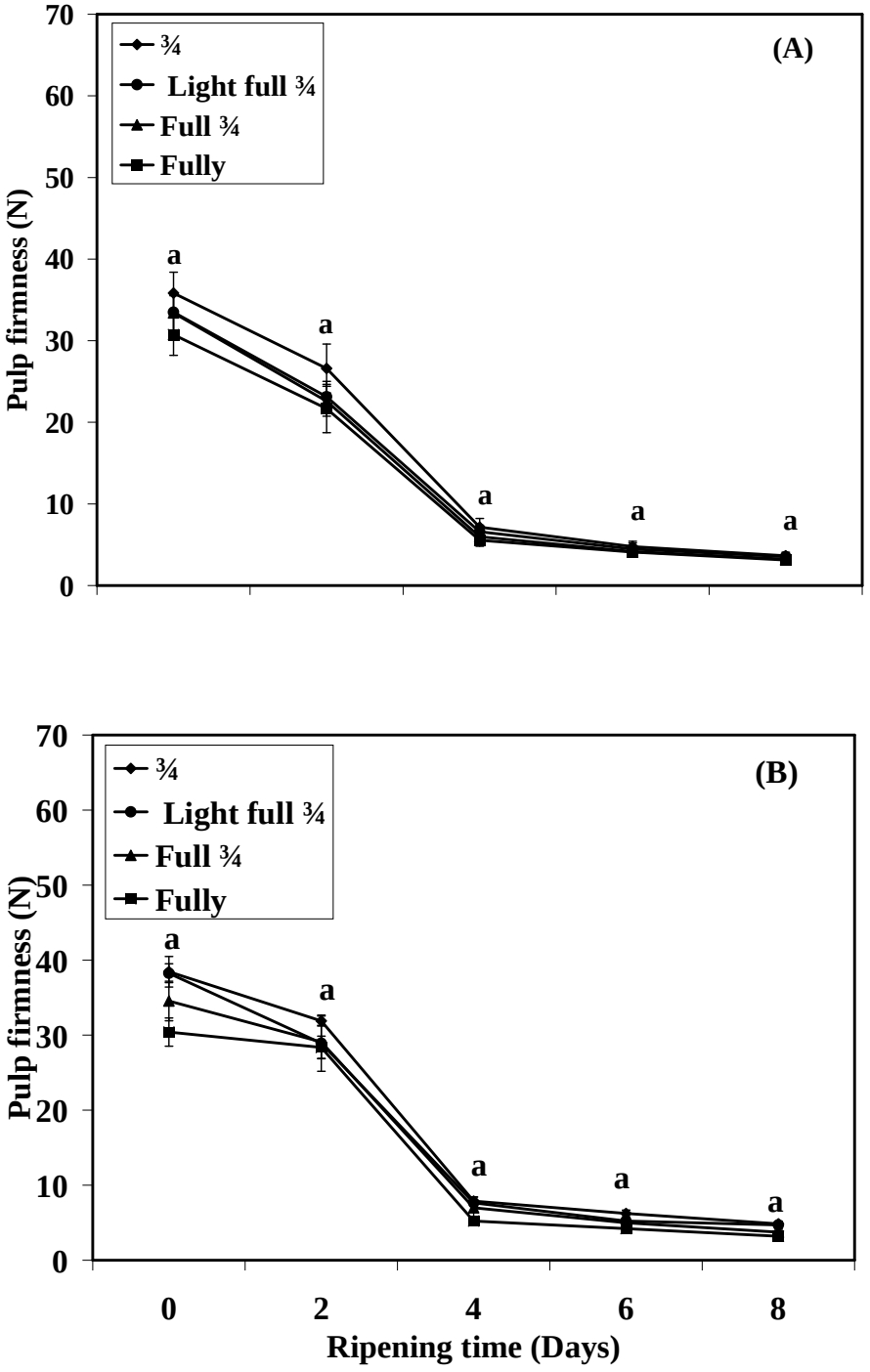

Figure 4.8: Effect of ripening time on pulp firmness (N) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Generally, both peel and pulp firmness decreased from less mature fruits to the most mature fruits at harvest and during ripening irrespective of the method of measurement used. Regardless of initial firmness, bananas harvested at different maturities had similar pulp and peel firmness after 6 days of ripening. This differs with the findings of Mustaffa *et al*. (1998) who indicated that fruits of different maturities had significantly different fruit firmness at harvest. However, the difference could have been due to differing maturities under comparison. Fruit firmness has been shown to decrease with maturity possibly due to starch being hydrolysed to sugars as the fruit matures rendering them less firm compared to the less mature ones (Seymour *et al.,* 1993). Firmness of both peel and pulp for both banana cultivars decreased on ripening. This could have been due to some compositional changes such as conversion of propectin to pectin that occurred in the cell wall and also due to starch degradation and loss of turgor (Seymour *et al.,* 1993; Ratule *et al*., 2007).

In banana fruit, cell walls in both peel and pulp have been shown to undergo modifications during ripening with the most apparent changes occurring in the pectic polysaccharides which may become more soluble and show a reduction in molecular weight (Seymour et al., 1993). This may be due to the action of such enzymes as polygalacturonase and pectin methyl esterase which are involved in the pectin degradation in the cell wall and middle lamella (Mustaffa et al., 1998). Also it has been shown that the disassembly of the fruit cell wall is largely responsible for softening and textural changes during ripening although the roles of particular cell

wall altering and/or of cell modifying enzymes that bring about these changes are not clearly understood (Brummel and Harpster, 2001).

4.3.6 Starch content

Effect of harvest maturity on starch content at harvest and during ripening is shown in Fig. 4.9 A for banana cultivar Grand Nain and Fig. 4.9 B for cultivar Williams. There was no significant difference ($p>0.05$) at harvest and during ripening in the starch content for both banana cultivars. However, fruits from more mature bunches had less starch at harvest and during ripening compared to those from less mature bunches.

Banana fruits accumulate starch during growth and as they mature, it is gradually hydrolysed to sugars. This hydrolysis continues during ripening hence the reduction of starch during ripening (Mustaffa *et al.*, 1999). Starch which constitutes 20-25% of fresh weight of unripe bananas is almost entirely converted to sugars. This conversion to sugars occurs rapidly during the ripening process which allows the fruits to attain desired sweet taste for consumption. A very small percentage of about 2-5% is also lost as carbon dioxide in respiration (Hubbard *et al.*, 1990). Enzymes for both hydrolytic and phosphorolytic breakdown of starch have been identified in the banana (Seymour *et al.*, 1993; Omonkhua *et al.*, 2006). The primary product of starch breakdown is sucrose via sucrose phosphate synthase (Seymour *et al.*, 1993). The hexose sugars then arise from sucrose hydrolysis perhaps by the action of acid invertase in the vacuole whose activity has been shown to increase during banana ripening. The conversion of starch to sucrose and sucrose turnover creates a high

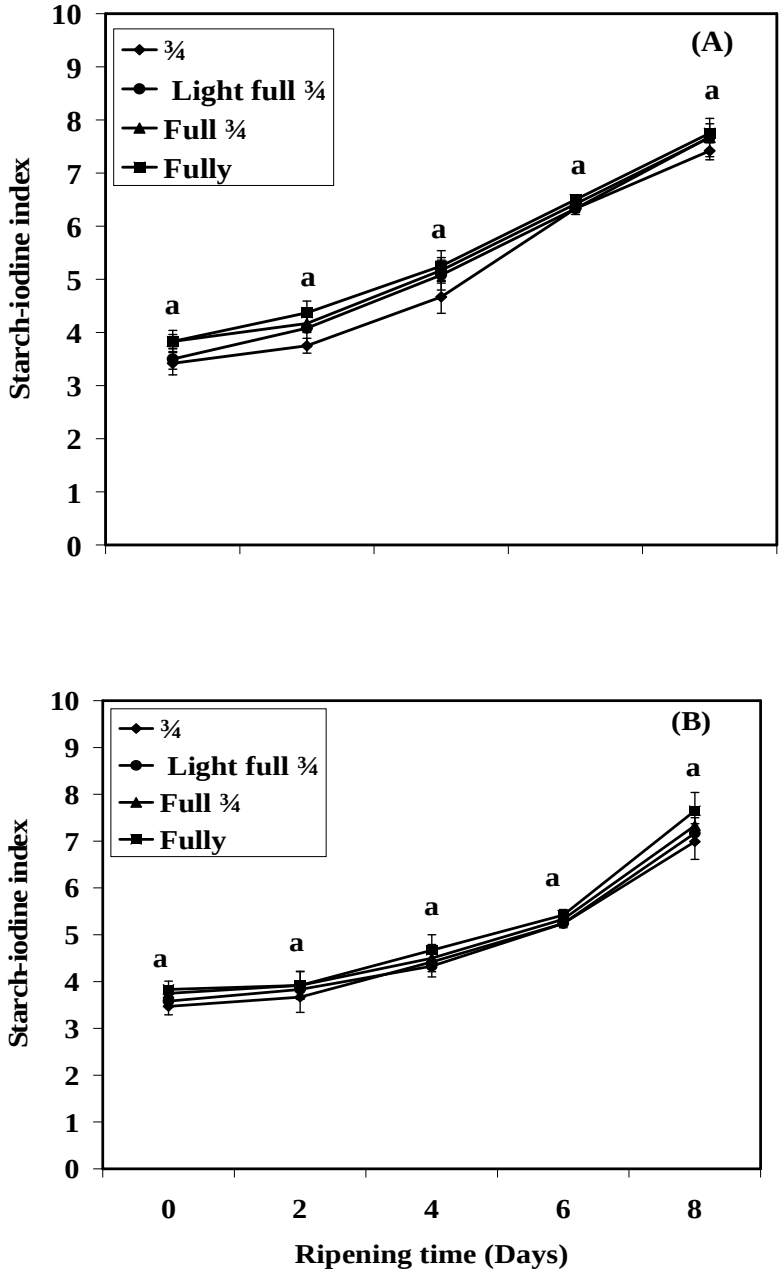

Figure 4.9: Effect of ripening time on starch content (starch-iodine index according to the Cornell starch chart with a scale of 3-8 with 3=all starch and 8=no starch) of Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

demand for ATP, and sugar accumulation and respired carbon dioxide were highly correlated (Hubbard *et al.*, 1990; Seymour *et al.*, 1993).

4.3.7 Total soluble solids (TSS) and sugar content

There was a significant difference in total soluble solids (TSS) content for cultivar Grand Nain (Fig. 4.10 A). Total soluble solids contents of cultivar Williams was also affected significantly ($p \leq 0.05$) (Fig. 4.10 B). This difference occurred at the early stages of ripening for cultivar Grand Nain and at harvest for cultivar Williams but none during ripening and at eating ripe stage for both banana cultivars. Generally, total soluble solids increased from harvest through ripening for both banana cultivars. Fruits from more mature bunches generally had higher total soluble solids content at harvest and during ripening compared to those from less mature bunches although at eating ripe stage the values were similar. For Grand Nain at harvest, full mature fruits had higher TSS content of about 8.39% compared to fruits harvested at full ¾ with 7.94%, followed by those harvested at light full ¾ at 7.72% and the lowest content was of those harvested at ¾ mature at 6%. At fully ripe stage, the TSS content was 26.05%, 25.84, 25 and 24 % for fully mature, full 3/4, light full ¾ and ¾ mature respectively although there were no significant ($p > 0.05$) differences.

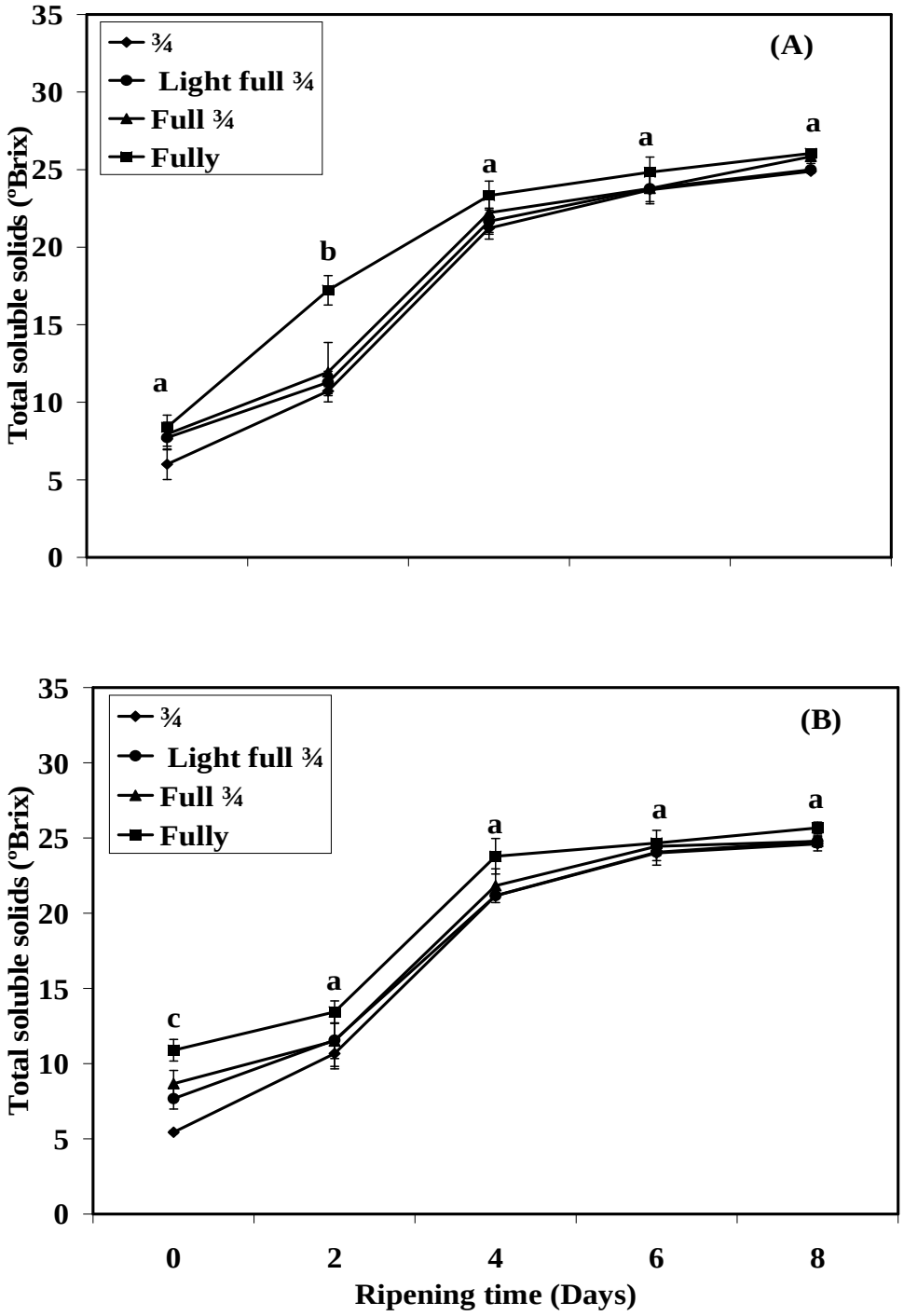

Figure 4.10: Effect of ripening time on TSS (ºBrix) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Total soluble solids have been shown to increase gradually during fruit development and maturity as reported for banana cultivar 'Montel' and 'Berangan' (Mustaffa *et al.*, 1998). As maturity increases, starch is converted to sugars and hence the higher TSS content as the banana fruits matured. Increased TSS during maturation and ripening could also have been due to partial breakdown of pectins, celluloses (Roe and Bruemmer, 1981; De Lima *et al.*, 2001). In most ripe fruits including banana, sugar is the main component of TSS resulting from starch breakdown to complex sugars and later to simple sugars (Marriot, 1980; Seymour *et al*., 1993). The rise in TSS content was rapid in the early stages of ripening and slowed down during the later stages of ripening probably due to reduced starch content as ripening proceeded. Similar trends have been found in other fruits such as mango (Peter *et al*., 2007). Total soluble solids content correlated well with harvest age and may be used as a maturity index (Hilaire, 2003) but has limitations as this method of evaluation is destructive.

Sugar content as fructose, glucose, sucrose and total sugars increased from harvest throughout ripening up to eating ripe stage for both cultivar Grand Nain (Figs. 4.11, 4.12) and Williams (Figs. 4.13, 4.14). There was a significant effect ($p \leq 0.05$) of maturity stage at harvest on fructose for cultivar Grand Nain at harvest but not during ripening. Other sugars were not affected significantly ($p > 0.05$) at harvest and during ripening for cultivar Grand Nain. Fructose, glucose, sucrose and total sugars content were not influenced significantly ($p > 0.05$) by maturity stage at harvest and during ripening for banana cultivar Williams. However, for both banana cultivars, the most mature fruits had higher sugar contents from harvest to ripening. The total sugar

content of about 24% for both banana cultivars and respective contents of sucrose, glucose and fructose at fully ripe stage compares well with values reported by John and Marchal (1995), Robinson (1996), Laylieam and Kosittrakun (1998) and Bugaud *et al*. (2006).

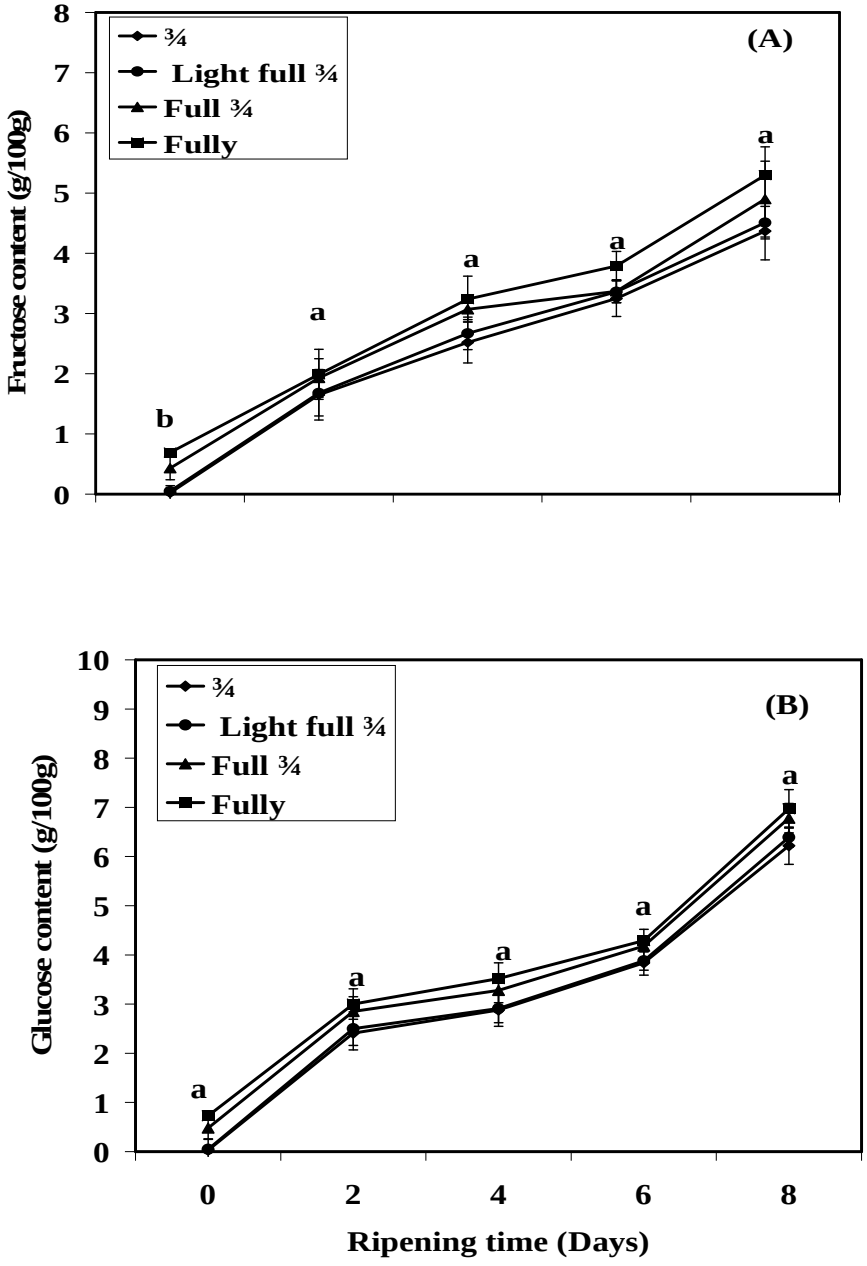

Figure 4.11: Effect of ripening time on fructose (A) and glucose (B) content (g/100g FW) of banana fruit pulp of banana cultivar Grand Nain harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

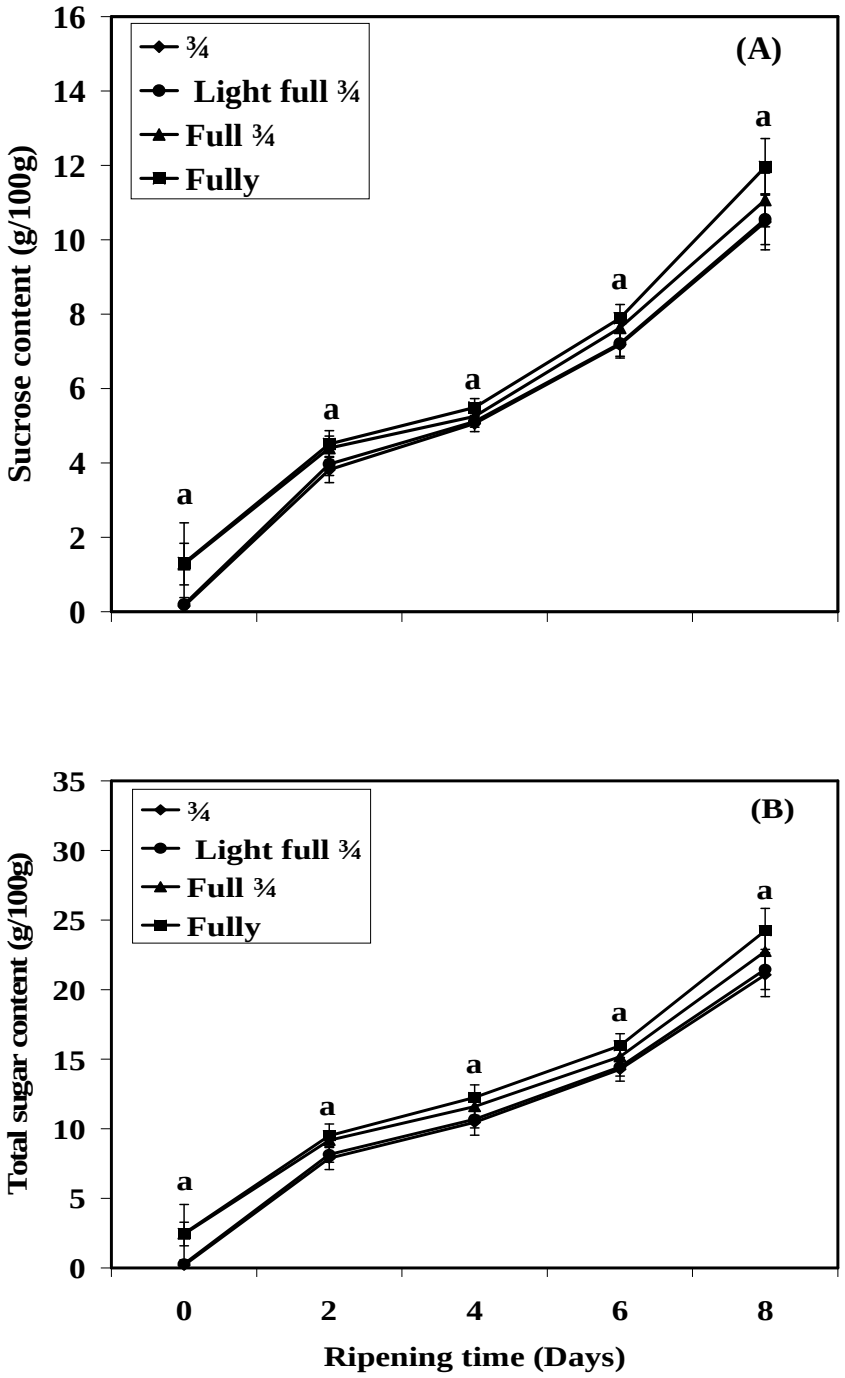

Figure 4.12: Effect of ripening time on sucrose (A) and total sugar (B) content (g/100g FW) of banana fruit pulp of banana cultivar Grand Nain harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

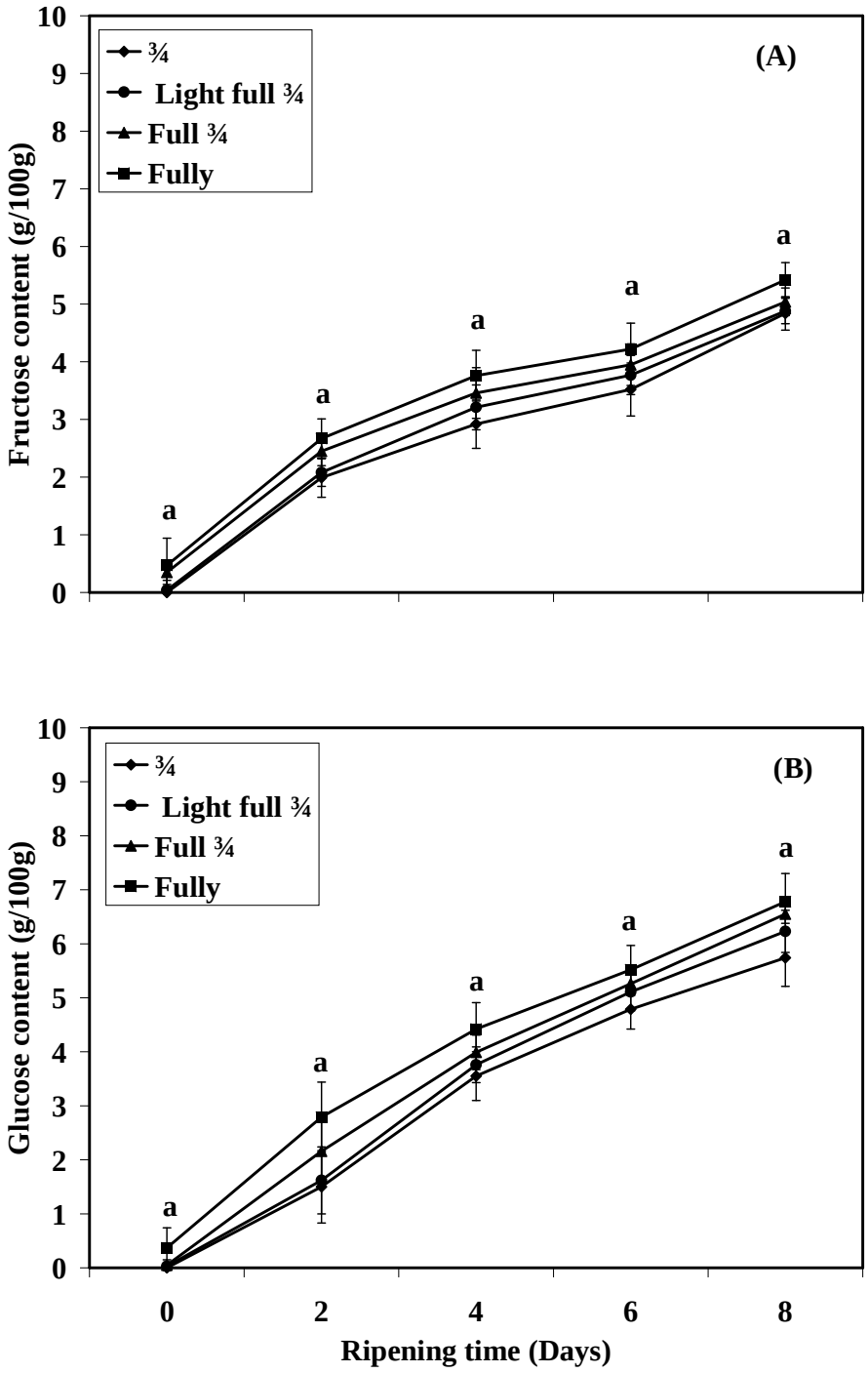

Figure 4.13: Effect of ripening time on fructose (A) and glucose (B) content (g/100g FW) of banana fruit pulp of banana cultivar Williams harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

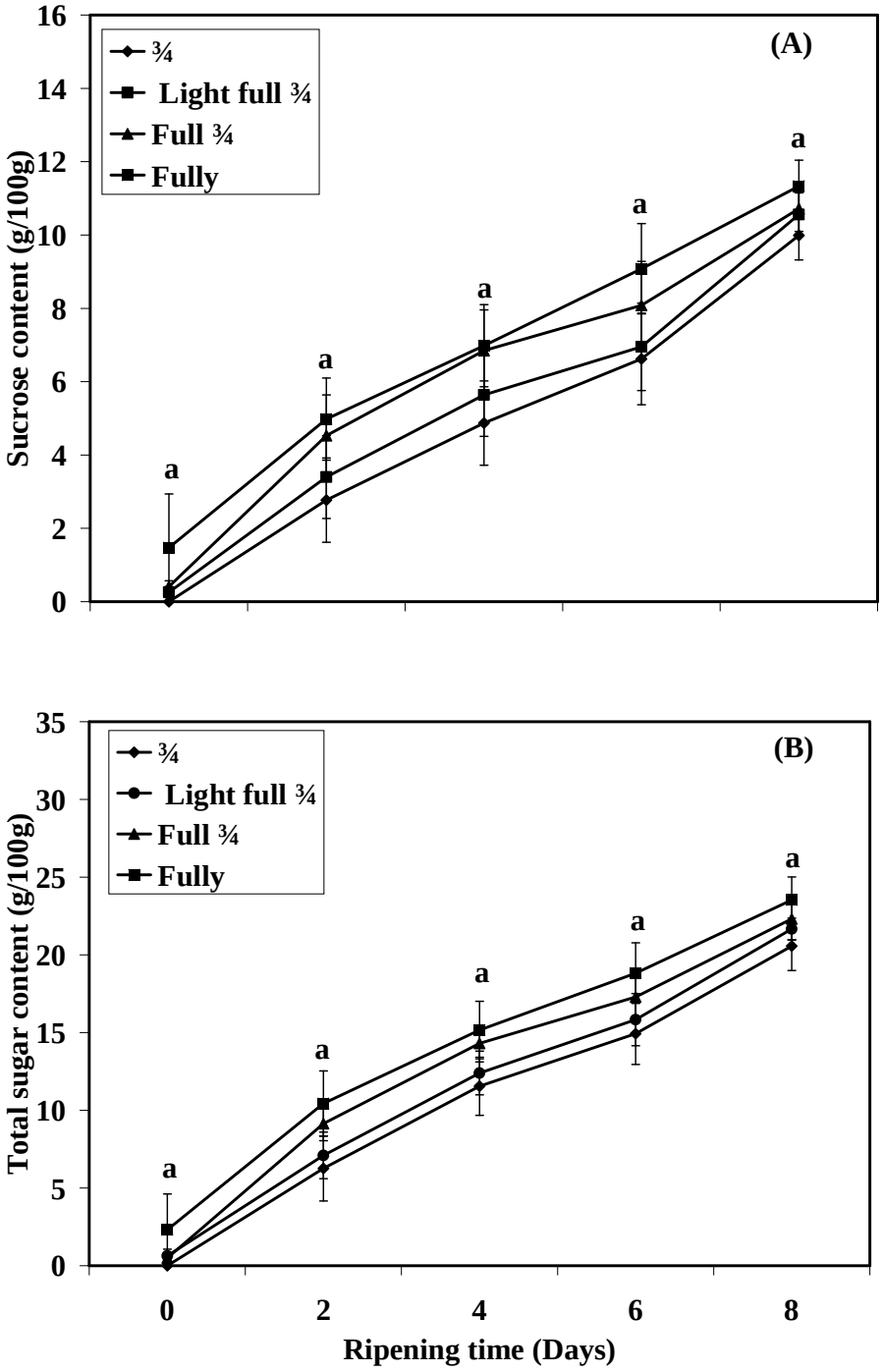

Figure 4.14: Effect of ripening time on glucose (A) and total sugar (B) content (g/100g FW) of banana fruit pulp of banana cultivar Williams harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

As the banana fruit matures starch is hydrolysed to sugars hence the increase in sugar content in more mature fruits at harvest. Starch hydrolysis continues during ripening hence the increase in sugars throughout the ripening period (Seymour *et al*., 1993; Turner, 1997). In bananas, the main change during ripening is the change of starch to sugars and the peel colour is closely correlated with starch/sugar ratio (Robinson, 1996). Complex sugars are also converted to simple sugars during ripening (Omonkhua *et al*., 2006). During early stages of ripening, the ratio of sugars is about 65 sucrose: 20 glucose: 15 fructose which indicates that sucrose appears earlier and hexose sugars at later stages. Several enzymes are involved in starch hydrolysis with fructose 2,6-biphosphate initiating the hydrolysis during the ethylene peak (Robinson, 1996). However, starch degeneration in banana appears to be mediated mainly by amylase and starch phosphorylase (Omonkhua *et al*., 2006). Specifically, starch phosphorylase and β-amylase are important in the initial stages of banana ripening while α-amylase is highest at the climacteric peak. Other enzymes involved include invertase which has been shown to reduce the sucrose pool (Omonkhua *et al*., 2006). Amylases and phosphorylase therefore participate in starch hydrolysis while invertase catalyses the reaction that converts sucrose into glucose and fructose.

4.3.8 Total titratable acidity (TTA)

Total titratable acidity increased with ripening for all maturity stages from harvest although not significantly for both cultivars Grand Nain and Williams (Figs. 4.15 A and 4.15 B, respectively). Other workers reported that TTA differed for different maturity stages at harvest through to ripening (Laylieam and Kosittrakun, 1998).

The difference could have been due to difference in the stages of maturity under study or due to cultivar differences. The acid content of fully ripe bananas of 0.45% and 0.49% for banana cultivars Grand Nain and Williams, respectively, compares well with what is reported for ripe bananas (Marriot, 1980).

Malic acid, citric and oxalic acids are the main acids found in ripening banana (Marriot, 1980; Seymour et., 1993; Turner, 1997). The ratio between sugars and acid in the ripening banana contributes a lot to the taste and it increases with ripening (Turner 1997) giving the fruit a good banana taste. The astringent taste of unripe banana is probably due to the oxalic acid content, which undergoes significant decarboxylation during ripening probably due to the action of oxalate oxidase (Seymour *et al*., 1993).

Organic acids normally decrease in several fruits except in bananas as they are respired or converted to sugars (Wills *et al*., 1989; Seymour *et al*., 1993). Citric and malic acids are the most significant acids at the green stage. However, the increase in TTA during ripening may be due to increase in malic acid whose content has been shown to rise from 1.8 to 6.2 meq/100g with ripening (John and Marchal, 1995). Several enzymes can have an influence on the levels of organic acids in bananas; malate synthase, activity of which decreases during ripening; malic enzyme, which is involved in the decarboxylation of malic acid and phosphoenolpyruvate carboxylase, which plays a part in the formation of malic acid (John and Marchal, 1995).

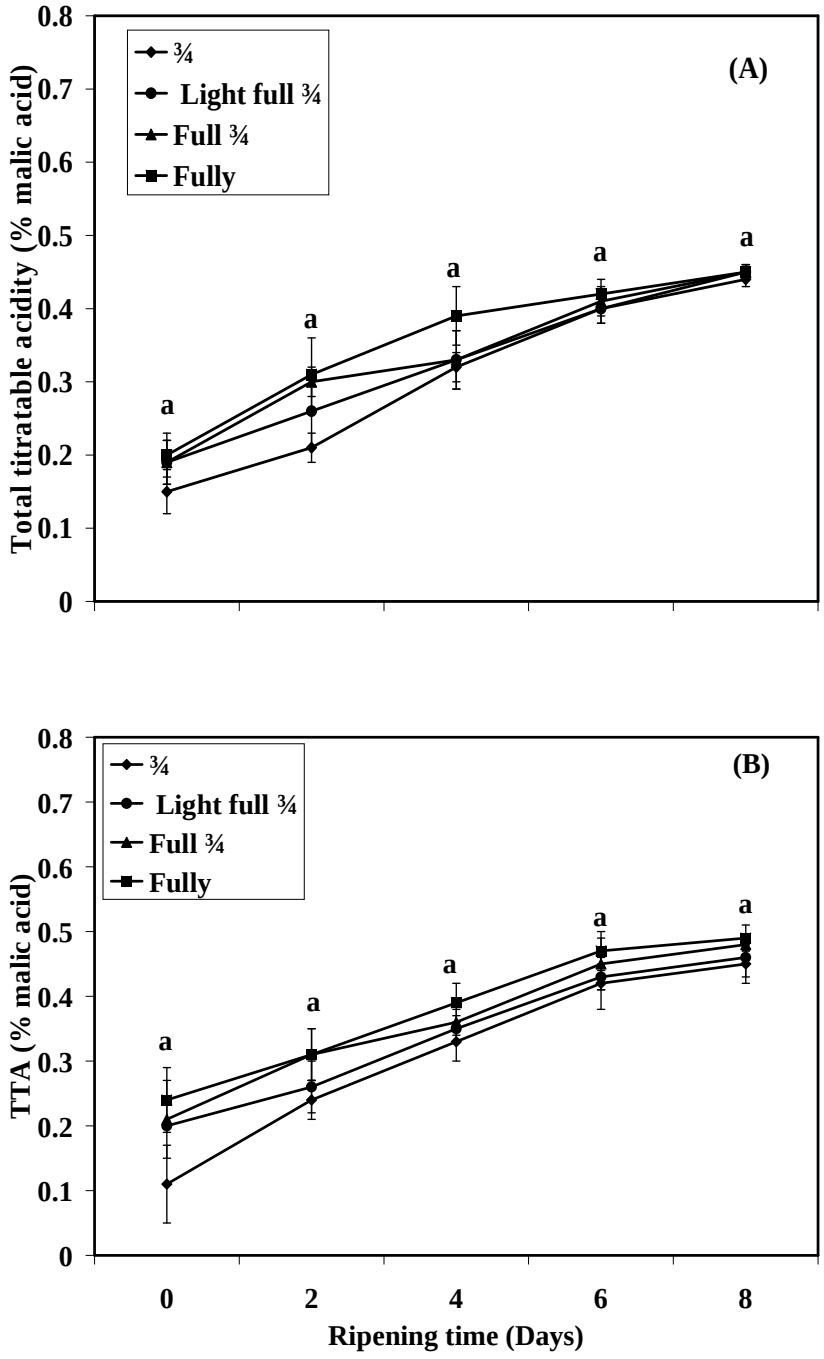

Figure 4.15: Effect of ripening time on total titratable acidity (%TTA) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

4.3.9 Vitamin C (ascorbic acid) content

Vitamin C (ascorbic acid) content increased with maturity and with ripening although not significantly ($p>0.05$) for both banana cultivars Grand Nain and Williams (Figs. 4.16 A and 4.16 B). Ascorbic acid content of 19.94-22.12 and 19.98-22.42 mg/100g for fully ripe banana fruits of cultivars Grand Nain and Williams respectively were in agreement with the expected levels of 20 mg/100g of banana fruits (Stover and Simmonds, 1987).

At all stages, the most mature fruits had the highest ascorbic acid content while the least mature had the least ascorbic acid content. Ascorbic acid is more stable in acidic conditions (Nagy, 1980) and hence more mature fruits with higher TTA had slower ascorbic acid degradation compared to the less mature ones, although the differences were not significant. Unripe banana fruits have been shown to have less ascorbic acid compared to ripe ones (Robinson, 1996). Ascorbic acid plays a role in the browning reactions where it inhibits the process (Turner, 1997).

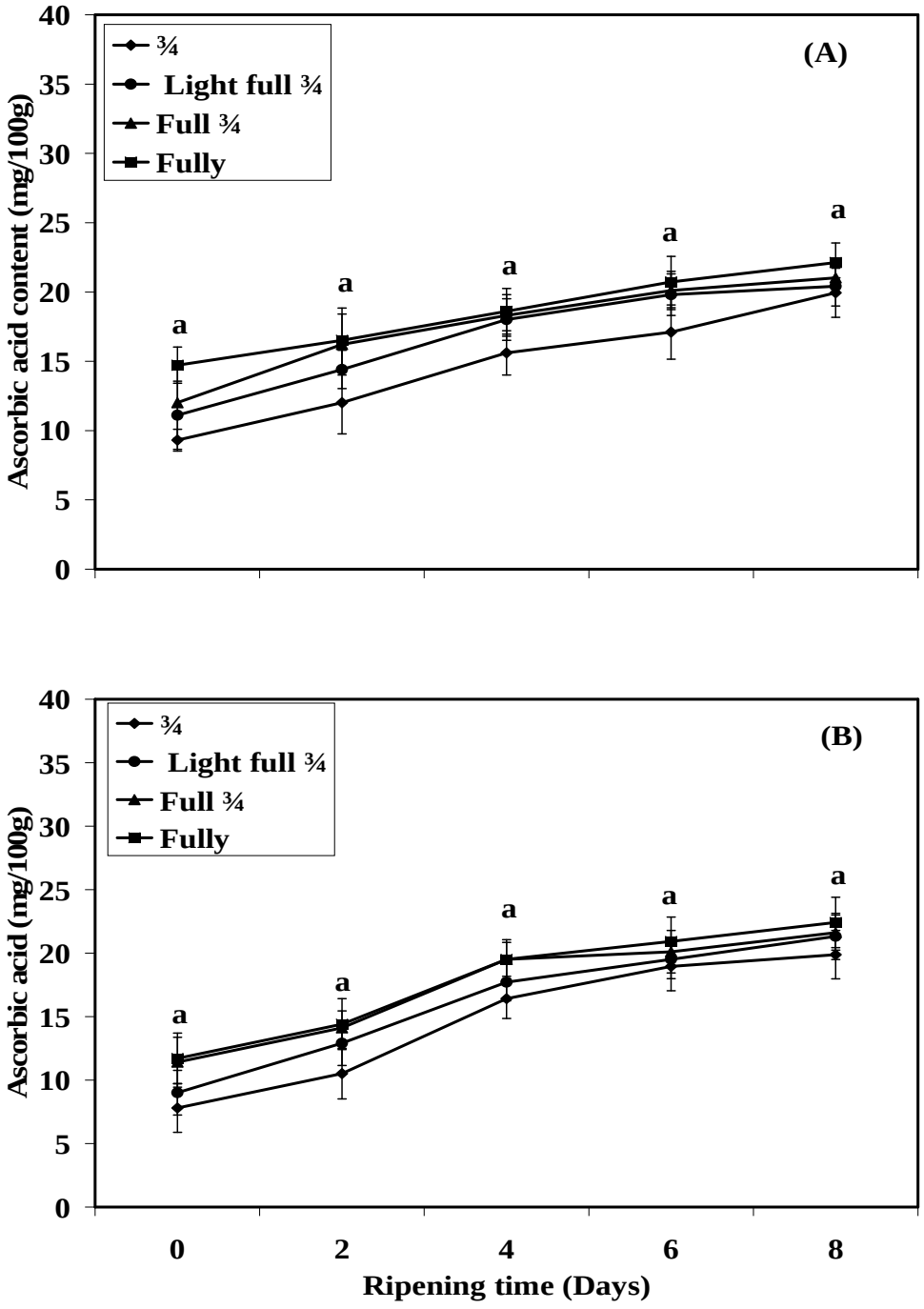

Figure 4.16: Effect of ripening time on vitamin C (mg/100g FW) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

4.3.10 Peel colour

Colour measured subjectively gradually changed from green to light green and rapidly to yellow as the fruits ripened for both banana cultivars (Figs. 4.17 A and 4.17 B). The more mature fruits had lighter green colour compared to the less mature fruits at harvest and during ripening although insignificant ($p>0.05$) for cultivar Grand Nain but significant ($p\leq0.05$) for cultivar Williams at the eating ripe stage where the full mature fruits were more yellow than fruits from all other maturity stages. As the fruits mature on the plant, they turn lighter green and this could be used as a maturity index as the more mature fruits have light green colour compared to the immature ones (Dadzie and Orchard, 1997). However, this is a highly subjective method and would require a lot of experience. During ripening, the peel colour changes from light green to bright yellow due to the destruction of chlorophyll and unmasking of carotenoid pigment also present in the unripe peel (Robinson, 1996).

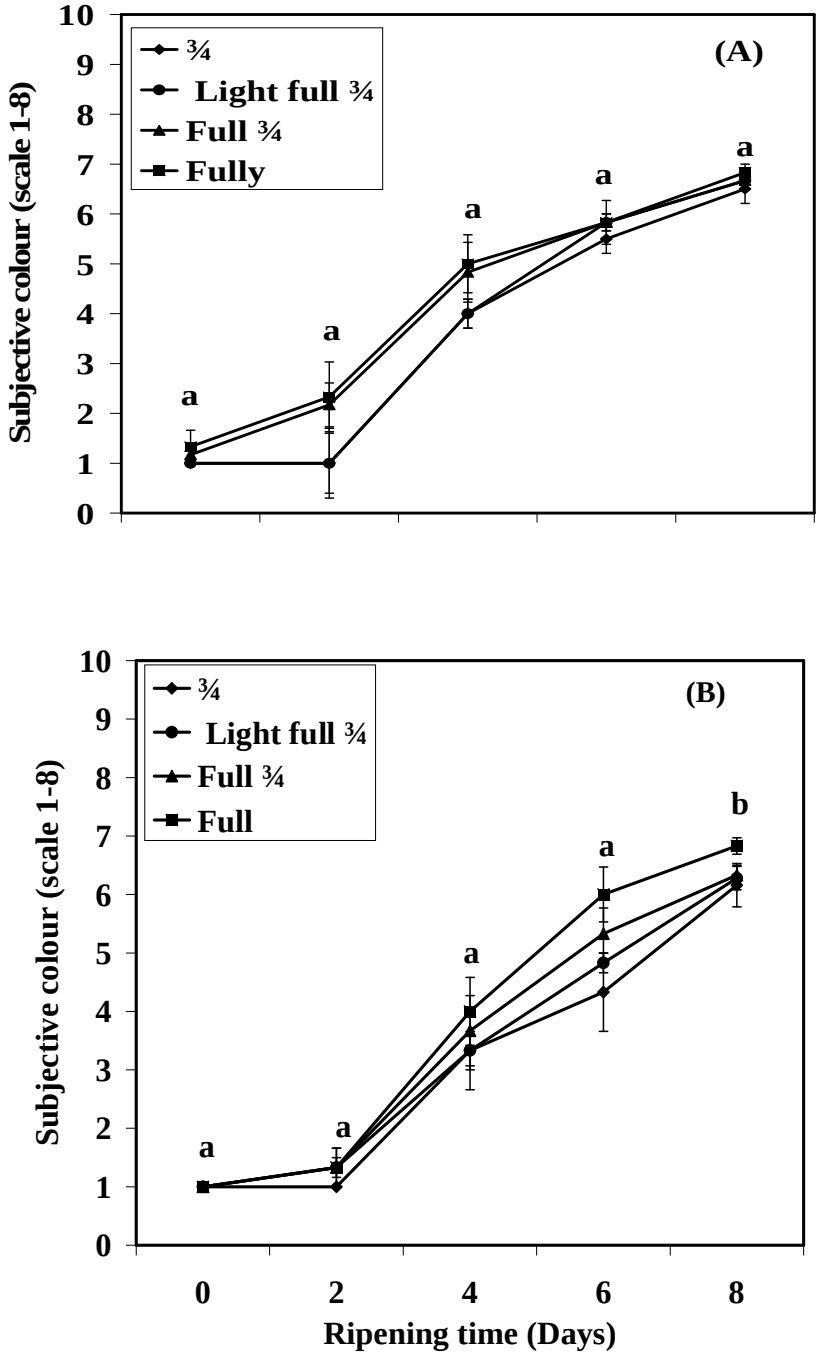

Figure 4.17: Effect of ripening time on subjective peel colour of banana Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

4.3.1 Hue angle and lightness

The hue angle of the peel decreased from harvest to ripening although the differences were not significant ($p>0.05$) for all maturity stages for both banana cultivars Grand Nain and Williams (Figs. 4.18 A and 4.18 B). However, the hue angle for more mature fruits was always lower compared to that of the less mature fruits due to the lighter tones of their green colour at all stages. The hue angle of the pulp was not influenced significantly ($p>0.05$) by the maturity stages at harvest and during ripening for both banana cultivars (Figs. 4.19 A and 4.19B). This may indicate that the fruits were harvested at similar physiological maturity. The hue angle of the peel decreased as the peel changed colour from green to yellow. The hue angle values ranged from 115.59º-113.43º, 94.90º-92.25º and 88.38º-83.23º for unripe, half-ripe and ripe peels, respectively, for banana fruits of cultivar Grand Nain for all maturity stages. The hue angle values for peels were, 119º-109.31º, 101.38º-94.81º and 90.69º-82.89º for unripe, half ripe and ripe peels, respectively, for cultivar Williams. This compares well with reported values of 122, 109 and 97 for unripe, half-ripe and ripe peels of banana fruits of banana cultivar Gran Enana (Hernadez *et al*., 2006).

The hue angle of the pulp decreased slightly after harvest as the fruit turned from whitish to cream and later to yellowish cream on ripening. Values representing this change were 95.81º-94.23º, 92.37º-90.92º and 89.84º-88.38º for unripe, half-ripe and ripe pulp, respectively for banana fruits of cultivar Grand Nain and 95.76º-94.19º, 92.28º-90.65º and 89.93º-88.53º for unripe, half-ripe and ripe pulp respectively, for fruits of cultivar Williams. Other workers (Hernadez *et al*., 2006) have reported

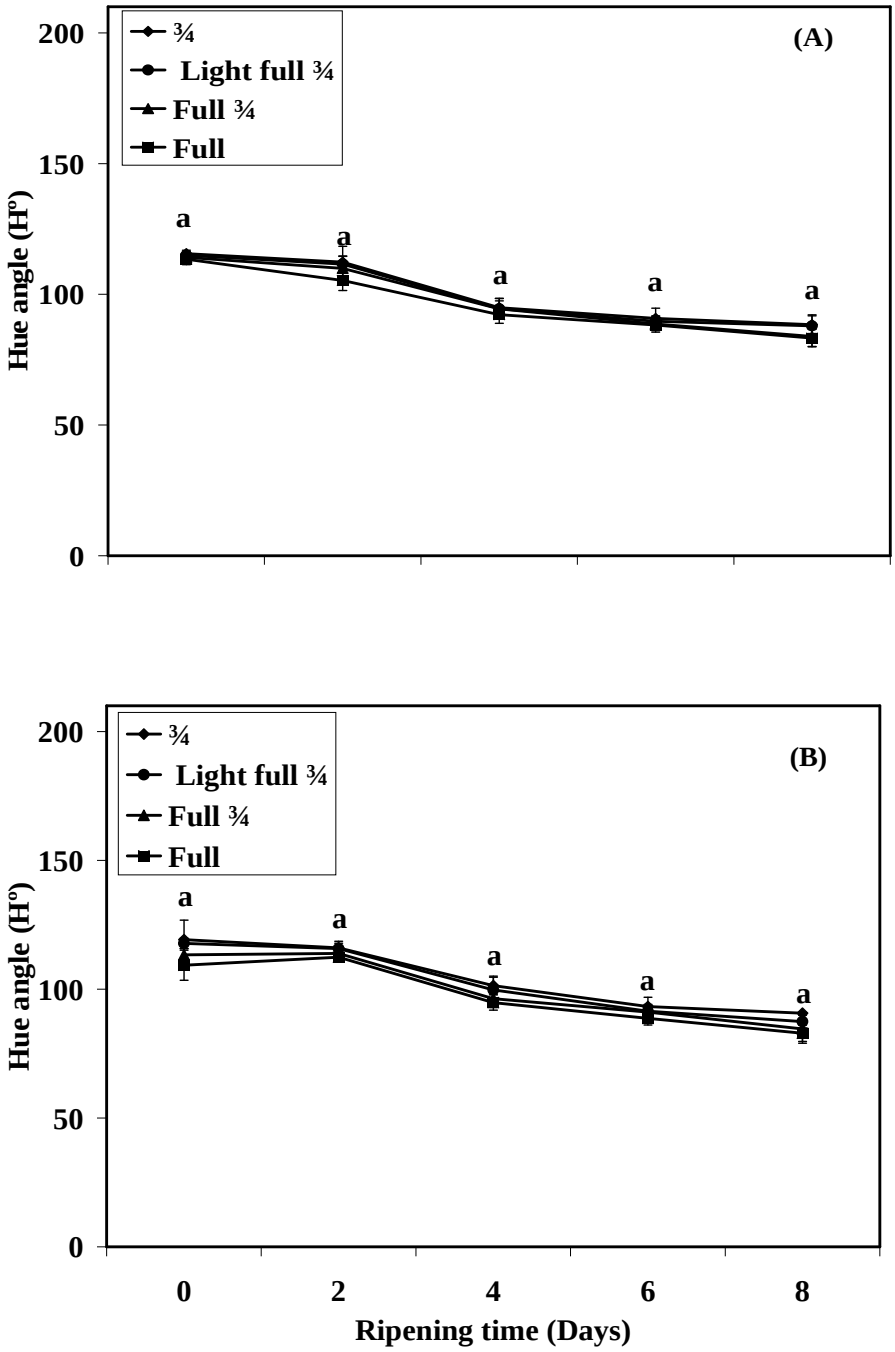

Figure 4.18: Effect of ripening time on hue (ºH) of banana peel of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

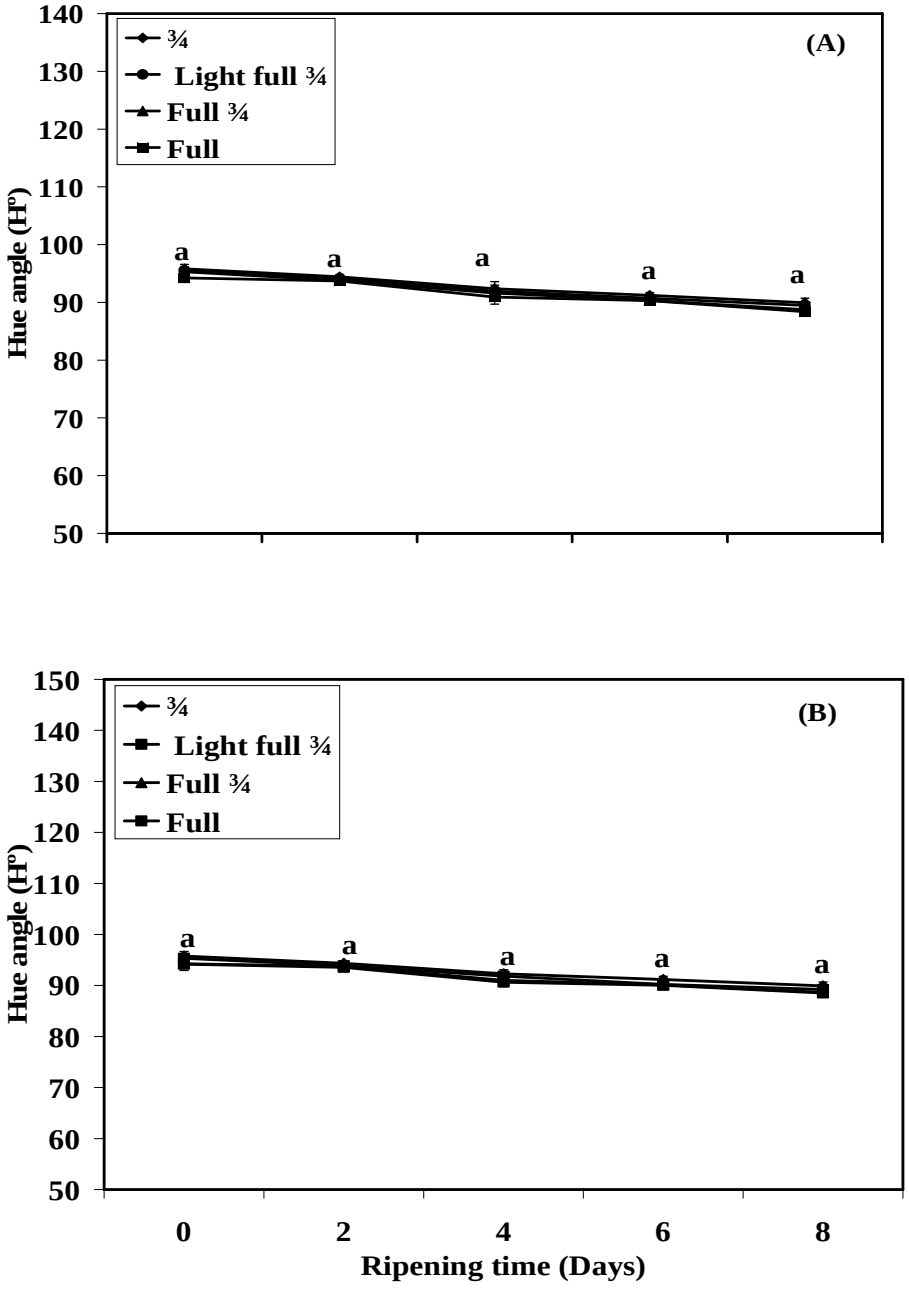

Figure 4.19: Effect of ripening time on hue (ºH) of banana pulp of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

values of 95º, 90º and 92º for unripe, half ripe and fully ripe pulp of fruits of banana cultivar Gran Enana, which compares well with findings of the current study. Pulp hue angle values of 58.04º and 64.7 º have also been reported for fully ripe banana fruits of Berangan and Mas cultivars, respectively. The difference with values observed could be due to cultivar effect.

Lightness of the peel followed an opposite trend to hue angle of the peel and increased from harvest to ripening for both banana cultivars (Figs. 4.20 A and 4.20 B). However, the maturity stages did not influence the lightness of the peel significantly ($p>0.05$) at harvest and during ripening for both banana cultivars although the more mature fruits generally had higher L* values at all stages which is a result of lighter peel colour compared to the less mature ones.

Peel colour of maturing banana fruits has been shown to turn light green (Mustaffa *et al*., 1998). The peel L∗ values in the current study were 54.9-52.1, 64.42-60.97 and 68.54-64.99 for unripe, half-ripe and ripe peels, respectively, for banana fruits of cultivar Grand Nain while for banana cultivar Williams, the values were 54.9-52.1, 64.42-60.97 and 68.54-64.99 for unripe, half-ripe and ripe fruits, respectively. This compares with L∗ values reported for banana cultivar Gran Enana of 54, 66 and 75 for unripe, half-ripe and ripe fruits, respectively (Hernadez et al., 2006). The pulp lightness decreased from harvest through to ripening for both banana cultivars (Figs. 4.21 A and 4.21B) although there was no significant difference ($p>0.05$) due to maturity stages. Lightness values were 84.54-83.88, 82.18-81.02 and 80.14-78.54 for unripe, half-ripe and ripe fruit pulp, respectively, for cultivar Grand Nain and

86.25-84.1, 83.04-82.29 and 81.31-80.45 for unripe, half-ripe and ripe pulp, respectively for banana cultivar Williams. Other workers have reported pulp L* values of 84, 74 and 75 for unripe, half-ripe and ripe pulp of fruits of banana cultivar Gran Enana (Hernadez et al., 2006) which compare well with values in the current study. Also, pulp lightness values of 70 for cultivar Montel (Mustaffa *et al*, 1998), 52 for Berangan and 50 for Mas have been reported (Yousaf *et al*, 2006). Pulp lightness value reported differs for different banana cultivars and may therefore be due to cultivar effect.

Banana pulp colour in most bananas cultivars has been shown to turn from white to cream or pale yellow during the latter stages of development on the plant (Dadzie and Orchard, 1997). The hue angle represents the colour perceived by the human eye and is a criterion used to measure state of banana fruit maturity and ripeness (Dadzie and Orchard, 1997; Turner, 1997). The peel of the green fruit has higher hue angle and low lightness values, while ripe fruit with a yellow colour has low hue angle and high lightness values. This is because of the chlorophyll degradation and/or unmasking of carotenes and xanthophylls and synthesis of new pigments during ripening (Turner, 1997). The changes in the peel and pulp colour could signify the onset of physiological maturity and may be used in the estimation of fruit maturity of banana varieties (Dadzie and Orchard, 1997).

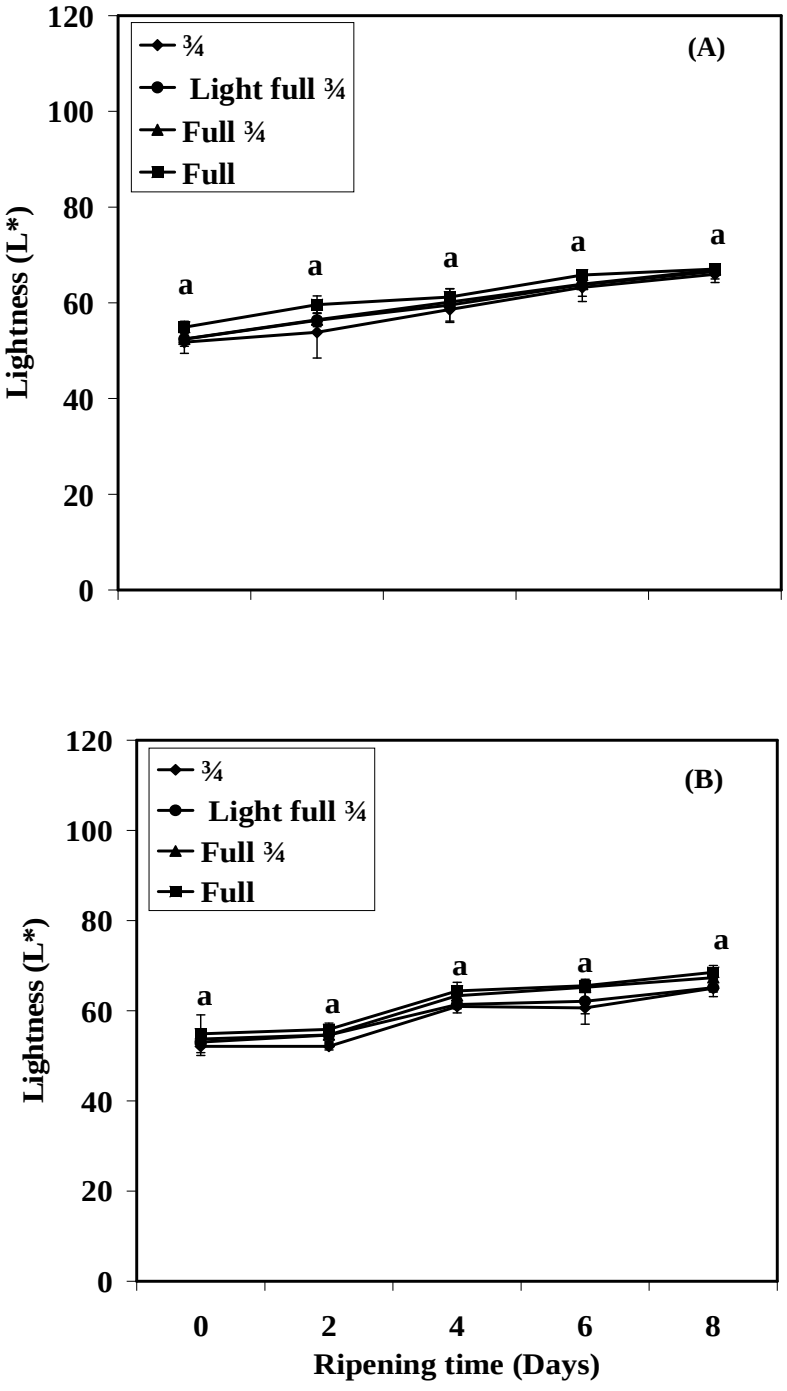

Figure 4.20: Effect of ripening time on lightness of banana peel of cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

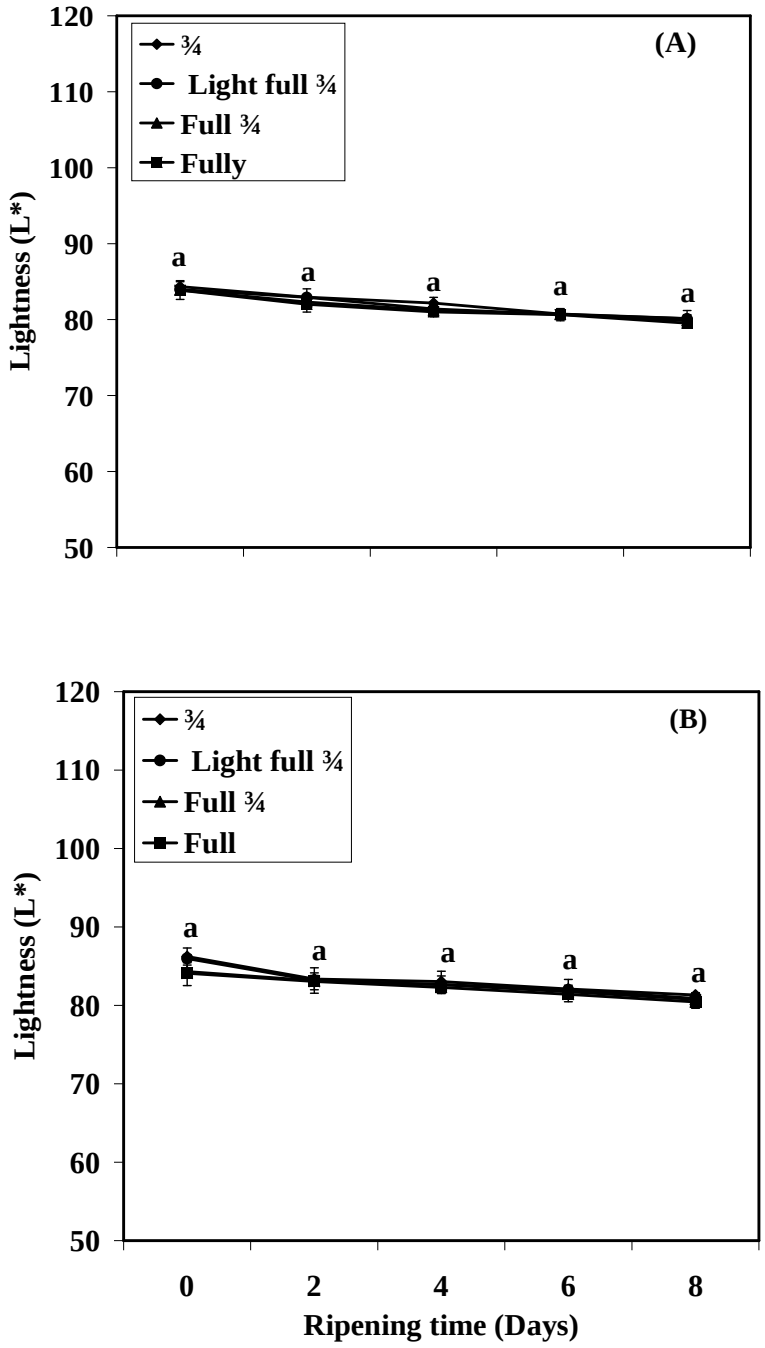

Figure 4.21: Effect of ripening time on lightness of banana pulp of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

4.3.12 Total chlorophyll content

Total chlorophyll content decreased from harvest to end of ripening (Fig. 4.22). The less mature fruits had more total chlorophyll compared to the more mature fruits at harvest and during ripening for both banana cultivars although the differences were not significant ($p>0.05$). As the fruit matures, the colour changes from dark green to light green (Mustaffa *et al*., 1998), probably due to start of chlorophyll degradation. Chlorophyll content also declined during ripening in this study. Chlorophyll content of banana peels of Grand Nain fruits decreased from 91.52 at the green state to 6.84 μg/g at the ripe state while that of banana cultivar Williams decreased from 92.76 to 5.05 μg/g. Chlorophyll content has been shown to decline from 50-100 μg/g fresh weight to almost zero from harvest through ripening (Stover and Simmonds, 1987; Seymour *et al*., 1993; Li *et al*., 1997). The values in the current study compare well with those reported by others of 94 μg/g at the green state and zero at the fully ripe state (Marriot, 1980).

During ripening, the chlorophyll in the peel degrades revealing the yellow carotenes and xanthophylls (Turner, 1997). This change is temperature dependent as the chlorophyll degradation is suppressed below 15˚C and above 40˚C (Turner, 1997). In banana, carotenes are synthesized during development stages in the plant and remain masked by the chlorophyll to be revealed during ripening when the chlorophyll is degraded (Wills *et al*., 1998).

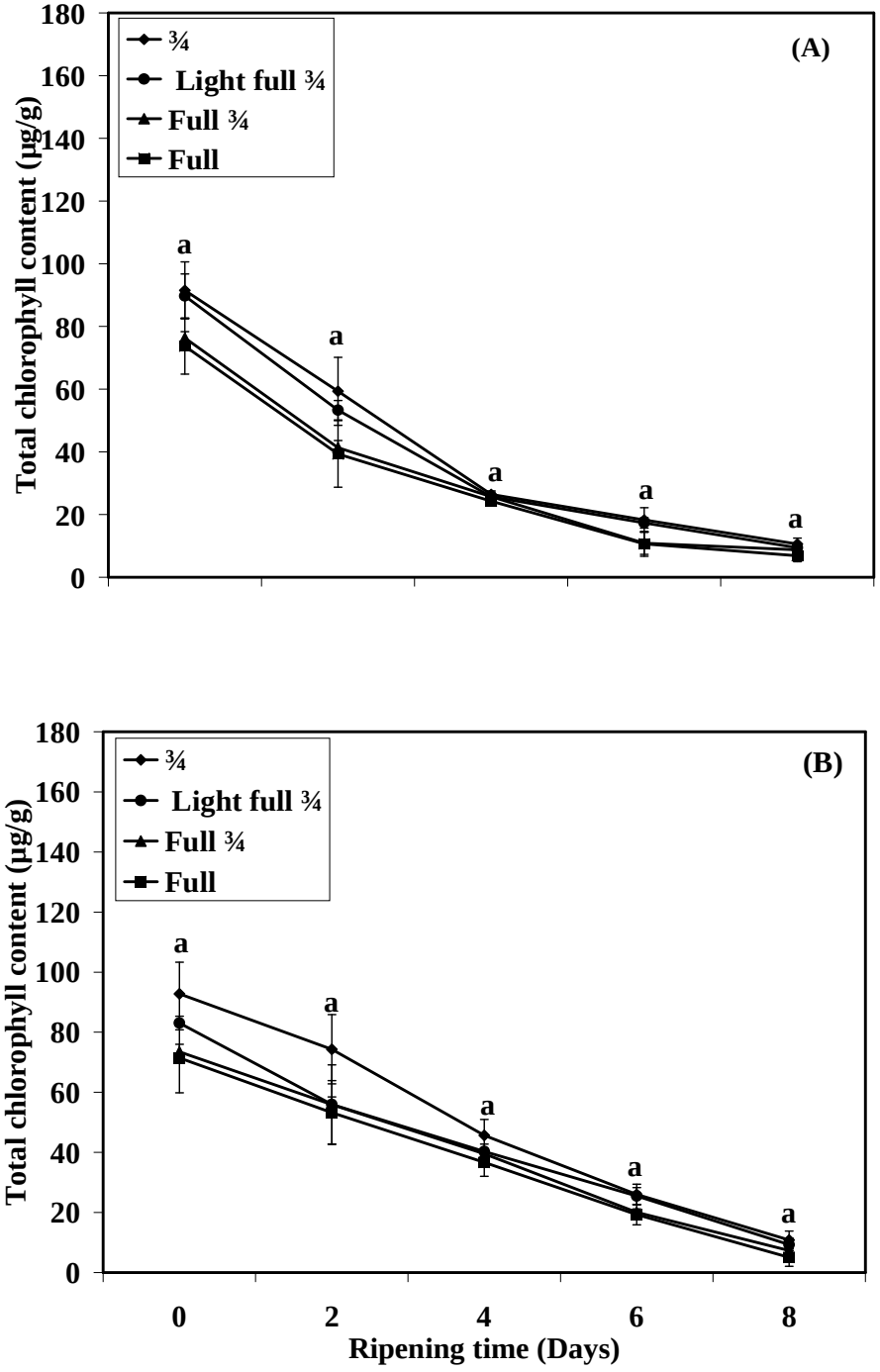

Figure 4.22: Effect of ripening time on total chlorophyll content (µg/g) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

4.3.13 Mineral content

Magnesium, iron, zinc and potassium content of the pulp of Grand Nain and Williams fruits is shown in Figs. 4.23, 4.24, 4.25 and 4.26 A and B respectively. There was a gradual but insignificant ($p>0.05$) increase of the minerals at harvest through ripening for all maturity stages for both banana cultivars. However, more mature fruits had more mineral content compared to the less mature fruits at all stages, although the differences were not significant. The increase of the mineral content during ripening is probably due to movement of minerals with the moisture as it moves from peel to pulp during ripening (Turner, 1997).

Mineral nutrient flow into the developing fruit is usually not linear. There is a rapid increase in mineral uptake in the initial phases of cell division in the fruit, a pattern that depends on the nature of the mineral, whether mobile or immobile and the uptake pathway (Ferguson and Boyd, 2002). Mobile mineral nutrients such as potassium and magnesium and even the not so mobile zinc have been shown to increase during fruit development and maturation (Ferguson and Boyd, 2002). The results of our study agree with this where the more mature fruits had relatively higher amounts of the minerals though the differences were not significant ($p>0.05$) compared to the less mature ones. It is important to understand mineral movement especially in developing fruits so that the fruits are harvested with optimal mineral concentrations for postharvest quality.

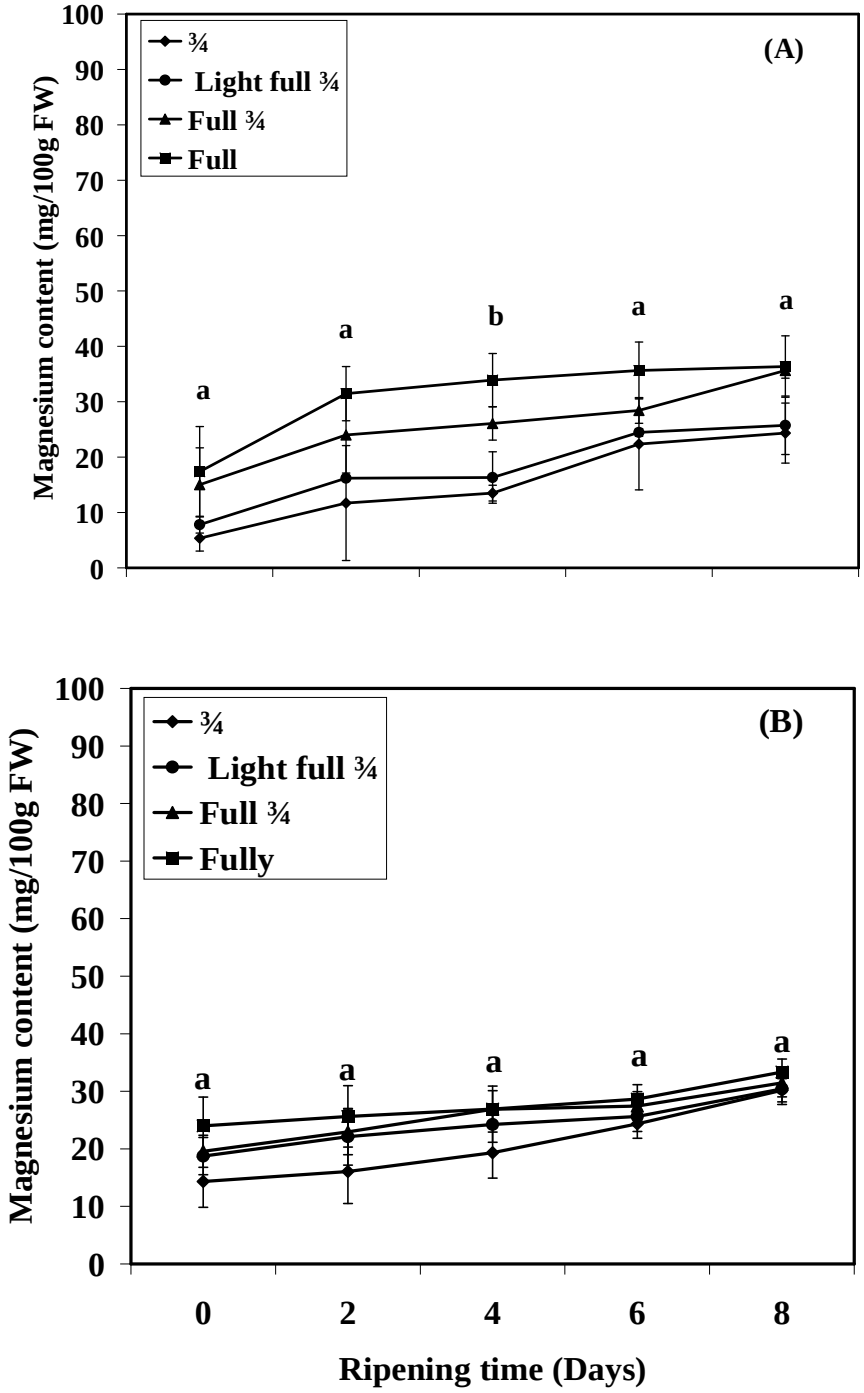

Figure 4.23: Effect of ripening time on magnesium (Mg) content (mg/100gFW) of banana pulp of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

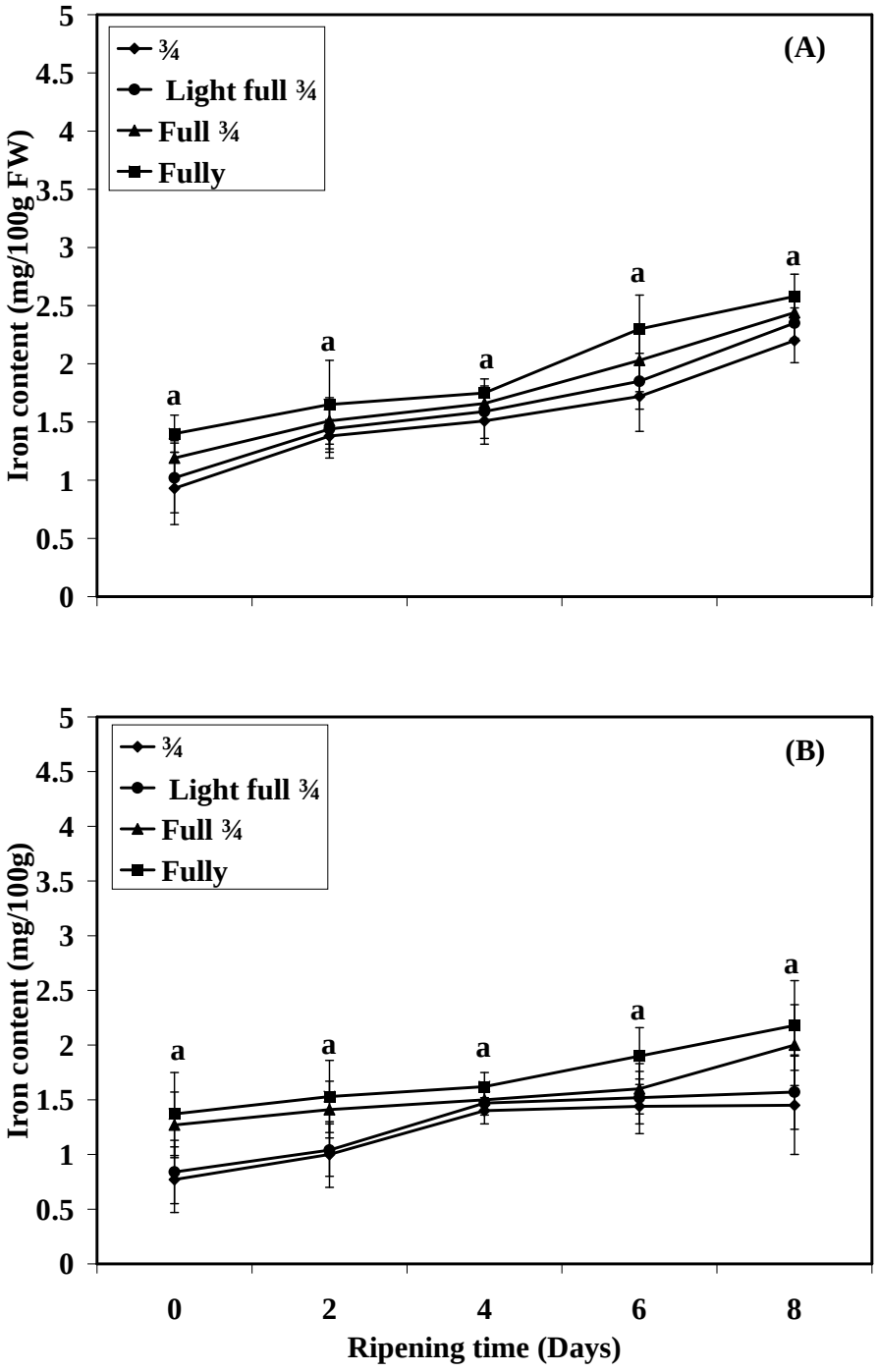

Figure 4.24: Effect of ripening time on iron (Fe) content (mg/100gFW) of banana pulp of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

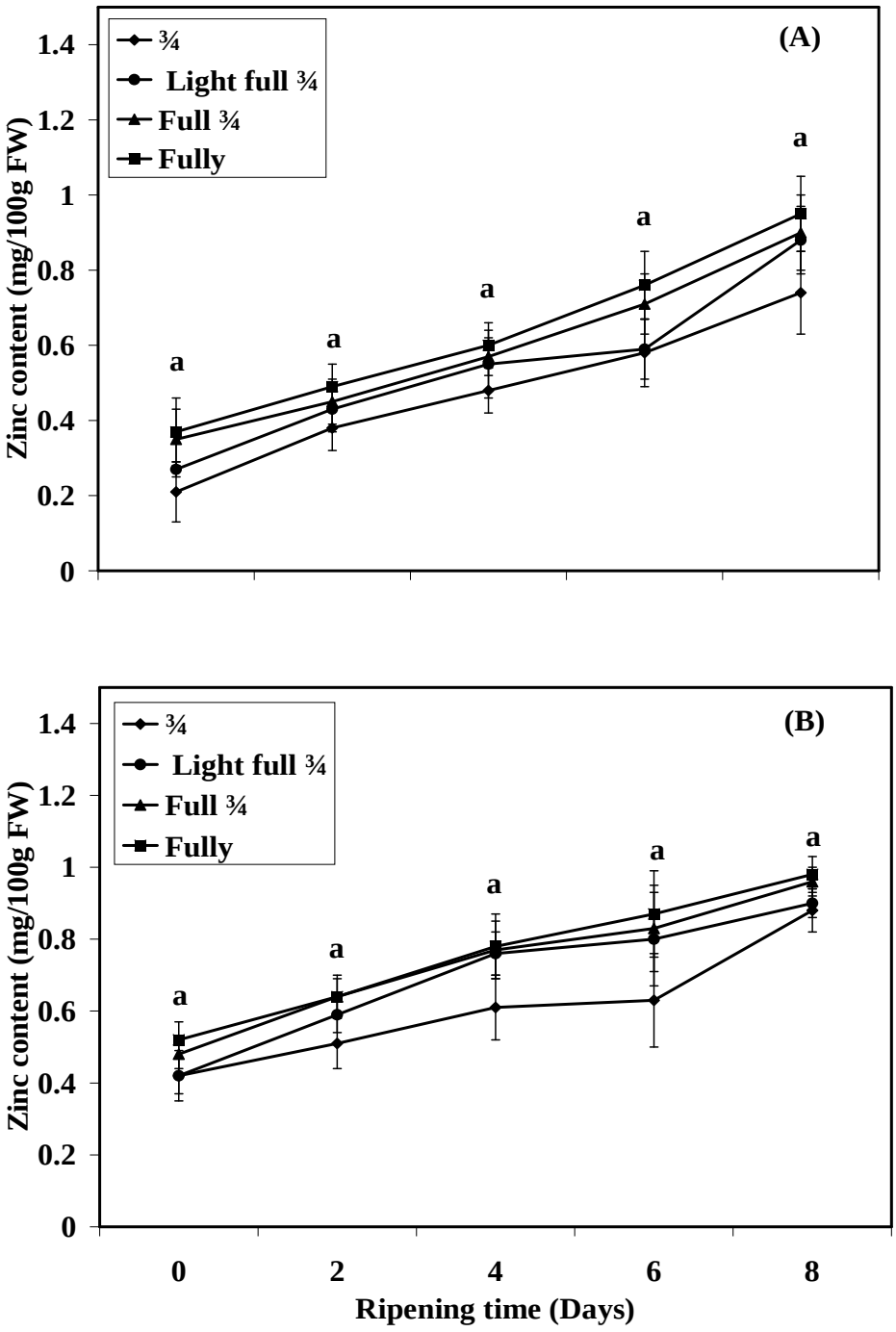

Figure 4.25: Effect of ripening time on zinc (Zn) content (mg/100gFW) of banana pulp of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

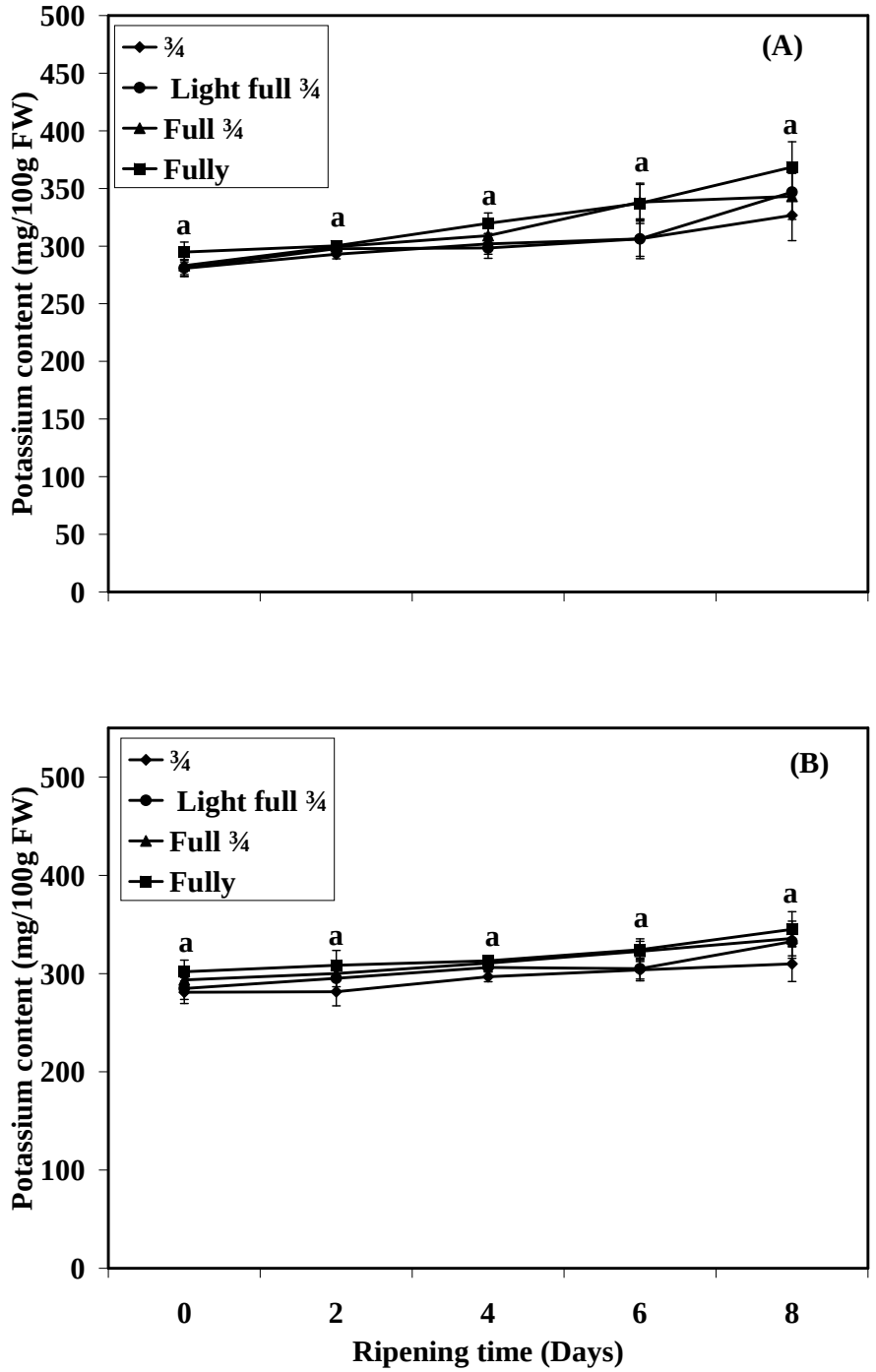

Figure 4.26: Effect of ripening time on potassium content (K) content (mg/100gFW) of banana pulp of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Increase in mineral content in the banana fruit pulp during ripening has been reported earlier by Gamlath (2008) who found that calcium, magnesium and potassium levels were significantly higher for fruits at ripening stage 6 compared to those at stage 4.

The results from the current study may indicate that by the time the banana fruits reach ¾ stage of maturity, they have accumulated enough minerals from the plant. Contents of K in the current study for ripe fruits were 368.52 for cultivar Grand Nain and 345.10 mg/100g FW for Williams. Reported levels for K range from 358-460 mg/100g FW (Robinson, 1996; Ferguson and Boyd, 2002; Vicente *et al*., 2009). Magnesium contents for ripe fruits were 36.35 and 33.33 mg/100g FW for cultivar Grand Nain and Williams, respectively. These results were in agreement with expected levels of 27-36 mg/100g FW (Robinson, 1996; Ferguson and Boyd, 2002; Vicente *et al*., 2009). Zinc contents were 0.95 and 0.98 mg/100g FW for Cultivar Grand Nain and Williams, respectively. These were higher than values reported for bananas of 0.15-0.16 mg/100g FW (Ferguson and Boyd, 2002; Vicente *et al*., 2009). Iron content of pulp of ripe fruits were 2.58 and 2.18 for Grand Nain and Williams, respectively, while other workers reported values of 0.26-0.31(Ferguson and Boyd, 2002; Vicente *et al*., 2009). Mineral contents of banana fruits may vary according to varieties, environmental and ecological conditions.

4.3.14 Total polyphenol content

The effect of maturity stages on total polyphenol content at harvest and during ripening for banana cultivar Grand Nain and Williams is shown in Figs 4.27A and

4.27B, respectively. Total polyphenol content was not significantly ($p>0.05$) different for all maturity stages at harvest and during ripening. This shows that at ¾ maturity stage, the banana fruits have accumulated almost all the necessary phenolics.

Bananas harvested at different harvest ages but similar physiological stages have been shown to have similar biochemical qualities (Laylieam and Kosittrakun, 1998). Generally, more mature fruits had less total polyphenol content at harvest and during ripening although there was no significant difference. The polyphenol content in the current study for fruit pulp of banana cultivar Grand Nain was 75.93-67.5, 64.97-60.92 and 21.5-13.7mg/100g for unripe, half-ripe and ripe fruits, respectively, while for banana cultivar Williams the content was 75.45-68.1, 67.25-56.85 and 18.5-12.5 mg/100g for unripe, half-ripe and ripe fruits respectively. This compares closely to what other workers found for bananas of 103.0, 53.2 and 44.2 mg/100g for unripe, half-ripe and ripe banana fruits (Kiyoshi and Wahachiro, 2003).

Phenolic content has been shown to decrease as the banana fruit matures on the plant (Turner, 1997; Mura and Tanimura, 2003) and may be a useful indicator of maturity. Total polyphenol content decreased from harvest through ripening. Dopamine which forms about 80% (Robinson, 1996) of tannins has been shown to decrease during ripening from about 50mg/g fresh weight in the pulp at harvest to about half during ripening (Turner, 1997). Tannins are water soluble phenols found in the peel and the pulp of bananas (Turner, 1997). There is a strong astringency in the pulp of mature

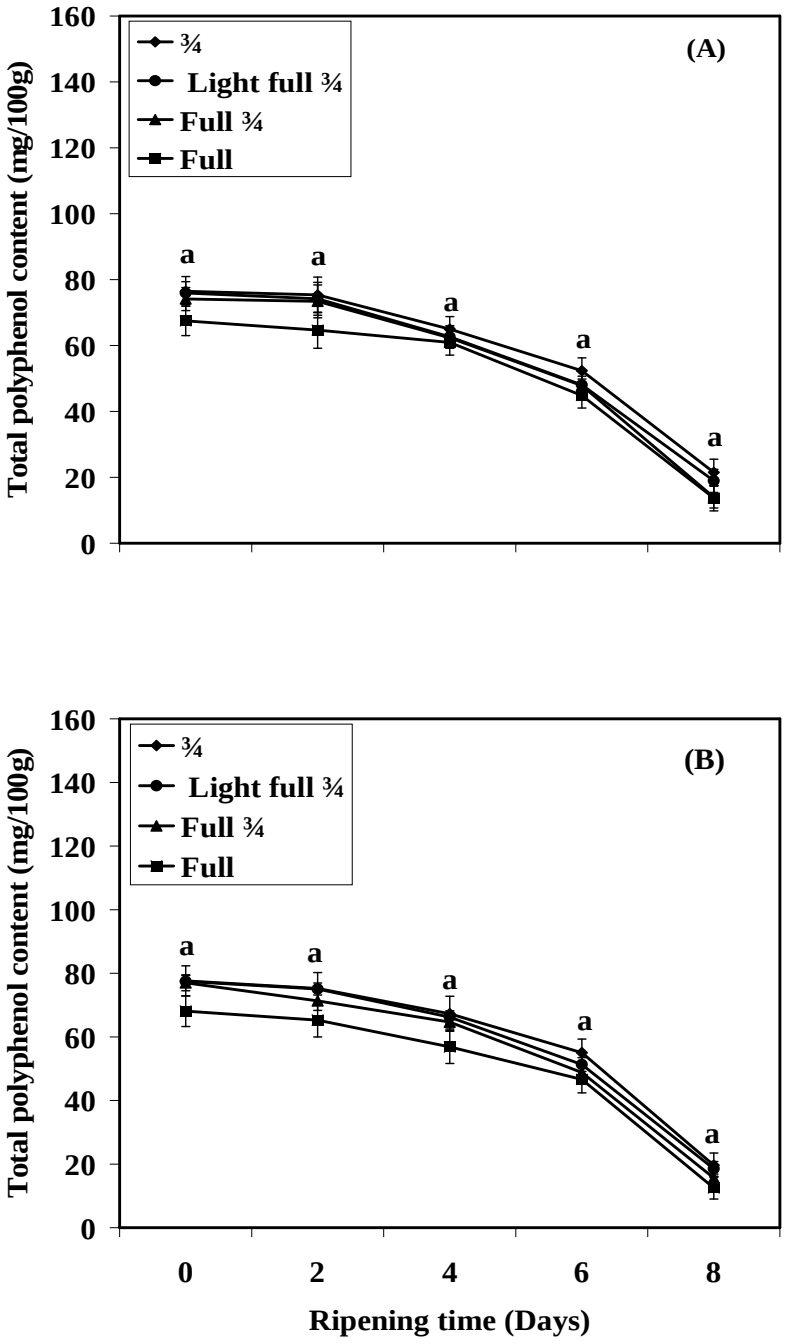

Figure 4.27: Effect of ripening time on total polyphenol content (mg/100g) (mg/100gFW) of banana pulp of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

green bananas before ripening and this has been associated with the polyphenol compounds contained in the idioblasts called tannin cells (Kiyoshi and Wahachiro, 2003). Phenolics have been shown to be responsible for astringency of bananas before ripening and also for certain browning reactions (Stover and Simmonds, 1987; Seymour *et al.*, 1993; Robinson, 1996).

Loss of astringency during ripening has been shown to be due to polymerization of the phenolics in the pulp. Browning of both pulp and peel is linked to enzymatic oxidation of phenolics by polyphenoloxidase (Seymour *et al.*, 1993; Robinson, 1996: Turner, 1997) and also to the concentration of ascorbic acid which inhibits the browning process (Turner, 1997). The primary substrate for browning reactions of which latex staining is commercially important is dopamine (Stover and Simmonds, 1987). The latex cakes during ripening and becomes brittle and withdrawn from the walls giving a beaded appearance (Kyamuhangire, 2006) indicating a change of chemistry of the contents. Japanese persimmon was reported to have reduced astringency on ripening probably due to the formation of tannin-pectin and tannin-protein complexes. Tannins have been shown to form insoluble complexes with polysaccharides (Kyamuhangire, 2006). Polyphenols have been shown to have an inhibitory effect on bioavailability of iron from the food consumed (Gillooly *et al.*, 1983) and hence the importance of ripening bananas as this reduces its content.

4.3.15 Respiration rate

Effect of maturity stages on respiration is shown in Fig. 4.28 below. Carbon dioxide produced at harvest was low for fruits of all maturity stages but increased till day 3

and then reduced throughout the ripening period. At harvest there was no significant ($p>0.05$) effect of maturity stages on carbon dioxide produced whereas at the climacteric peak, there were significant ($p\leq0.05$) differences with more mature fruits producing more carbon dioxide for both banana cultivars. The full mature fruits of banana cultivar Grand Nain had a respiration rate of 89.86 mL/Kg/hr and the ¾ mature had 70.04 mL/Kg/hr while for cultivar Williams, the full mature fruits had a respiration rate of 93.98 mL/Kg/hr and ¾ mature had 67.91 mL/Kg/hr. Other workers have reported peak respiration rates of 80 mL/Kg/hr for banana fruits (Pelayo *et al*., 2003) which compares well with the results of the current study. Carbon dioxide in Bartlett pears was higher in more mature fruits compared to the less mature fruits (Agar *et al*., 1999). The climacteric phase in this study was followed by a decrease in respiration for fruits in all maturity stages irrespective of the banana cultivar. This pattern is typical of climacteric fruits (Wills *et al*, 1998).

Banana is a climacteric fruit and a pronounced increase in respiration coincides with ripening (Wills *et al*., 1998; Jiang *et al*., 2000). Carbon dioxide increased during the respiratory climacteric probably due to increased flux of carbon through the glycolytic pathway to the mitochondria (Beaudry *et al*., 1987). A high respiration rate may indicate the high rate of breakdown of respiratory substrates such as starch, sugars and organic acids (Jiang *et al*., 2000). This may have an indication on the storage life of the fruits where more mature fruits have shorter green life compared to less mature ones.

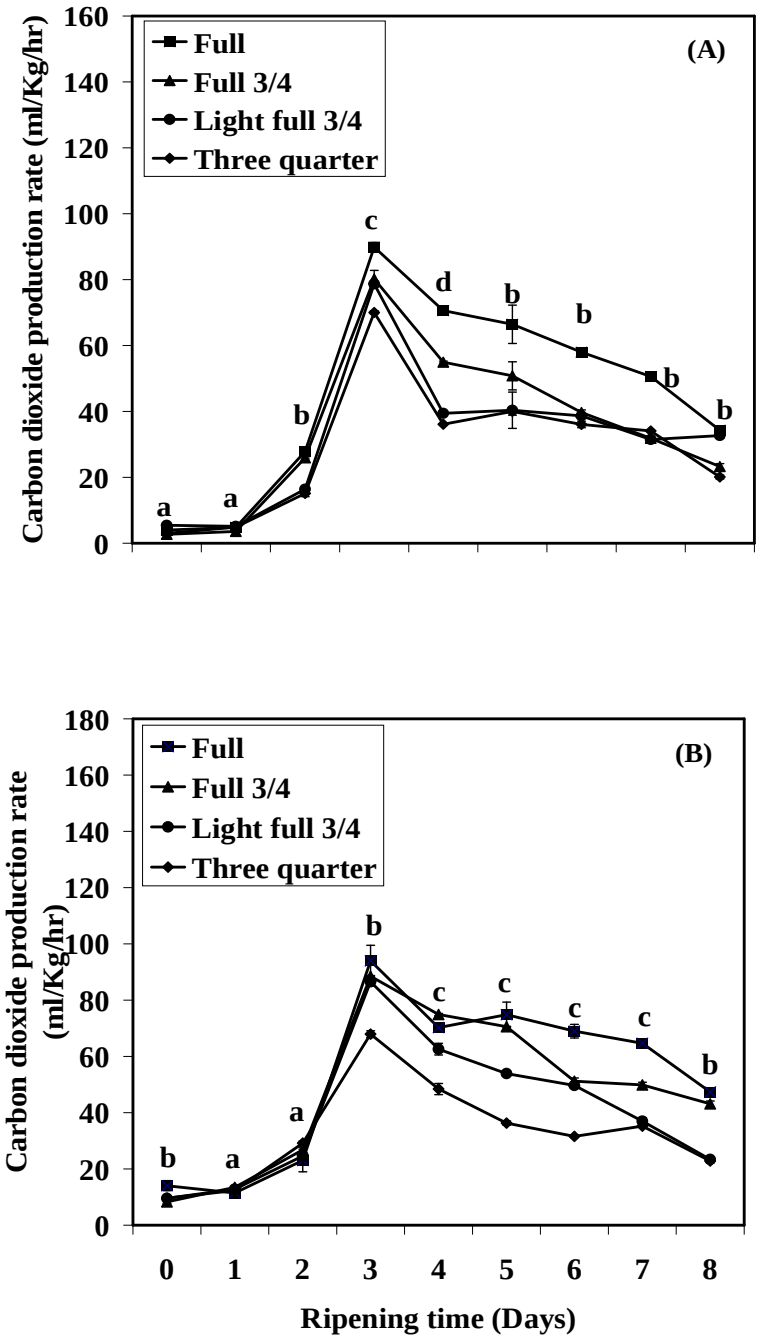

Figure 4.28: Effect of ripening time on total carbon dioxide production (ml/kg/h) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Fruits with high respiration rates ripen faster and also deteriorate faster and have lower keeping quality than those with lower rates (Wills *et al*., 1998). The rate of respiration and ethylene production usually depends on storage, age of fruit and also cultivar (Kader, 1992).

4.3.16 Ethylene production rate

Effect of maturity stages on ethylene production rate is shown in Figs. 4.29 A and 4.29 B for banana cultivars Grand Nain and Williams, respectively. Maturity stages affected the ethylene produced significantly ($p \leq 0.05$) and more mature fruits generally had higher ethylene production. There was a rapid increase in ethylene production until day 2 when the fruits reached their climacteric peak production which was followed by a decline in production. In banana, a climacteric fruit, ethylene production rises before the onset of ripening which has been defined as the initial respiratory increase (Wills *et al*., 1998). More mature fruits are more sensitive to ethylene and produce more ethylene on ripening (Wills *et al*., 1998). Findings of this study differ with those of Agar *et al*. (1999) who found that harvest dates did not affect ethylene production for Bartlett pears at harvest. This could be due to differences in the period between the harvest date and also in the fruits as ripening has been shown to be under genetic control in the cells of the fruit (Wills *et al*, 1998). Ethylene production plays a major role in the postharvest storage life of several horticultural commodities as deterioration gradually occur on exposure to even low levels of the gas (Wills *et al*., 1998).

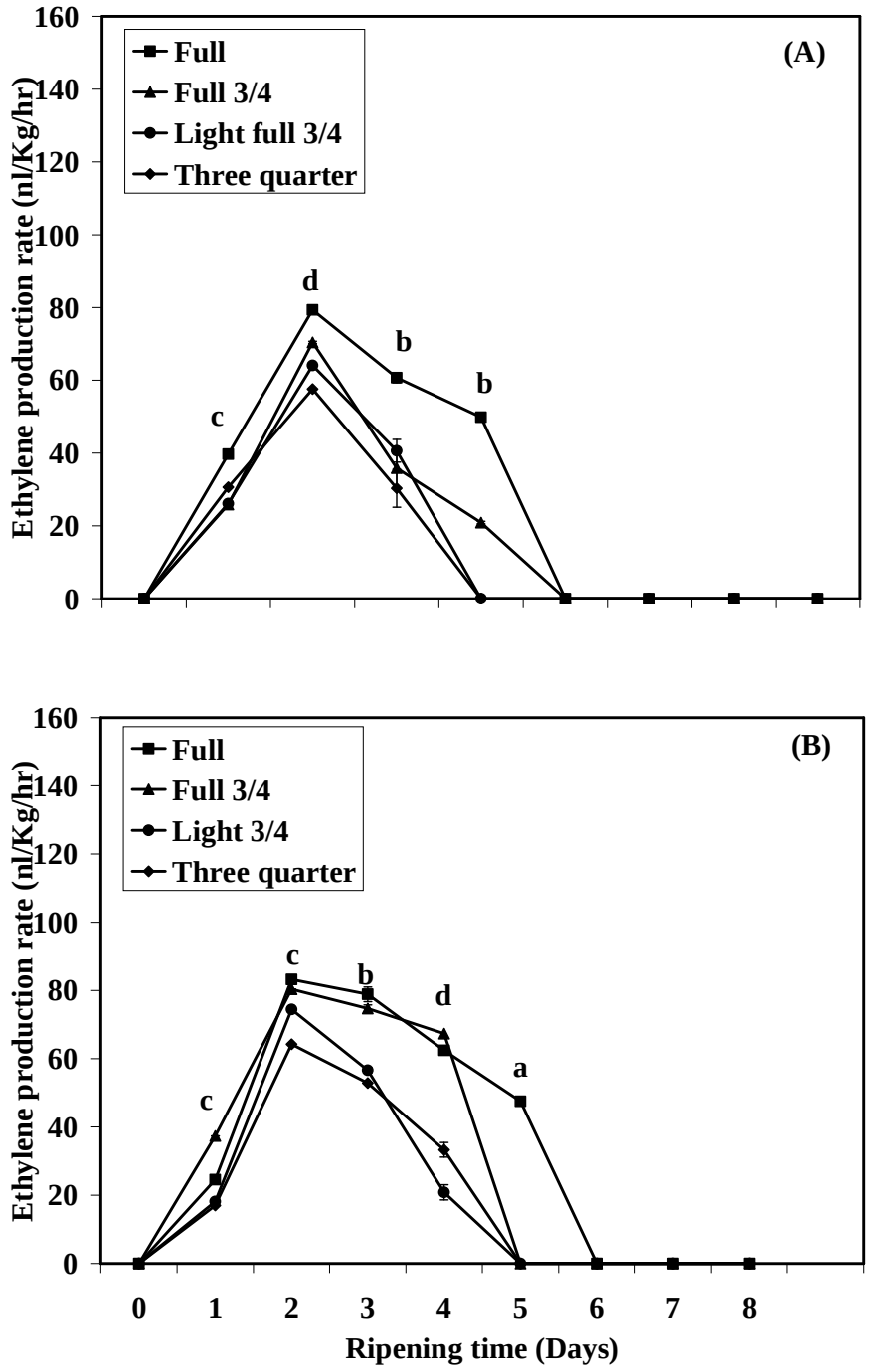

Figure 4.29: Effect of ripening time on ethylene production (nl/Kg*h) of banana cultivar Grand Nain (A) and Williams (B) harvested at different maturity stages. Vertical bars represent SE of the means of 3 replicates. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Deterioration of banana fruits is rapid once ripening which is initiated by ethylene starts, which poses a challenge to the marketers and consumers. The values in this study were slightly higher than those found for fruits of banana cultivar Giant Cavendish of 60 nl/kg/hr (Miriti, 2009) which may be attributed to cultivar and growing conditions effect.

4.3.17 Correlation coefficient (R) of maturity indices

Table 4.2 shows correlation coefficients of selected maturity indices for banana cultivar Grand Nain and Williams.

Table 4.2: Correlation coefficients (R) of maturity indices for banana cultivars Grand Nain and Williams

Correlation coefficients (R) of maturity indices		cultivar Grand Nain	cultivar Williams	Stage of ripeness (CSIRO, 1972)
Bunch age vs	Finger diameter	+0.99	+0.92	1
Bunch age vs	Finger weight	+0.99	+0.98	1
Bunch age vs	Finger length	+0.99	+0.99	1
Bunch age vs	Pulp/peel ratio	+0.97	+0.98	1
Bunch age vs	Peel diameter	-0.99	-0.99	1
Bunch age vs	TSS	+0.81	+0.86	6
Bunch age vs	TTA	+0.77	+0.99	6
Bunch age vs	Finger firmness	-0.94	-0.89	6
Bunch age vs	Green life	-0.99	-0.92	N/A
Bunch age vs	Shelflife	-0.96	-0.99	N/A
Finger diameter vs	TSS	+0.85	+0.75	6
Finger diameter vs	TTA	+0.72	+0.87	6
Finger diameter vs	Pulp/peel ratio	+0.98	+0.86	1

N/A denotes not applicable.

The results indicate a strong positive correlation between bunch age and finger diameter (grade), finger weight, finger length and pulp/peel ratio of the middle fingers of the outer whorl of the second hand of cultivars Grand Nain and Williams

at harvest (Table 4.2). There was a strong negative correlation between bunch age and peel diameter at harvest. Bunch age also correlated positively with TSS and TTA at eating ripe stage 6 and negatively with finger firmness at the same stage. Green life and shelflife correlated negatively with bunch age for both banana cultivars. Finger diameter correlated positively with postharvest parameters such as TSS and TTA at the eating ripe stage and positively with pulp/peel ratio at harvest ripeness stage 1.

Most of the maturity indices studied here could be used for predicting banana fruit maturity since they had high correlations. However, not all are applicable since some are destructive. Hence, finger diameter which correlated very well with bunch age and several postharvest quality characteristics such as TSS, TTA and pulp/peel ratio, and is not destructive, may be the best to use for both banana cultivars. Finger diameter at physiological maturity for cv. Grand Nain was 34.5 mm and 32.5 mm for cv. Williams. Bunch age also correlated highly with all the parameters studied (Table 4.2) for both banana cultivars and may also be used in combination with finger diameter, since it is not destructive and is easy to measure.

4.4 Conclusions and recommendations

Finger grade increased with age of the bunch for both cultivars. Green life was negatively correlated to bunch age with 24, 26 and 28 weeks old bunches corresponding to finger diameter (grade) of about 33-35, having a green life of about 17, 16 and 15 days, respectively for cultivar Williams while for cultivar Grand Nain, 22, 24 and 26 weeks old bunches had a green life of 14, 12 and 9 days, respectively

compared to 11 days and 6 days for fully mature fruits for cultivar Williams and Grand Nain, respectively. Longer green life may allow for transportation to far off local markets and also for export market. Fruits harvested at full maturity had significantly shorter green life compared to those harvested at all the other stages. The maximum achievable green life for cv. Grand Nain was 14 days and 17 days for cv. Williams. However, shelflife was not influenced by the stage of maturity at harvest. In Kenya, most commercial banana handlers purchase the fruits while still green from the farms and transport them on pick-up trucks to markets where they ripen them for sale. Green life is a very important parameter as it influences the storage and ultimately the marketing of the fruits. Bananas are often sold in far off places from their areas of production. Longer green life is therefore preferred to short green life. A longer green life would allow fruits to be distributed to far off markets without ripening which compromises marketability as ripe fruits are easily damaged compared to green ones. Shelflife was not affected by the maturity stage. A longer shelflife is preferred as it allows the retailers and consumers to have fresh fruits at their disposal for marketing and consumption respectively.

Further, finger angularity, finger grade and bunch age may be used as optimum maturity indices for the tissue-cultured banana cultivars Grand Nain and Williams. It may be concluded here that the best stage of harvest maturity is ¾ mature, light full ¾ and full ¾ as the fruits had acceptable grades and kept well. This corresponded to a flower emergence to harvest (E-H) interval of 24 to 28 weeks respectively for cultivar Williams and 22 to 26 weeks for cultivar Grand Nain. However, both cultivars could still be harvested at full maturity although this reduces the

handling/storage period to ripening. The fruits had acceptable postharvest quality and may be sold locally or consumed within the household. This would give a range of 22-28 weeks for cultivar Grand Nain and 24-30 weeks for cultivar Williams. Fruits harvested for commercial dessert purposes are normally cut at three quarters round when the angles are still clearly visible and fruit has only about 75% of its potential size and mass (Robinson, 1996) but have acceptable postharvest quality. Previous research (Ramma *et al.*, 1999) has also indicated that finger diameter should be used in combination with bunch age. Other workers have indicated that angularity of the fruit although subjective could also be used in combination with the other indices (Dadzie and Orchard, 1997; Turner. 1997).

Bunch age can be easily recorded by simply tagging the fruits with coloured ribbons on emergence. Finger diameter can also be easily measured using a veneer caliper on a pre-determined finger on the bunch. It is, therefore, important to introduce this simple equipment in areas growing bananas for commercial purposes. However, it should be noted that this will only be helpful to the farmers who follow the recommended growing practices as the parameters being measured are influenced by cultural practices. Pulp/peel ratio and TSS may also be reliable maturity indices but may not be applicable being destructive methods. Fullness of the fruit commonly referred to as finger angularity has been shown in this study to correlate well with finger grade and emergence to harvest interval allowing prediction of postharvest quality of banana cultivars Grand Nain and Williams.

CHAPTER FIVE

5.0 EFFECT OF BUNCH COVERS ON POSTHARVEST QUALITY OF TISSUE-CULTURED BANANA (*Musa* spp.)

5.1 Introduction

External appearance, internal and market quality of bananas are influenced by several factors, including pre-harvest production practices. The external appearance includes key attributes such as colour, shape, size and freedom from defects. The internal attributes such as taste, texture, sweetness, aroma, acidity, flavour, shelflife and presumed nutritional values of the fruit are important in ensuring repeat buys for sustained repeat purchase (Hewett, 2006; Shewfelt, 2009). The physical appearance of the peel is especially important in the highly competitive export markets and in some local niche up-markets like the supermarkets. Buyers in these premium markets require consistent supplies of uniform coloured fruit with blemish-free peels.

Banana bunch covers may allow for production of high quality banana fruits that are not bruised, and hence have acceptable visual appearance. Consumers use visual quality to purchase fresh produce (Shewfelt, 1999; Shewfelt, 2009). Market returns for bananas in international markets are generally greatest for large fruit that are blemish-free (Johns, 1996). The supply of blemish-free fruit is difficult due to various types of mechanical injury and insect damage imparted on the delicate peel surface during growth and development, with wind and insects being the principal agents of this damage (Anon, 2003). Indeed, pre-harvest insect feeding has been shown to be a main cause of peel damage to banana fruits (Shanmugasundaram and

Manavalan, 2002). However, bagging of bananas with bags impregnated with insecticides has been shown to protect fruits from insect attack (Amarante *et al*., 2002).

Wind blows dust and debris which hits the delicate outer skin causing cellular damage and subsequent fruit scarring. Considerable physical injury and damage to the fruit peels can also be caused by the blowing of adjacent leaves and rubbing of leaf petioles onto the developing bunch (Anon, 2003). This chaffing from leaves during growth has also been reported to be eliminated by bunch covers (Weerasinghe and Ruwapathirana, 2002). The covers have also been effective against sunburn and blemishes caused by birds and wind-blown dust (Weerasinghe and Ruwapathirana, 2002).

Bunch covers of various colours and condition (perforated and non-perforated) have been extensively used in both tropical and subtropical banana growing countries with the aim of improving yield and quality (Stover and Simmonds, 1987; Robinson, 1996). Improved quality include appealing skin colour, reduced sunburn, reduced fruit splitting, increased finger length and bunch weight among others (Robinson, 1996; Amarante *et al*, 2002). Bunch covers have also been used to protect bunches from low temperatures, especially in temperate countries (Gowen, 1995; Robinson, 1996; Harhash and Al-Obeed, 2010). Indeed bagging has been shown to reduce winter stress under supra-optimal condition which resulted in early fruit maturation (Jia *et al.*, 2005). This is due to enhanced physiological and metabolic activities provided by the microclimate created by bagging (Johns and Scott, 1989a).

However, the effect of fruit bagging, especially in the tropics, on size, maturity and skin colour among other fruit quality parameters has been contradictory, with some workers reporting that bagging has an effect on these parameters while others report no effect (Amarante *et al*., 2002). This may reflect differences in the type of bag used, fruit age at bagging, fruit and cultivar response, prevailing climatic conditions and conditions of holding fruit after harvest (Johns and Scott, 1989a; Amarante *et al*., 2002; Weerasinghe and Ruwapathirana, 2002; Narayana *et al*., 2004).

Technologies such as bunch covering that enhance production and help realize the benefits of tissue culture technology would go a long way in boosting banana farming in Kenya where bunch covering has not been practiced extensively. Recently, a few farmers have attempted this practice in collaboration with importers of the bunch covers. However, the effect of the covers on the postharvest quality and behavior of tissue-cultured bananas in Kenya has not been studied in Kenya. The objective of this study therefore was to investigate the effect of bunch covering on postharvest quality and behavior of tissue-cultured banana fruits using cultivar Grand Nain and Williams as the test cultivars.

5.2 Materials and Methods

5.2.1 Study area, plant material and parameters measured

Banana bunches were tagged in an already existing banana orchard in 2008 in Maragua District, Central Kenya, Agro-Ecological Zone, upper midland 3 (AEZ UM3) (Jaetzold and Schmidt, 1983). The farm is located at latitude 00º 49’ 14”S and longitude 037º 08’ 34” E as marked by a Global Positioning Satellite (GPS)

instrument (Magellan,Triton, China) and the bananas had been grown using the recommended agronomic practices (Anon, 2002). Perforated dull blue and shiny blue polyethylene bunch covers were applied to the bunches when the flower bracts had hardened and the hands had started to curl upwards. For bananas growing in the tropics, translucent blue covers are recommended as they allow heat transmission but reduce sunburn damage compared to other colours (Robinson, 1993; Robinson, 1996). The bunch covers had perforations measuring 8 mm spaced at 10.5X9 cm and a thickness of 5µm and were left to hang for about 150 mm below the distal hands and were securely attached to the bunch stalk above the proximal hand using a rubber band. Some of the tagged bunches were not covered and they served as control. The three treatments were applied randomly in a completely randomized design and were replicated three times with three plants per replicate.

The bunches were allowed to grow to full ¾ maturity and were harvested carefully, dehanded and placed in plastic crates and then transported to the postharvest laboratory of Jomo Kenyatta University of Agriculture and Technology (JKUAT). Parameters measured at harvest were bunch weights, finger grade and finger length. The fruits were then washed with tap water to remove latex and dirt and were then subjected to fungicidal treatments by dipping for 1 min in 100 ppm sodium hypochlorite (Jik, Reckitt Benckiser-East Africa Limited, Kenya) in order to control spoilage during postharvest storage due to common fungal diseases such as anthracnose (*Colletotrichum musae*) and crown rot. The fruits were left to air dry and then ripened in a humidity chamber at 18˚C and 95% RH using passion fruit as the ethylene source. Five fingers per replicate were placed on the bench for green

life at ambient conditions of temperature and humidity. Parameters measured during ripening were: total soluble solids (TSS), starch, individual and total sugars, chlorophyll, respiration and ethylene production, moisture content and weight loss, colour, firmness and pulp/peel ratio. The fruits were also evaluated for green life, shelflife and visual appearance.

5.2.2 Analyses and Determinations

5.2.2.1 Fruit weight, diameter (grade) and length

Fruit weight and diameter measurements were carried out as previously described in section 3.2.3.1.

5.2.2.2 Pulp/peel ratio

Pulp: peel ratio was determined as previously described in section 3.2.3.2 with a few modifications. The measurements were done at harvest at ripeness stage 1, through the ripening stages, until the fruits were fully ripe at ripeness stage 6 (CSIRO, 1972, Marin *et al*., 1996).

5.2.2.3 Starch content

Starch content was determined as earlier indicated in section 3.2.2.3. However starch content was determined from stage 1 through to ripeness stage 6 (CSIRO, 1972, Marin *et al*., 1996)

5.2.2.4 Ripening

Banana fruits were ripened as previously described in section 4.2.1.5.

5.2.2.5 Moisture content

The pulp and the peel were analysed for moisture content from green stage through to fully ripe stage using oven drying method AOAC (1996) as described earlier in section 3.2.3.9.

5.2.2.6 Weight loss

Weight loss was determined by weighing three fingers every day from green to yellow stage. The initial weight (W_1) of each fruit at day 0 and weight of the same fruit (W_2) on each sampling day were noted. Weight loss was then calculated as a percent as follows:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight } (W_1) - \text{Final weight } (W_2)}{\text{Initial weight } (W_1)} \times 100$$

5.2.2.7 Firmness

Hand fruit firmness assessment was determined as described earlier in section 3.2.3.6 although the measurements were carried out at ripeness stage one to six (CSIRO, 1972; Joyce *et al*., 1993; Jiang *et al*., 1999). Objective firmness was determined as earlier described in section 4.2.1.6.

5.2.2.8 Colour

Both subjective and objective colour was determined at harvest and during ripening. The assessment methods are as described earlier in section 3.2.3.17.

5.2.2.9 Total soluble solids content

Total soluble solids content was measured at harvest and during ripening until colour stage 6 as described earlier in section 3.2.3.7.

5.2.2.10 Green life

Green life was determined as indicated earlier in section 3.2.3.12.

5.2.2.11 Shelflife

Shelflife was determined as described earlier in section 3.2.3.13.

5.2.2.12 Chlorophyll content

Chlorophyll content was determined using the method of Arnon, (1949) as indicated earlier in section 3.2.3.4 with a few modifications. Three fingers per treatment were used and chlorophyll determination was carried out at harvest and during ripening until ripeness stage 6 (CSIRO, 1972, Marin *et al*., 1996).

5.2.2.13 Ethylene production and respiration (carbon dioxide production) rates

Ethylene production and respiration rates were determined as described earlier in section 4.2.1.12.

5.2.2.14 Sucrose, fructose and glucose contents

Sucrose, fructose and glucose contents were determined as earlier indicated in section 3.2.3.15 for fruits at harvest through ripening until ripeness stage 6 (CSIRO, 1972, Marin *et al*., 1996).

5.2.2.15 Visual appearance

The fruits were checked for incidences of dirt, which included, dust, bird droppings and spider webs and mechanical injuries (blemishes). They were also checked for general visual appearance. Percentage surface area covered was rated based on the Merz 0 to 6 scale (Merz, 2000), adopted for surface area covered by dirt instead of lesions where, 0=0%, 1=0 to 2%, 2=2 to 5%, 3=5 to 10%, 4=10 to 25%, 5=25 to 50% and 6=>50% of the surface area covered by the blemishes, dust and spider webs.

5.2.3 Statistical analysis

Data were examined for normality using R software and outliers by scatter plot using the Ms Excel software. Data were then subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS statistical programme (SAS, 2001). The means were compared according to Student Newman Keul's (SNK) test ($\alpha = 0.05$) and LSD ($\alpha = 0.05$) to test for significant effects.

5.3 Results and Discussion

5.3.1 Effect of bunch covers on grade, finger length and bunch weight

Bunch bagging had no significant ($p>0.05$) effect on grade, for both tissue-cultured bananas cultivars Grand Nain and Williams (Table 5.1 and 5.2). Bunch bagging tended to increase bunch weight significantly ($p\leq0.05$) for cultivar Grand Nain while that of cultivar Williams was not influenced. Finger length of fruits for cultivar Williams was not influenced by bunch bagging, while for Grand Nain, fingers from bunches grown under shiny blue covers had significantly ($p\leq0.05$) longer fingers than those from the control while the length was similar to the fingers from bunches grown under dull covers (Table 5.1 and 5.2).

In South Africa, a 16.5% increase in 'Williams' bunch mass was recorded due to a 10% increase in finger length (Robinson, 1996). This may have been due to increased temperatures (0.5 ˚C) under blue covers that favoured growth (Robinson, 1996). Banana bunches sealed with polyethylene bags had increased fruit size at harvest (Amarante *et al.*, 2002; Weerasinghe and Ruwapathirana, 2002). However,

Table 5.1: Effect of bunch covers on finger grade (mm), finger length (cm) and bunch weight (kg) of tissue culture banana cultivar Grand Nain

Treatment	Grade (mm)	Finger Length (cm)	Bunch Weight (Kg)
Control	29.18[a]	18.47[b]	7.71[b]
Dull blue	34.92[a]	20.00[ab]	7.83[b]
Shiny blue	32.41[a]	22.00[a]	14.53[a]
LSD	8.25	2.52	5.68

Values in the column followed by the same letter are not significantly different according to LSD test (α=0.05).Values are means of 3 replicates.

Table 5.2: Effect of bunch covers on finger grade (mm), finger length (cm) and bunch weight (kg) of tissue culture banana cultivar Williams

Treatment	Grade (mm)	Finger Length (cm)	Bunch Weight (kg)
Control	30.94 [a]	19.07 [a]	8.62 [a]
Dull blue	33.33 [a]	20.03 [a]	10.44 [a]
Shiny blue	33.44 [a]	20.37 [a]	9.16 [a]
LSD	2.72	1.87	5.56

Values in the column followed by the same letter are not significantly different according to LSD test (α=0.05). Values are means of 3 replicates

bagging some fruits such as lychee and mangoes had no effect on fruit weights (Amarante *et al*., 2002). Research reports on bagging of fruits have given contradictory information on the effect of bagging on both physical and compositional quality of fruits (Amarante *et al*., 2002) which may reflect differences in cultivar, bagging material and climatic conditions.

5.3.2 Pulp/peel ratios

Pulp/peel ratio for cultivar Grand Nain banana fruits (Fig. 5.1A) was initially influenced by bagging with the fruits grown under shiny blue covers having

significantly ($p \leq 0.05$) higher values compared to those grown under dull blue covers and control. However, this difference was not there at the eating ripe stage. Bunch covering had no significant ($p \leq 0.05$) effect on changes in the pulp/peel ratios of fruits of cultivar Williams at harvest and during ripening (Fig. 5.1B). This agrees with the result of the bunch weights above (section 5.3.1) indicating that the shiny blue covers increased bunch weights and especially the pulp portion giving the higher values for cultivar Grand Nain at harvest. In bananas, the pulp portion continues to grow even in the later stages of maturation (Turner, 1997; Nakasone and Paull, 1998).

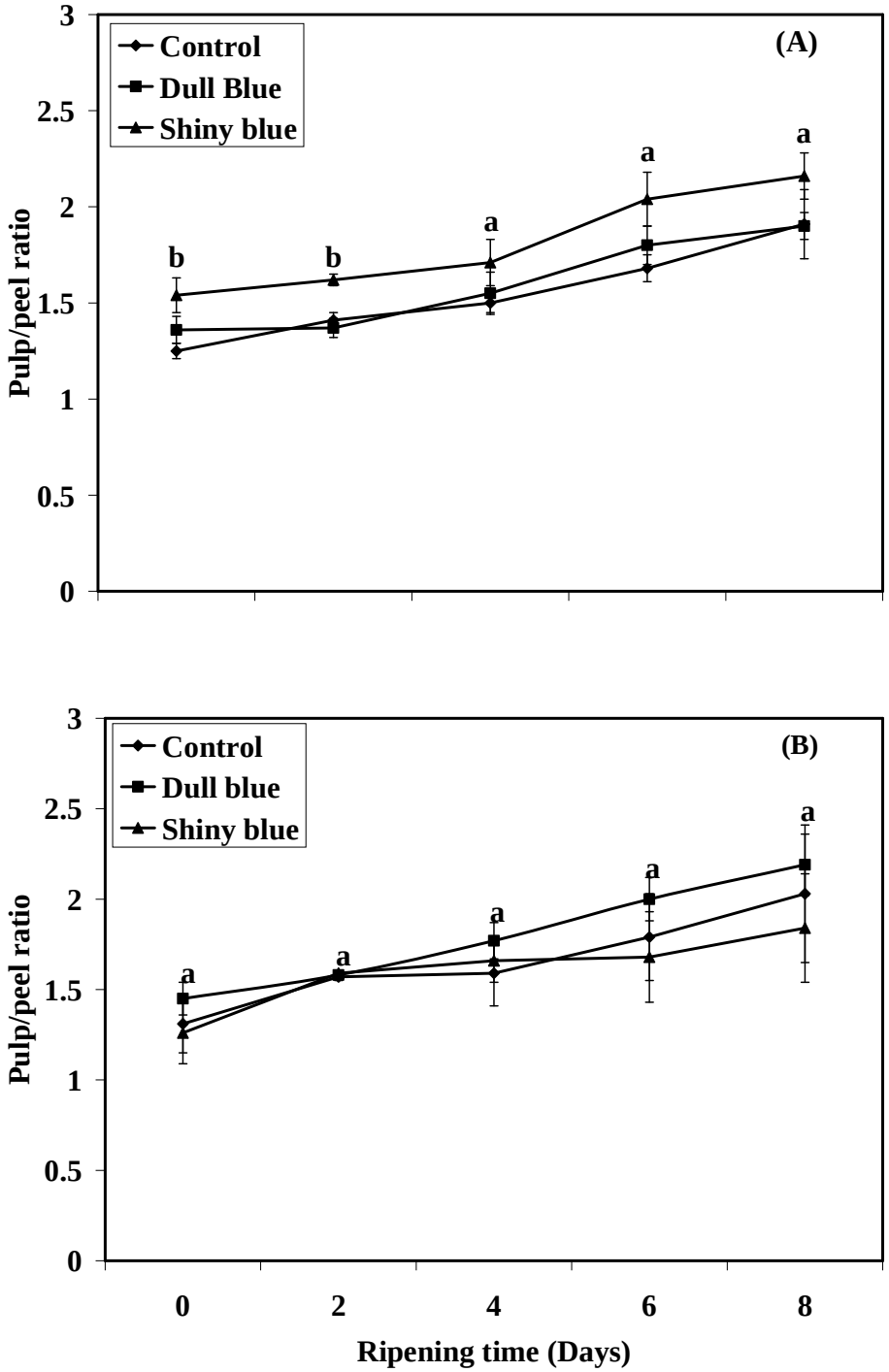

Figure 5.1: Effect of bunch covers on pulp/peel ratio during ripening of cultivars Grand Nain (A) and Williams (B) fruits. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

5.3.3 Starch and total soluble solids

Both starch and total soluble solids (TSS) at harvest and during ripening were not influenced significantly ($p \leq 0.05$) by bunch covers for both cultivars (Figs. 5.2 and 5.3). Starch reduced as ripening progressed while TSS increased. Unripe bananas have large amount of starch, with a content of 20-25% found in the pulp of the fruit (Nascimento *et al.*, 2006). During the climacteric, the accumulated polysaccharide is rapidly degraded and most of it is converted into soluble sugars which form a large proportion of TSS in the banana (Marriot, 1980; Seymour *et al*., 1993). Starch degradation to sugars in bananas occurs rapidly during the ripening process which allows the fruits to attain desired sweet taste for consumption. Enzymes for both hydrolytic and phosphorolytic breakdown of starch have been identified in the banana (Seymour *et al*., 1993). The primary product of starch breakdown is sucrose via sucrose phosphate synthase. The hexose sugars then arise from sucrose hydrolysis perhaps by the action of acid invertase in the vacuole whose activity has been shown to increase during banana ripening. The conversion of starch to sucrose and sucrose turnover creates a high demand for ATP, and sugar accumulation and respired carbon dioxide were highly correlated (Seymour *et al*., 1993).

Bagging, however, did not influence the starch formation during banana growth and starch degradation during ripening considerably in this study. Indeed, starch degradation in control fruits and those grown covered with dull and shiny blue covers proceeded normally in this study. However, in apples, bagging reduced starch content (Proctor and Lougheed, 1976) and fruit soluble solids at harvest (Proctor and Lougheed, 1976; Mattheis and Fellman, 1999). In other reports, panicle bagging of

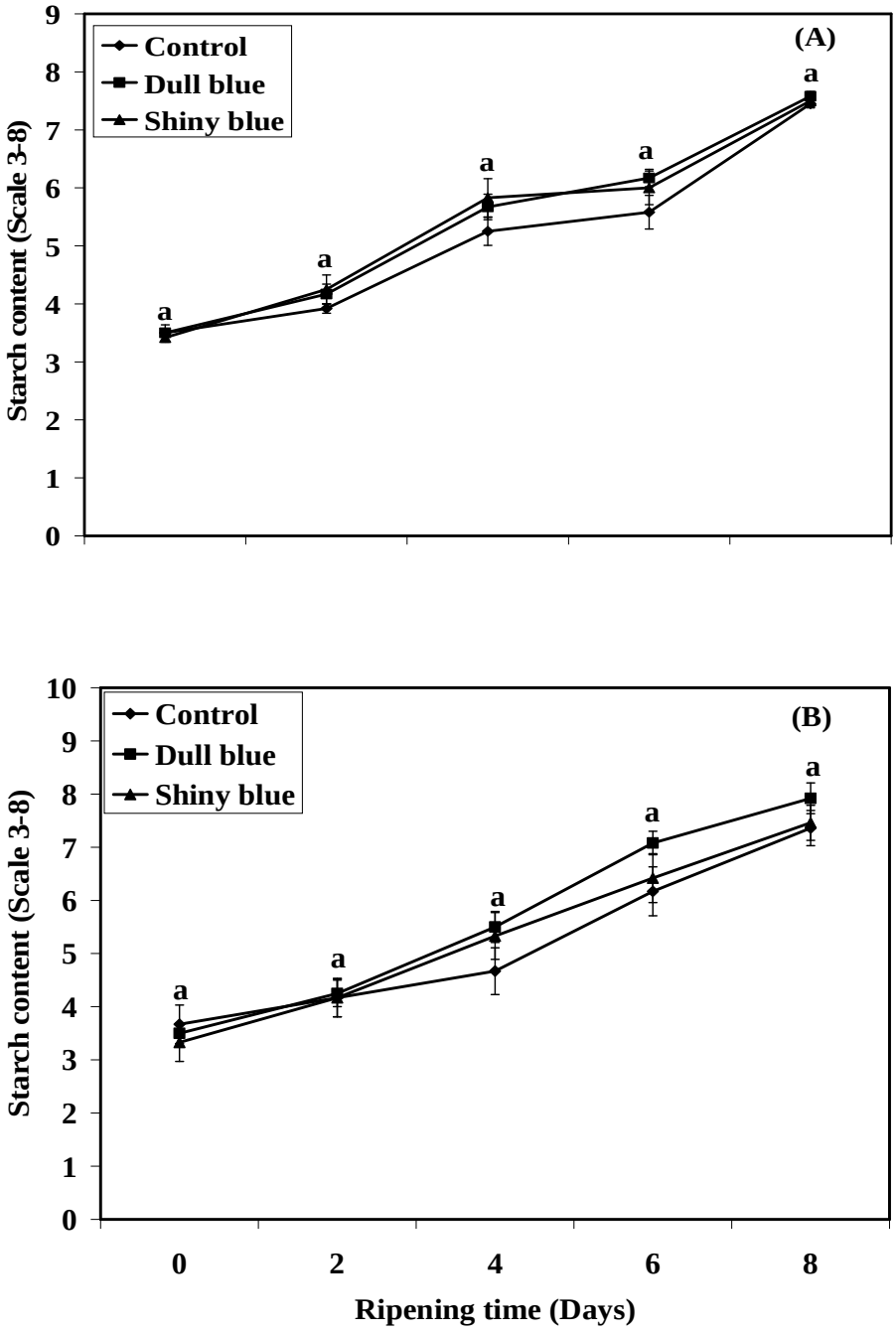

Figure 5.2: Effect of bunch covers on starch content of cultivars Grand Nain (A) and Williams (B) banana fruits using the Cornell Starch Chart scale 3-8 where 3= all starch and 8= no starch. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

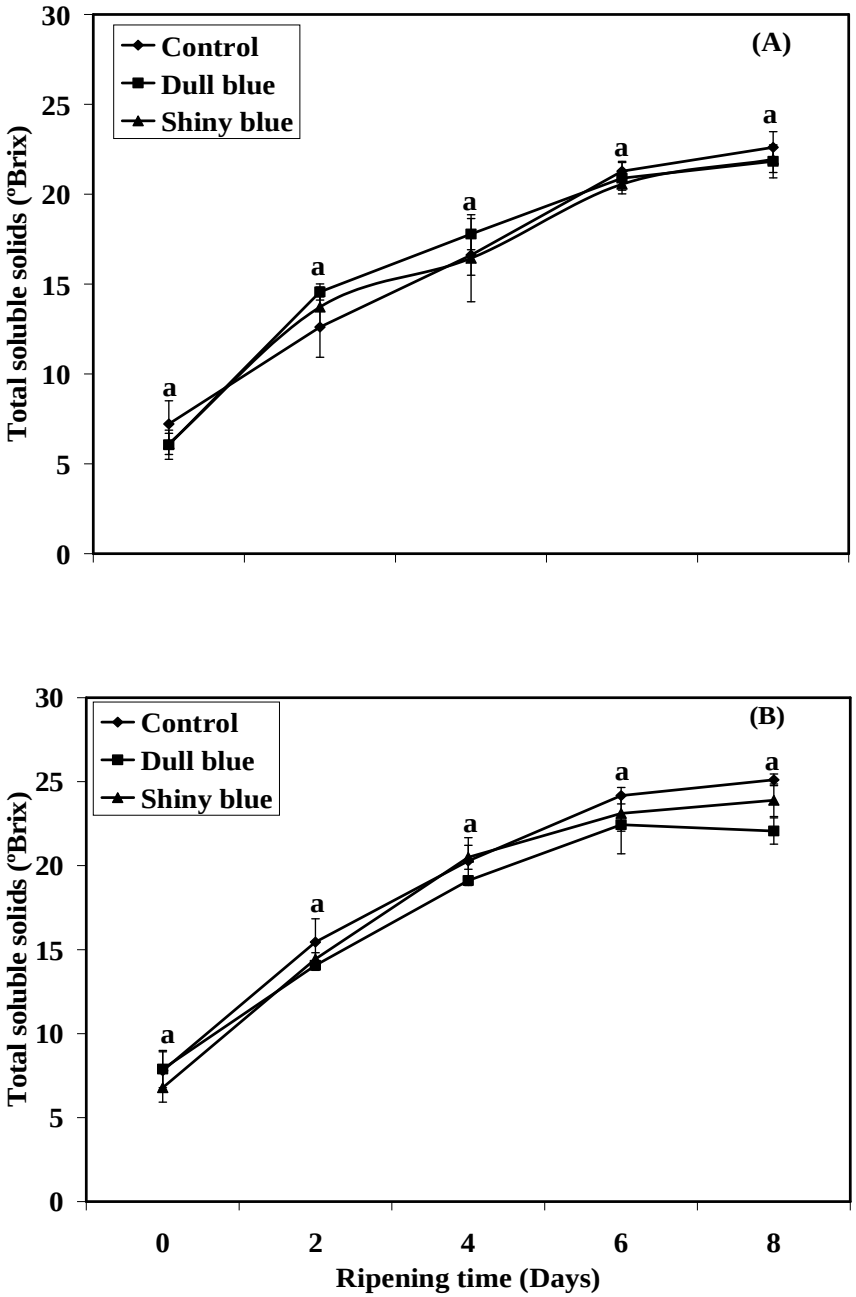

Figure 5.3: Effect of bunch covers on total soluble solids content (ºBrix) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

lychee was found to have no effect on total soluble solids (Tyas *et al*., 1998). Elsewhere, fruit ripening for mangoes was enhanced by preharvest bagging although there was no effect on TSS, acidity and sensory quality at the postharvest stage for the bagged and unbagged fruits (Hoffman *et al*., 1997).

5.3.4 Fruit moisture content and weight loss

Fruits from the bagged and non bagged treatments of both cultivars Grand Nain and Williams had similar moisture contents for peel (Fig. 5.4) and pulp (Fig. 5.5) at harvest and during ripening. Also, changes in weight loss of fruits of both banana cultivars during ripening were not significantly ($p>0.05$) influenced by bunch covers (Fig. 5.6). Moisture content of the peel reduced gradually during ripening while that of the pulp increased with ripening. Percentage fruit weight loss increased with days of storage in all the treatments. During normal ripening, the banana peel loses water to both the pulp and the atmosphere (Stover and Simmonds, 1987; Burdon *et al*., 1994). Fruit weight loss is attributed to physiological weight loss due to respiration, transpiration and other biological changes taking place in the fruit during ripening (Rathore *et al*., 2007). Fruit surfaces are covered by cuticle covers which restrict water loss through transpiration (Amarante *et al*., 2002). Fruits from the bagged and control bunches may have had similar cuticle structures (Amarante *et al*., 2002). Also, since the bunch covers in the current study had perforations, it is possible that the control and fruits grown under cover had similar humidity environment during growth and after harvesting. Similar observations were recorded in pears between fruits grown under perforated covers and control ones where both the moisture content and weight loss were not significantly ($p>0.05$) affected by pre-harvest

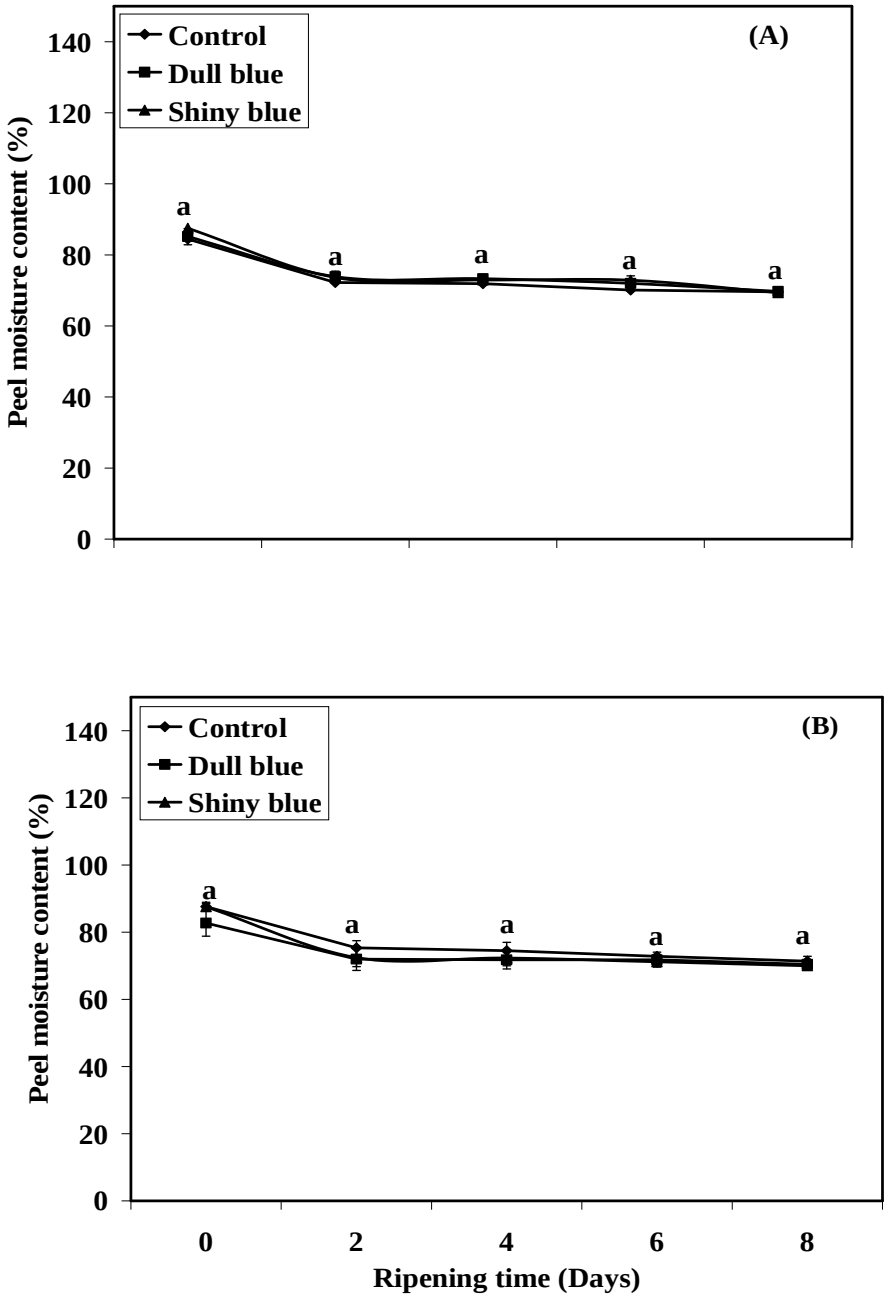

Figure 5.4: Effect of bunch covers on peel moisture content (%) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

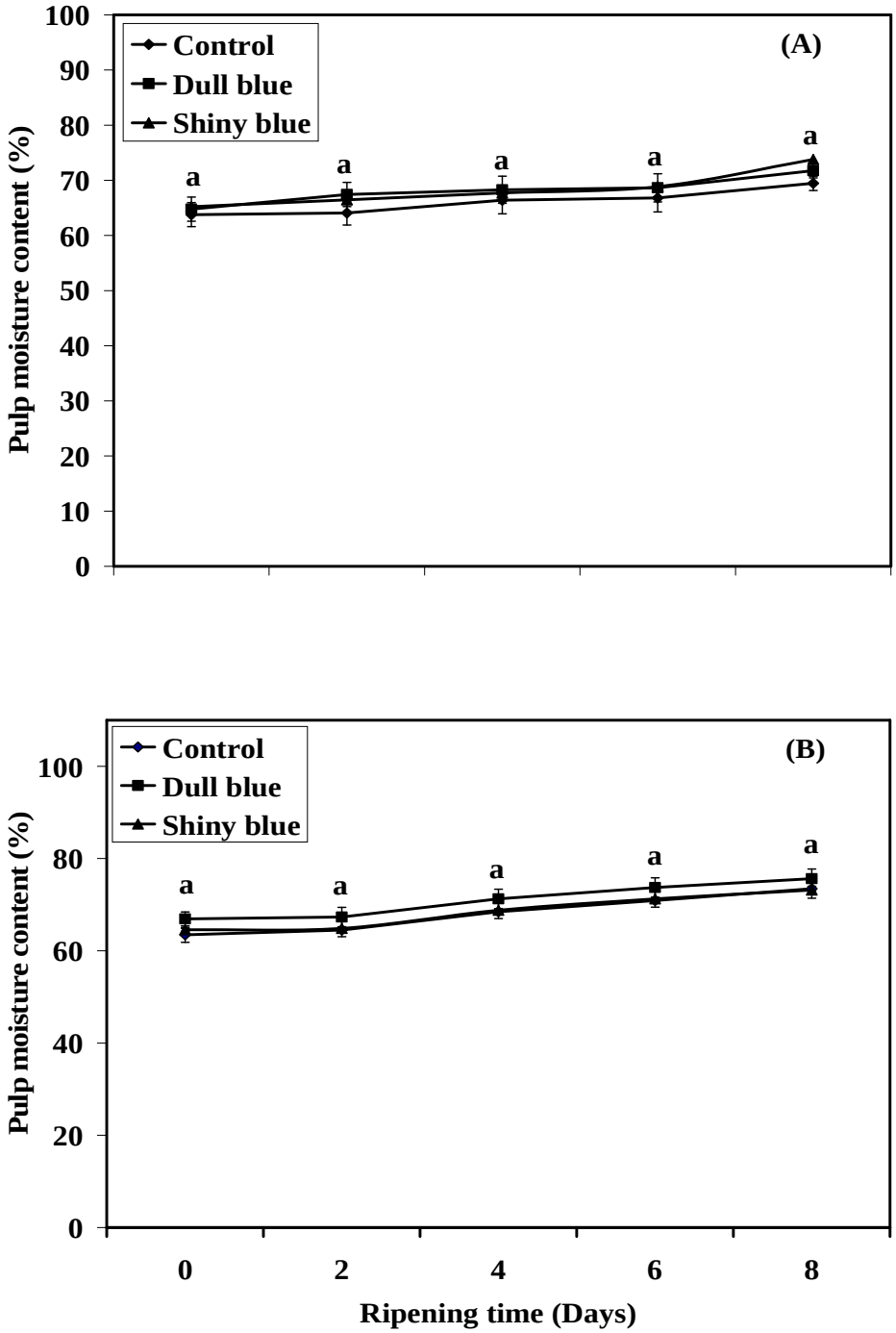

Figure 5.5: Effect of bunch covers on pulp moisture content (%) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

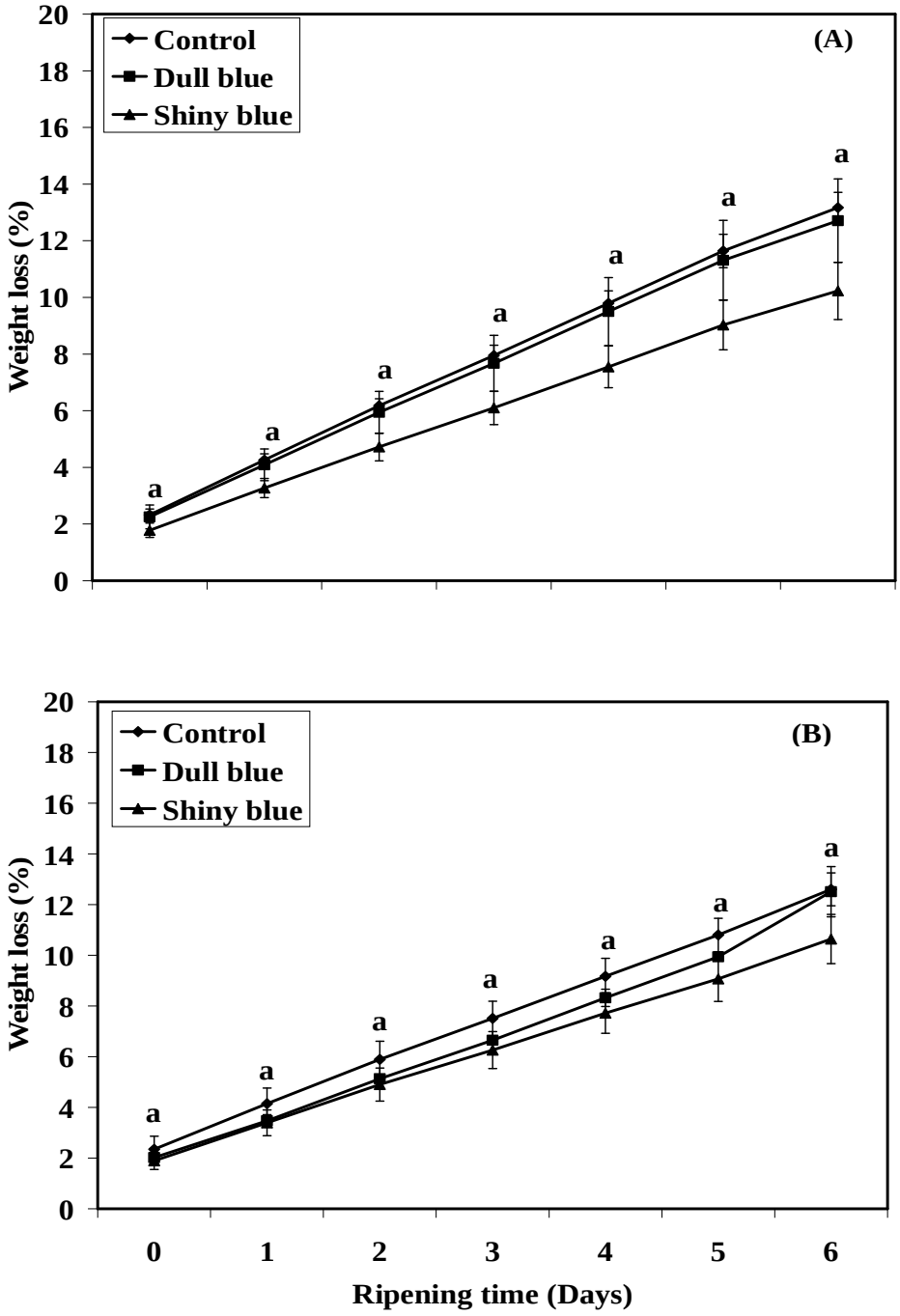

Figure 5.6: Effect of bunch covers on changes in percentage weight loss of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

bagging as they had similar skin permeability due to similar wax content of the cuticle (Amarante *et al*., 2002).

5.3.5 Total chlorophyll content

Effect of bunch covers on total chlorophyll content was not significant for fruits of cultivars Grand Nain and Williams at harvest and during ripening (Fig. 5.7). Chlorophyll content generally decreased on ripening as the fruits turned yellow. This is as a result of chlorophyll degradation and/or unmasking of the yellow carotenoids or synthesis of new pigments (Gray *et al*, 2004). Bunch bagging had no effect on the chlorophyll degradation which has been shown to be converted to colourless non-fluorescent chlorophyll catabolites in a pathway that is probably active in all higher plants (Gray *et al*., 2004). Variable results in pigment development in fruits due to bagging have been reported. Bananas grown under non-perforated blue transparent polyethylene, non-transparent blue polythene, non-transparent black polythene and without covers had green, pale green, glossy white and dark green peel which probably affected the chlorophyll content of the peel (Shanmugasundaram and Manavalan, 2002). Anthocyanin accumulation and red colour development of the skin was reduced by bagging (Hoffman *et al*., 1997; Joyce *et al*., 1997; Fan and Mattheis, 1998) while other reports indicate increased red colour development in apples (Wang *et al*., 2000) and pears (Amarante *et al*., 2002). This may reflect differences in the type of bagging material and whether perforated or not perforated. In this study, the bags were translucent blue and were perforated and hence allowed light penetration which may explain why bagging did not affect chlorophyll content.

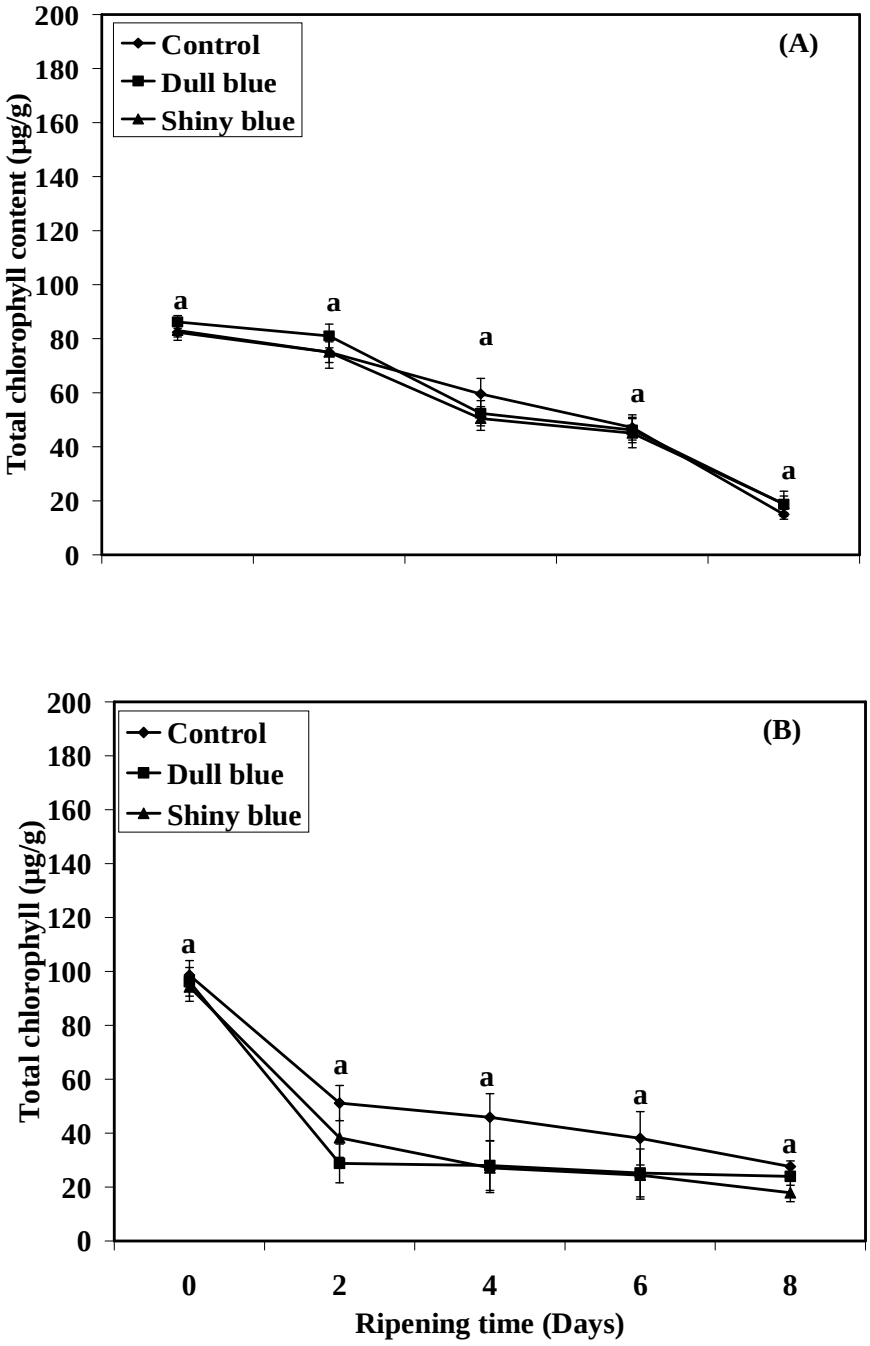

Figure 5.7: Effect of bunch covers on changes in peel chlorophyll degradation (µg/g) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

5.3.6 Peel and pulp firmness

Peel and pulp firmness measured objectively and subjectively were not significantly ($p>0.05$) different in all the treatments for both banana cultivars at harvest and during ripening (Figs. 5.8, 5.9 and 5.10). This may indicate that bagging did not change the peel and pulp properties considerably in this study. Firmness decreased rapidly during ripening, and gradually after ripening of the fruits. However, in other reports, bagging of fruit reduced fruit firmness in the postharvest stage for bananas (Amarante *et al.*, 2002) while it had no effect on firmness at harvest although it enhanced loss of firmness during cold storage for pears (Amarante *et al.*, 2002).

The variable results reported on the effect of bagging on fruit firmness at harvest and postharvest stage may reflect differences in the cultivar, type of bag, duration of cover, storage conditions and methods of testing for fruit firmness. In mangoes, opaque white plastic bags hastened softening of the skin while white waterproof paper bags did not have this effect (Joyce *et al.*, 1997). When non-destructive methods of assessing peel firmness are performed over the fruit skin, they mainly reflect the changes in skin properties. Differences in softening may reflect differences in skin composition and structure between treatments affecting loss of cell wall integrity (Amarante *et al.*, 2002). In this study, the bags were perforated and translucent and probably did not change the skin properties compared to the control.

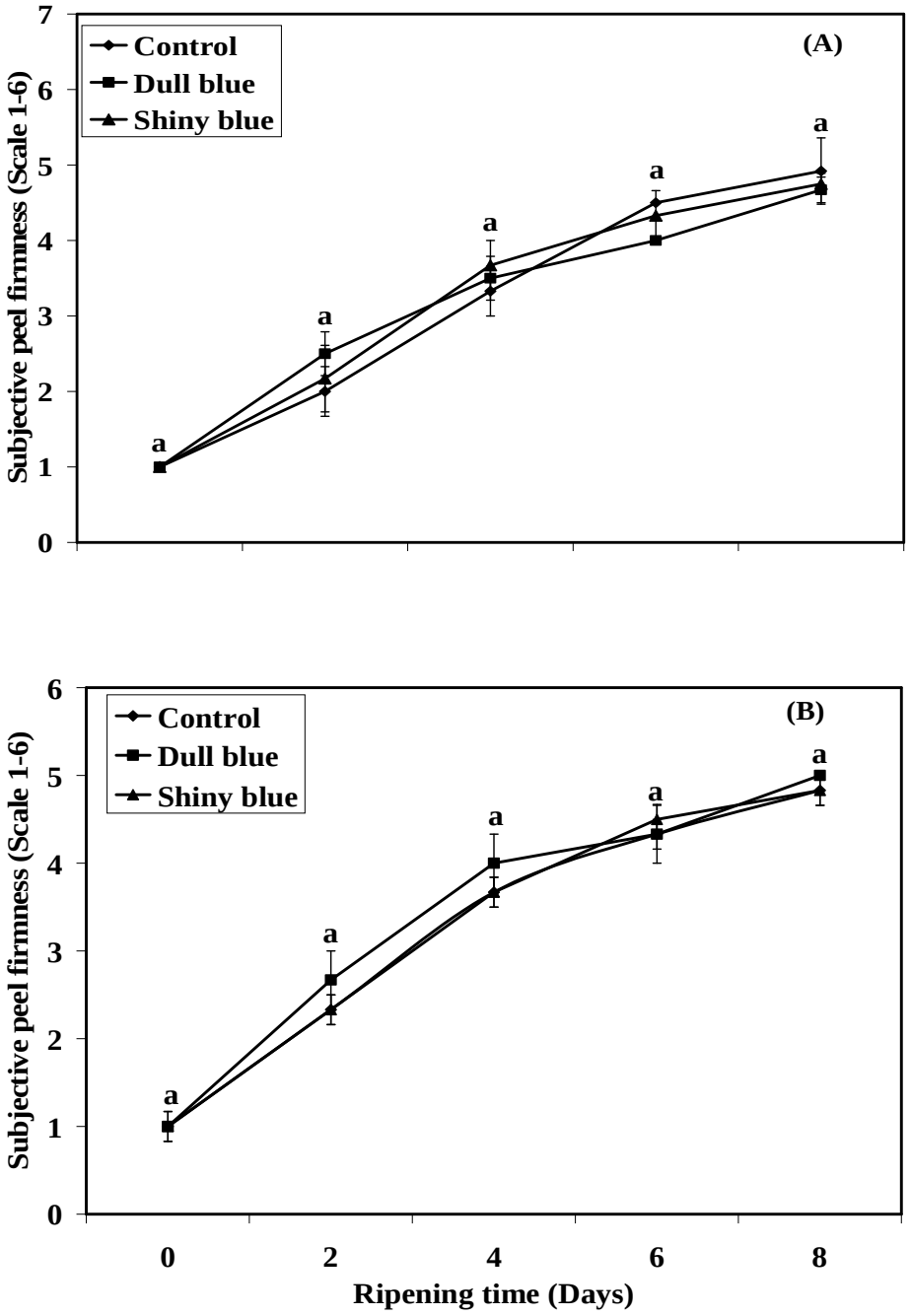

Figure 5.8: Effect of bunch covers on subjective firmness of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening using the scale 1-6 where 1 = hard, 2 = firm, 3 = slightly soft, 4 = moderately soft, 5 = soft and 6 = very soft (Joyce *et al*., 1993). Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

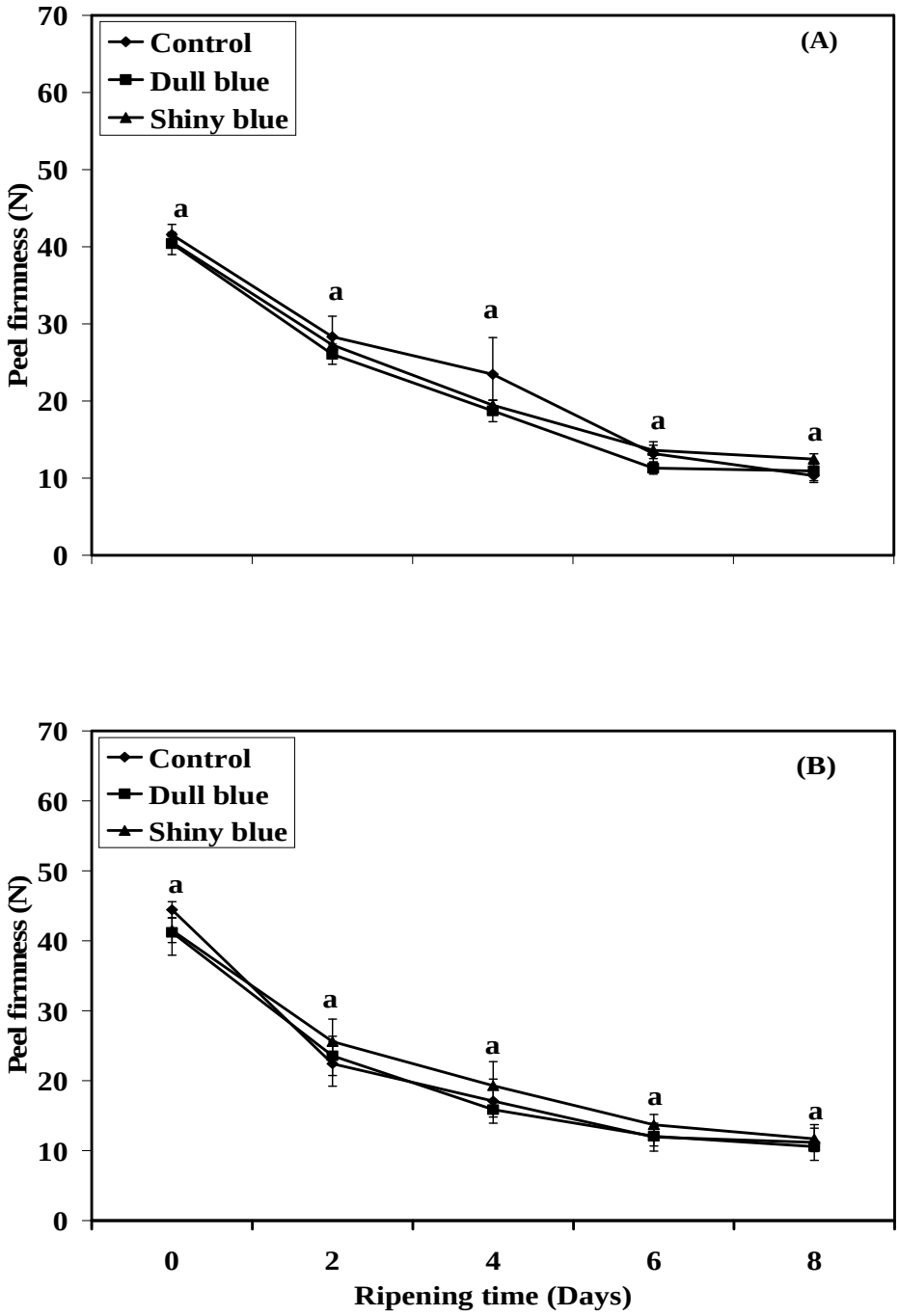

Figure 5.9: Effect of bunch covers on changes in objective peel firmness (N) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

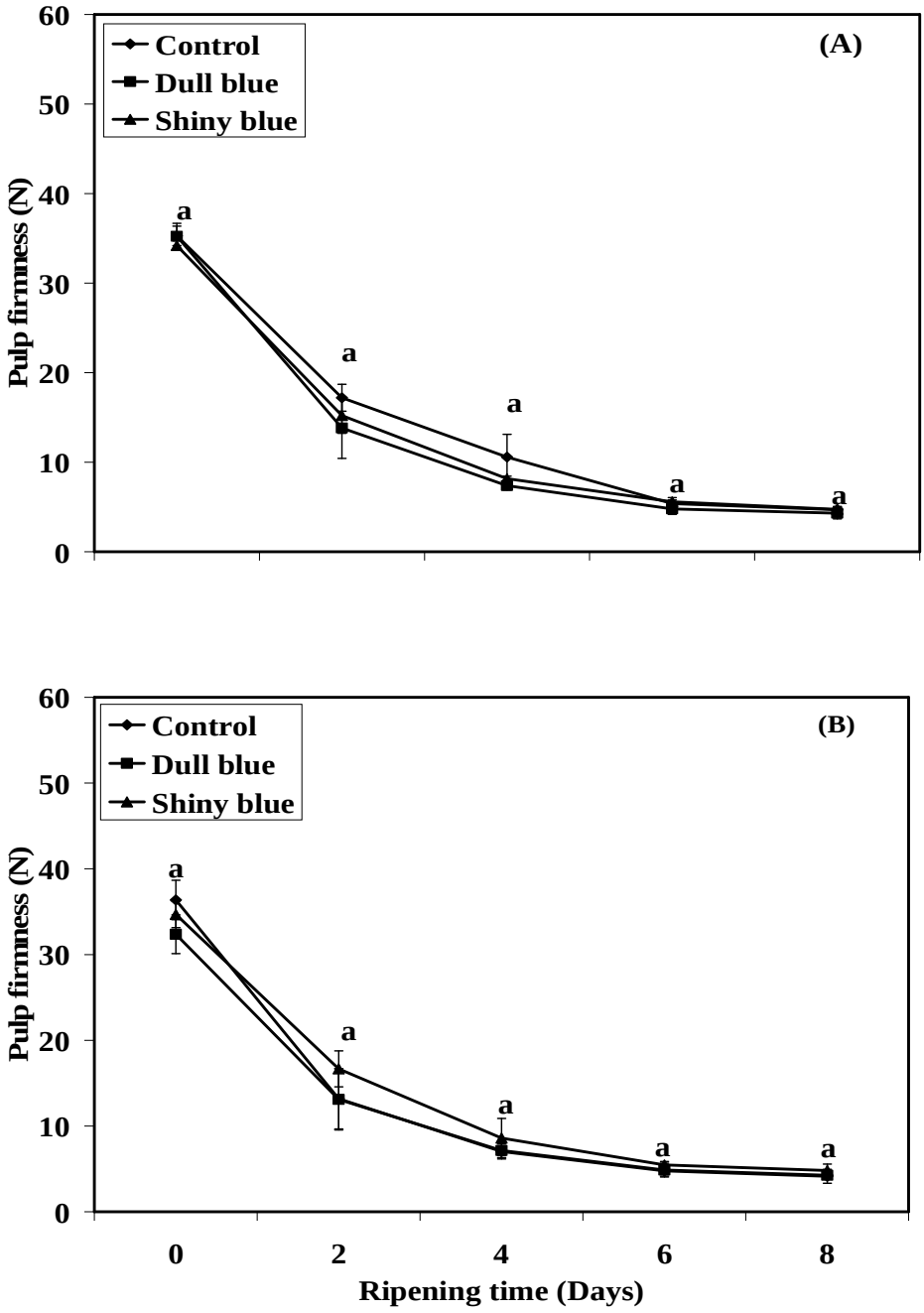

Figure 5.10: Effect of bunch covers on changes in objective pulp firmness (N) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

5.3.7 Colour

Colour measured subjectively at harvest and during ripening was not influenced significantly ($p>0.05$) by bunch covers for both banana cultivars (Fig. 5.11). Likewise, colour measured objectively (L* and hue angle values), of both peel and pulp were not affected by bagging in the current experiment (Figs. 5.12, 5.13, 5.14 and 5.15). The peel changed from green to yellow as the chlorophyll was degraded to unmask the yellow carotenoids (Gray *et al*., 2004) hence influencing the lightness of the peel positively on ripening. Therefore, L* value increased for the peel but decreased for the pulp on ripening as the peel degreened and the pulp turned from whitish to cream. Hue angle decreased for the peel also due to the change of the peel colour from green to yellow. Several reports have documented that bagging fruit increased skin lightness (Fan and Mattheis, 1998) which shows that bagging has different effects on different fruit cultivars.

The difference in effects on colour may also be dependent on type and duration of bagging. Other workers showed that preharvest bagging of pears with micro-perforated polypropylene bags resulted in fruits with a more attractive light green colour and did not reduce blush on the exposed side of the skin (Amarante *et al*., 2002). Unbagged lychee fruits had had lower intensity of colour (lower C*) than those bagged for 80 days but not different from those bagged for 20 and 42 days. The covers applied to the banana bunches in the current study were slightly translucent and perforated and therefore did not cause substantial modification of bag internal atmosphere to reduce chlorophyll accumulation as they allowed enough light to penetrate. Pear fruits bagged with micro-perforated transparent plastic bags

had similar anthocyanin content hence similar skin colour with control fruits probably due to the fact that the bags did not cause significant changes in internal atmosphere to reduce anthocyanin accumulation (Amarante *et al*., 2002). When bagging affects fruit colour components significantly, then the visual colour is also affected probably due to the influence of the bag on radiation and temperature and consequently pigment production (Tyas *et al*., 1998).

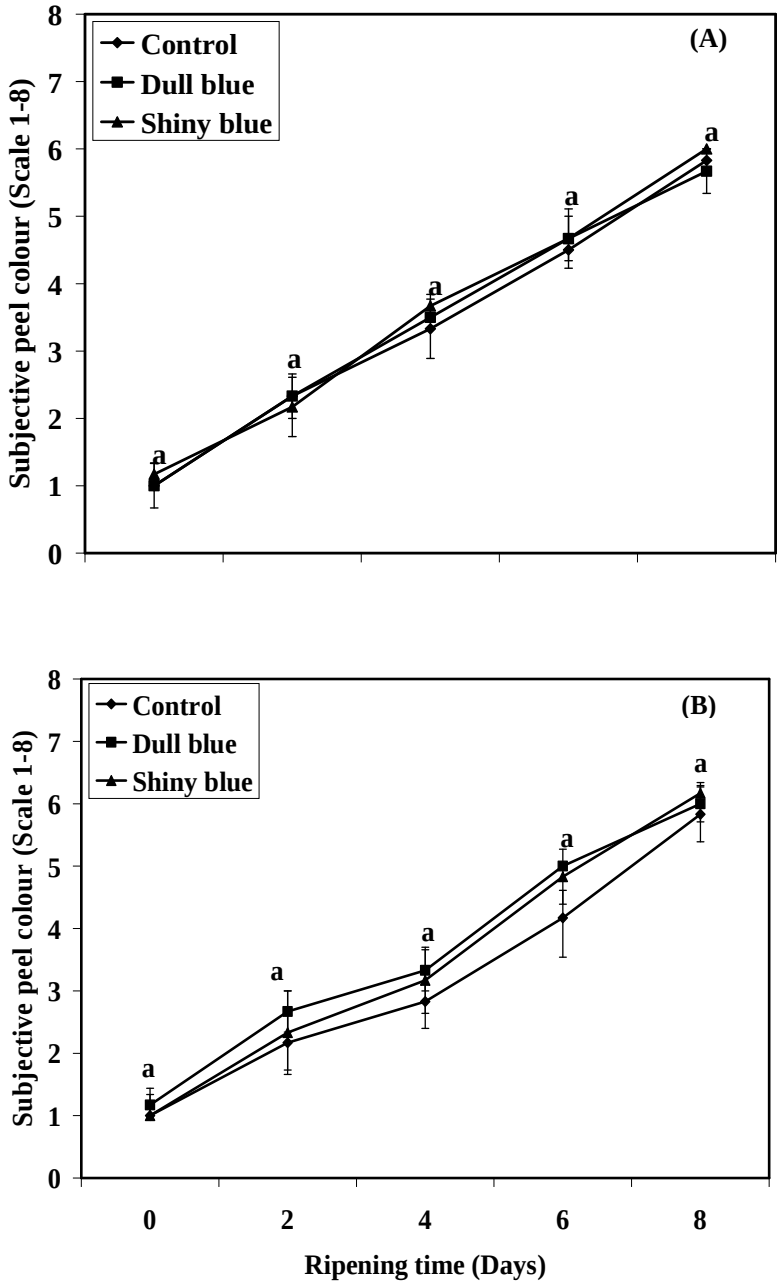

Figure 5.11: Effect of bunch covers on subjective peel colour of cultivars. Grand Nain (A) and Williams (B) fruits during ripening using the scale of 1-8 where 1=green, 2=light green, 3=half yellow half green, 4=3/4 yellow with green, 5=yellow with green tip, 6=fully yellow, 7=yellow with spots and 8=yellow with coalesced black spots (CSIRO, 1972; Turner, 1997). Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

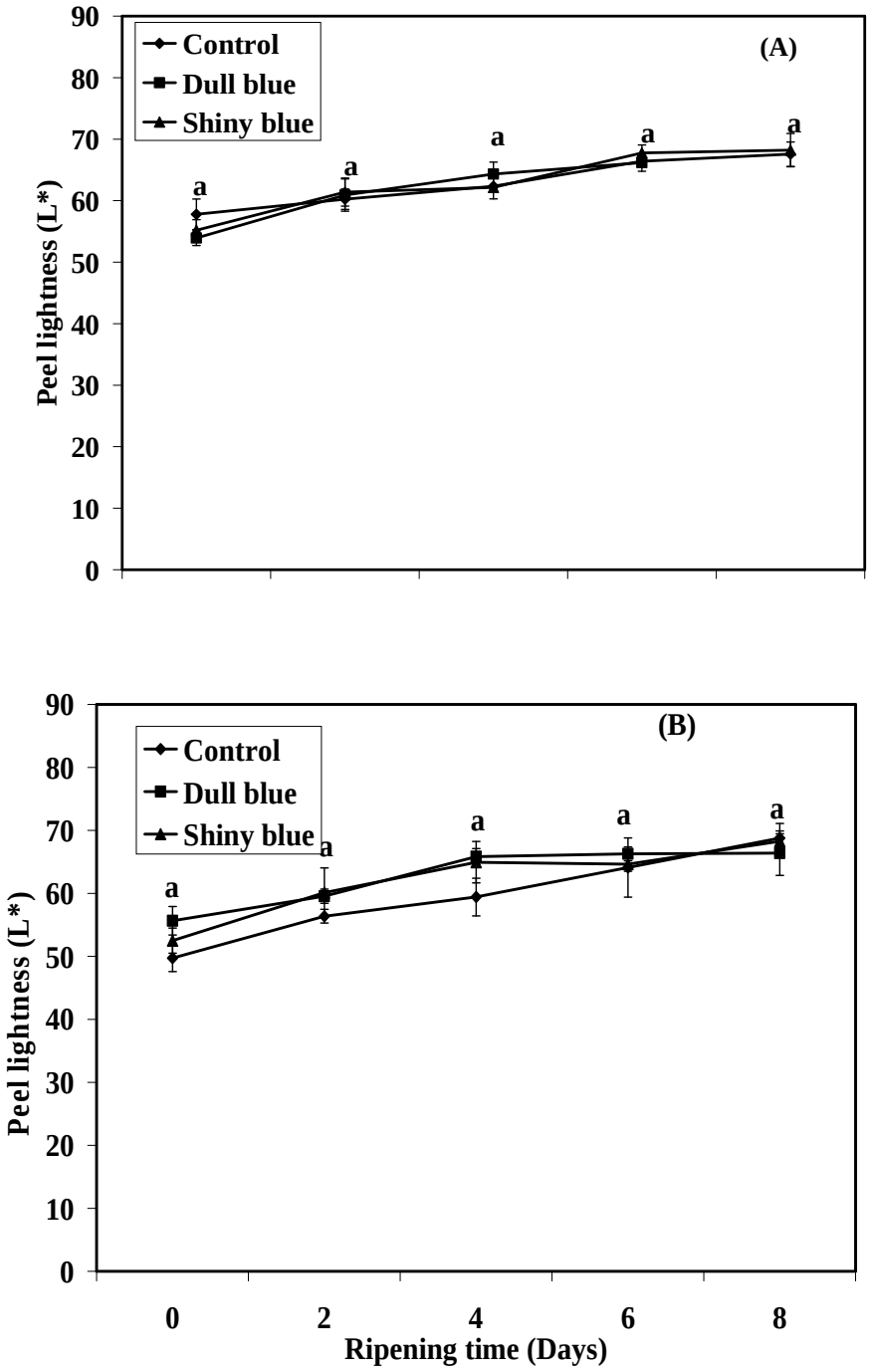

Figure 5.12: Effect of bunch covers on changes in peel lightness (L*) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

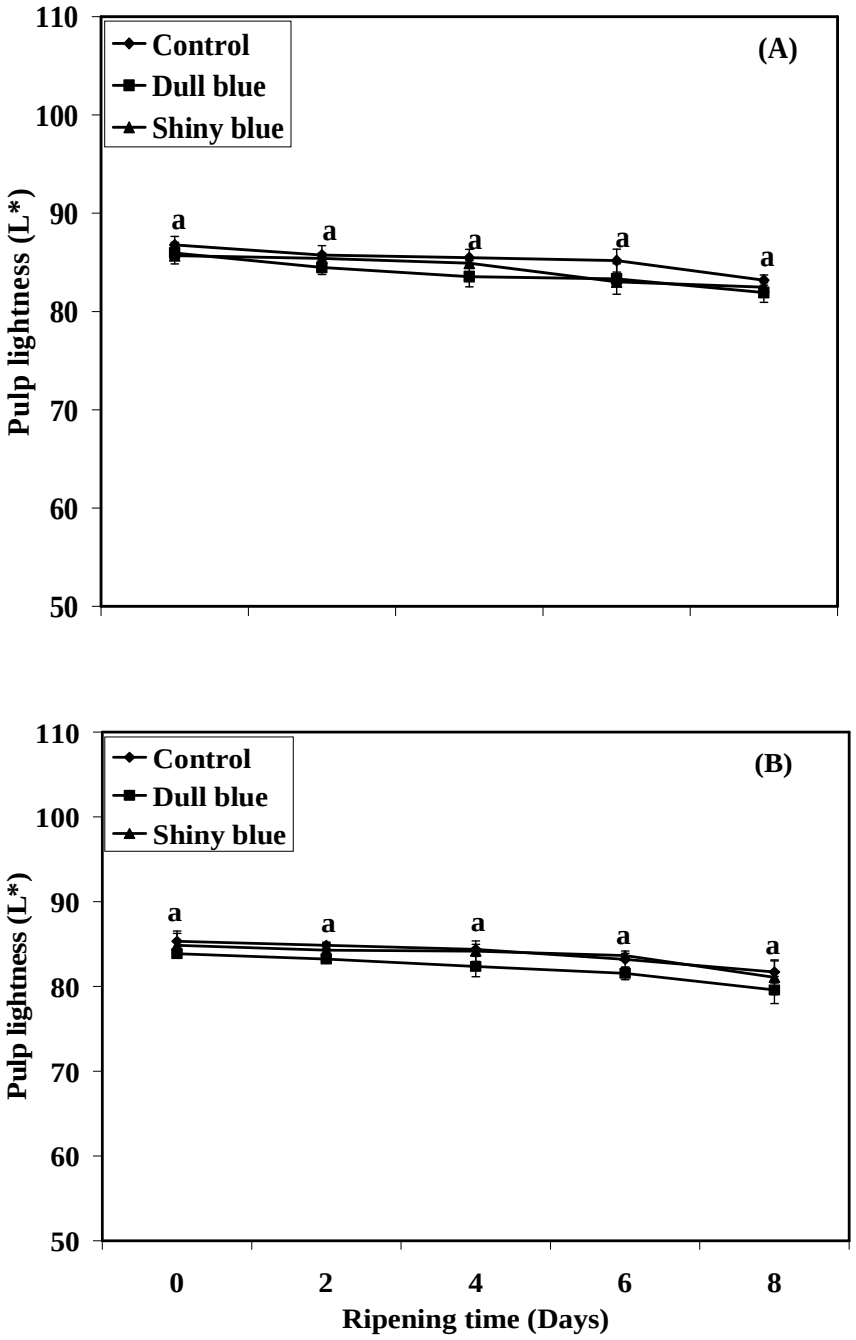

Figure 5.13: Effect of bunch covers on changes in pulp lightness (L*) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

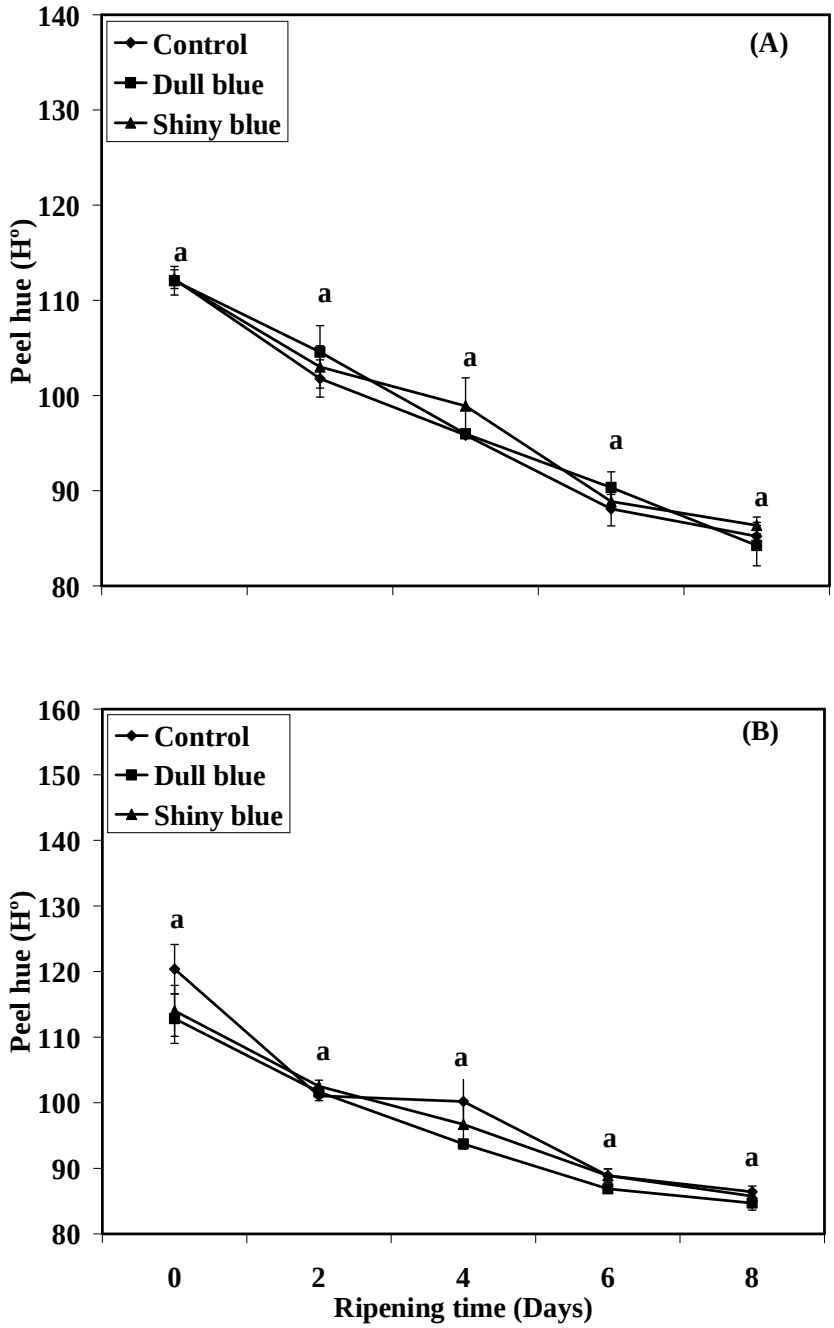

Figure 5.14: Effect of bunch covers on changes in peel hue (Hº) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

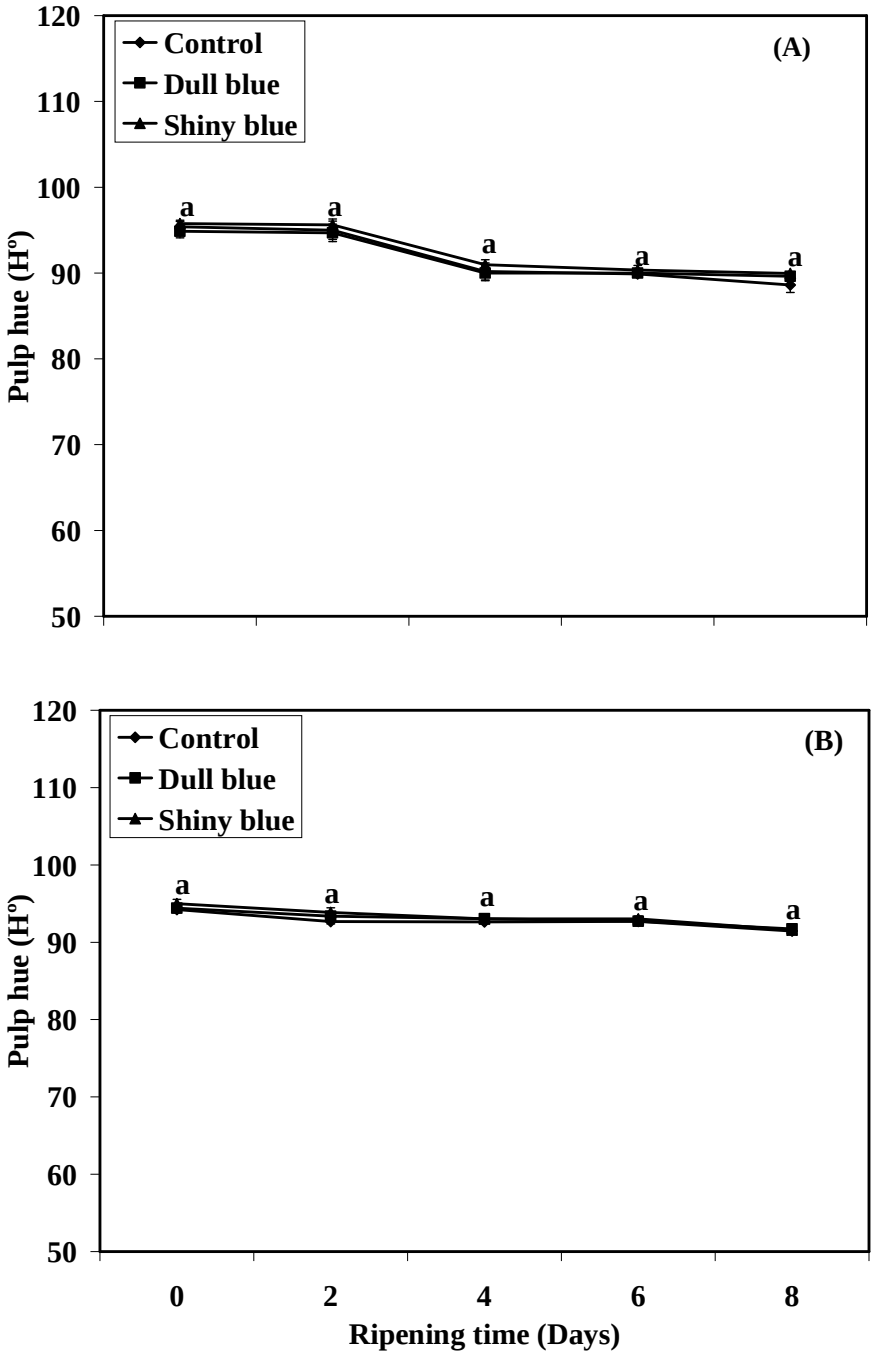

Figure 5.15: Effect of bunch covers on changes in pulp hue (Hº) of cultivars Grand Nain (A) and Williams (B) banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

5.3.8 Sugar content

Fructose and sucrose contents were not influenced by the bunch covers for cultivar Grand Nain, while glucose and total sugar content differed during early ripening but not at the fully ripe stage (Figs. 5.16 and 5.17). Both individual and total sugar contents were not influenced by covers for banana cultivar Williams (Figs. 5.18 and 5.19). Noro *et al.* (1989) reported results where only fructose was affected by bagging in apples with bagged fruits having higher content while other main sugars were not affected.

Other workers (Watson *et al.*, 2002) have reported that pre-harvest shading of strawberry fruits caused a significant reduction in sucrose and glucose/fructose contents compared to fruits from unshaded treatments. In the later experiment, shade netting was used which blocked some percentage of light from reaching the crop and hence may have affected such processes as photosynthesis and ultimately sugar synthesis. Light exposure of 'Sunscrest'/GF 677peaches resulted in increased reducing sugars content (Watson *et al.*, 2002). Covering grapes with cellulose bags was shown to reduce sugar content in the fruits compared to the uncovered control (Signes *et al*., 2007). The inconsistent result in effect of bagging on sugar content may be due to different cover materials, fruit cultivars and holding environment after harvest.

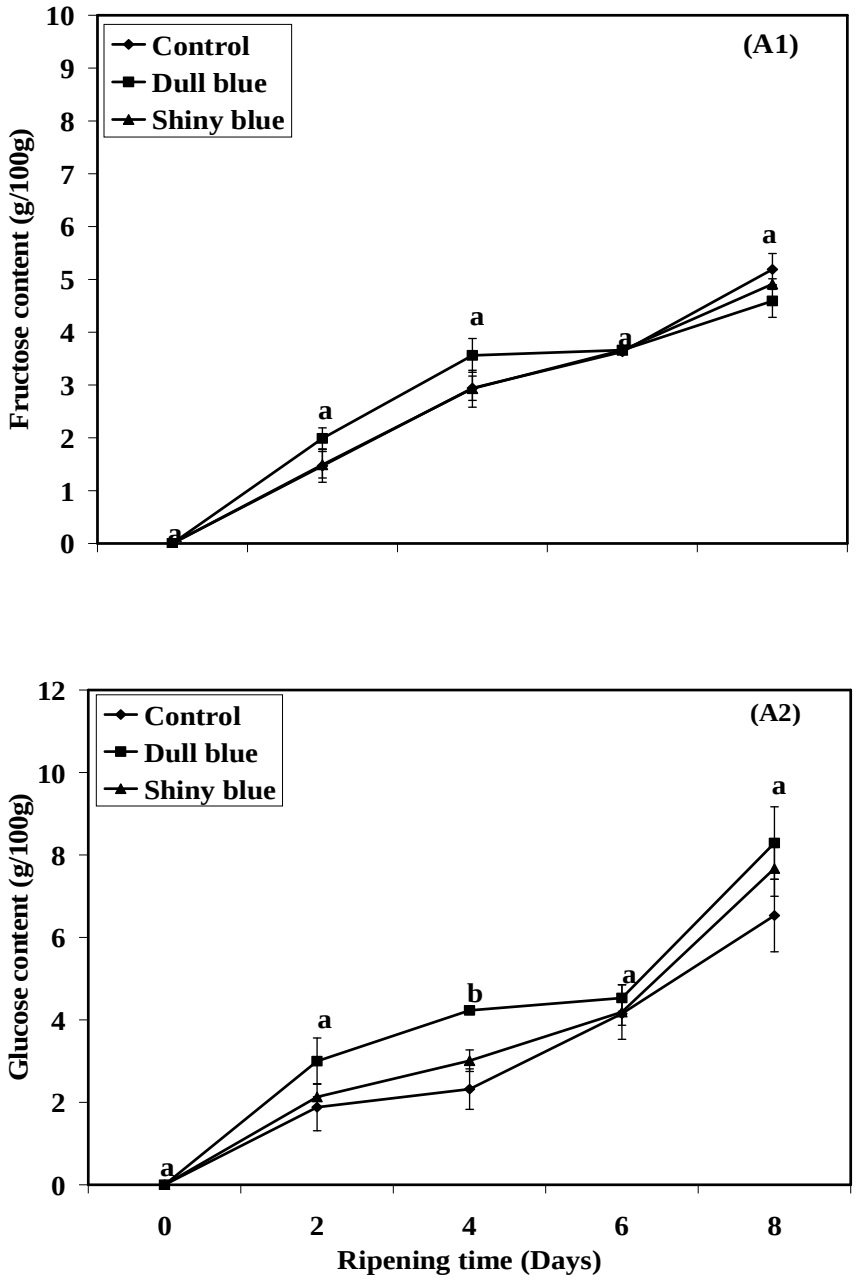

Figure 5.16: Effect of bunch covers on changes in fructose (A1) and glucose (A2) content of cultivar Grand Nain banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

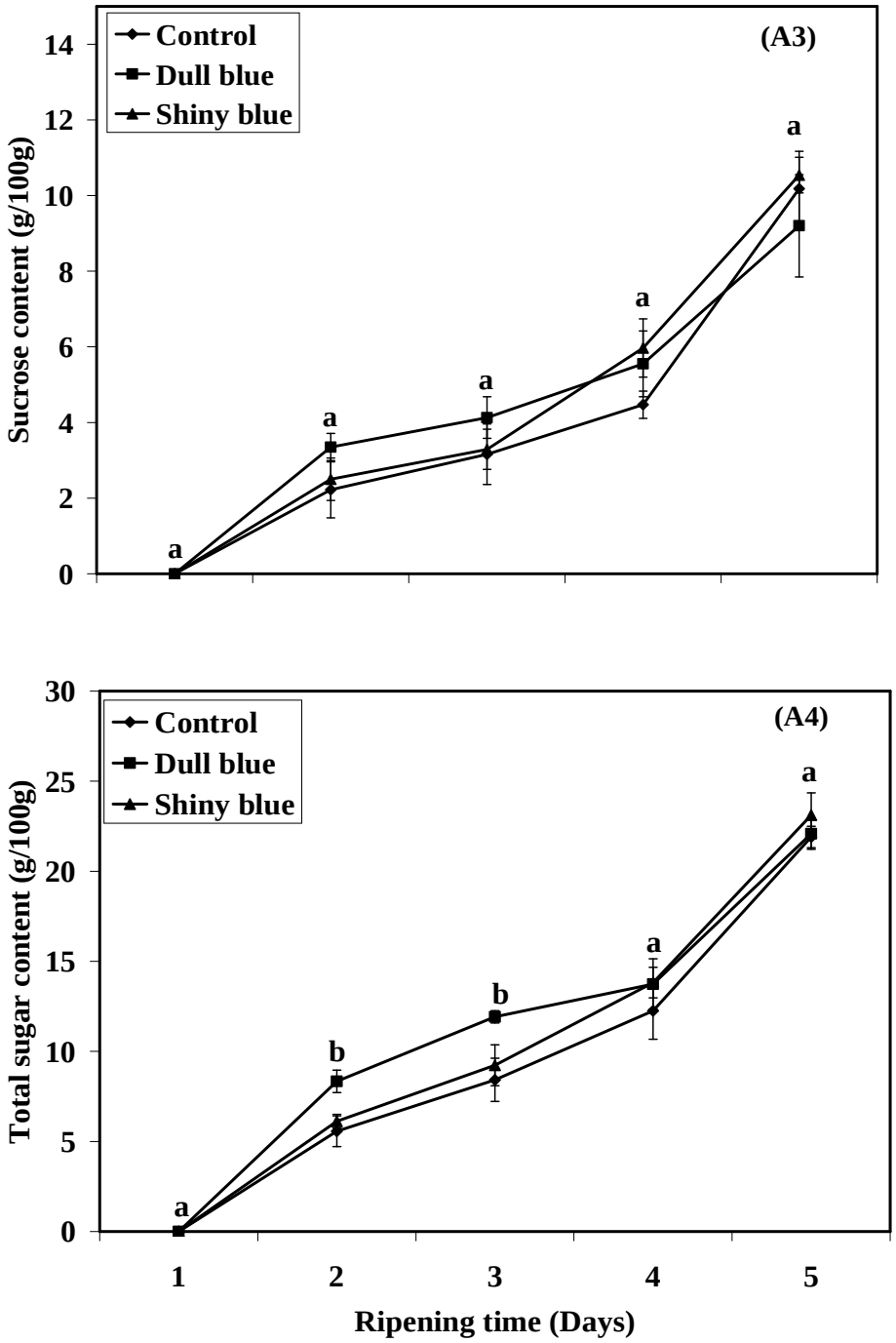

Figure 5.17: Effect of bunch covers on changes in sucrose (A3) and total sugars (A4) content of cultivar Grand Nain banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

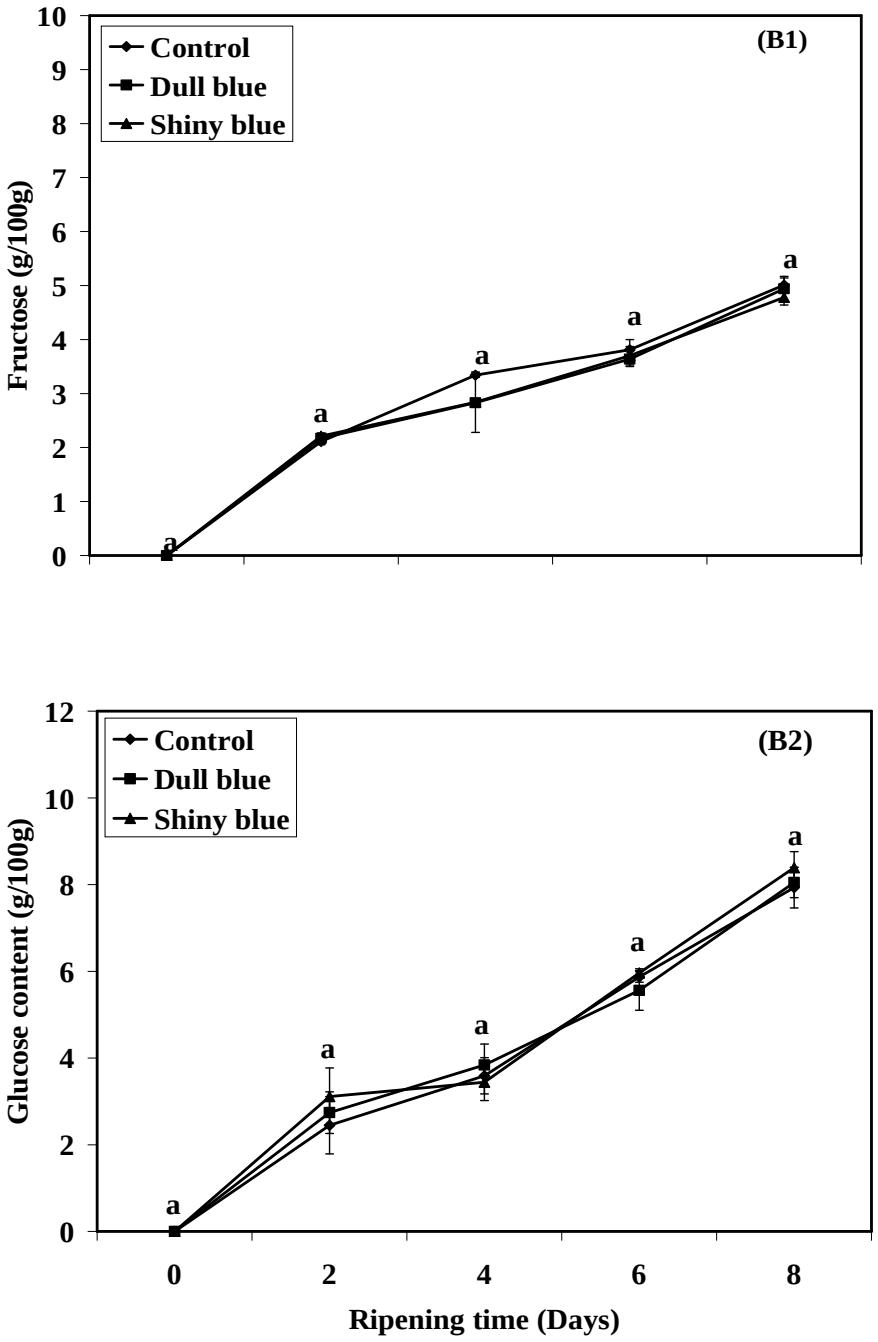

Figure 5.18: Effect of bunch covers on changes in fructose (B1) and glucose (B2) content of cultivar Williams banana fruits during ripening. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

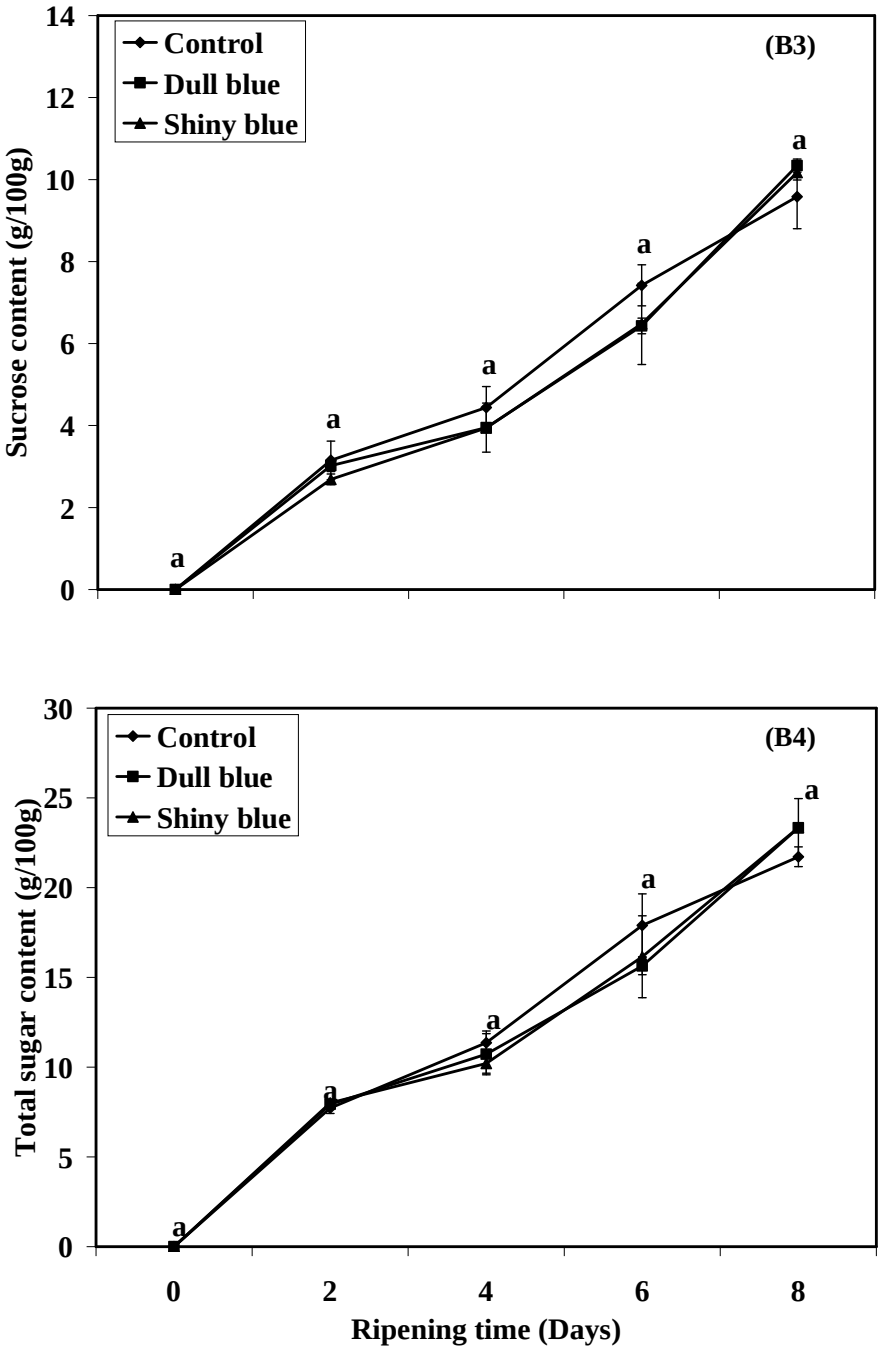

Figure 5.19: Effect of bunch covers on changes in sucrose (B3) and total sugars (B4) content of cultivar Williams banana fruits during ripening. Vertical bars represent SE of the mean of 3 replicates. When absent, the SE fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference at according to Student Newman Keul's (SNK) test (α=0.05).

5.3.9 Green life and shelflife

Green life and shelflife for both banana cultivars were not influenced significantly ($p>0.05$) by bunch covers (Tables 5.3 and 5.4).

Table 5.3: Storage life of tissue-cultured banana cultivar Grand Nain as influenced by bunch covers

Treatment	Green life (Days)	Shelf Life (Days)
Control	12.33[a]	4.33 [a]
Dull blue	11.00 [a]	4.33 [a]
Shiny blue	12.00 [a]	4.00 [a]
LSD	4.05	1.88

Values in the column followed by the same letter are not significantly different according to LSD test ($\alpha=0.05$). Values are means of 3 replicates.

Table 5.4: Storage life of tissue-cultured banana cultivar Williams as influenced by bunch covers

Treatment	Green life	Shelf Life
Control	14.67 [a]	5.33 [a]
Dull blue	10.33 [a]	3.67 [a]
Shiny blue	11.67 [a]	4.33 [a]
LSD	9.00	3.83

Values in the column followed by the same letter are not significantly different according to LSD test ($\alpha=0.05$). Values are means of 3 replicates.

Research reports on bagging of fruits have given contradictory information on the effect of bagging on both physical and compositional quality of fruits. Narayama *et al.* (2004) found bagging of bananas coupled with postharvest hot water treatment and storing with ethylene absorbent to be beneficial in extending shelflife. Elsewhere, banana grown under bunch covers had delayed ripening (Scott *et al*., 1971; Johns and Scott, 1989a) which may have possibly influenced green life. Fruit

bagging has also been shown to adversely affect fruit quality. Sealed plastic covers delayed bunch maturity of bananas (Scott *et al*., 1971). Fruit ripening for mangoes was enhanced by bagging (Hoffman *et al.*, 1997) which may have affected the green life. Banana bunches sealed with polyethylene covers during fruit growth had a longer green life compared to the control (John and Scott, 1989). The shorter green life of fruits from the control could be due to stresses caused by dust and blemishes among others during growth compared to fruit grown covered.

5.3.10 Ethylene production rate

Ethylene production followed a similar pattern for all treatments for both cultivars Grand Nain and Williams (Figs. 5.20 A and 5.20 B, respectively). The rates were, however, affected by the treatment and for cultivar Williams, fruits from the control treatment had significantly higher rates, followed by those from the shiny blue cover treatment which also had higher rates compared to fruits grown under dull blue covers. Fruits of cultivar Grand Nain from all treatments had similar ethylene production rates during peak production but fruits from the control produced higher rates later during ripening compared to the other treatments. However, ethylene production rate of both banana cultivars followed a climacteric pattern irrespective of the treatment. Banana fruits show a characteristic climacteric pattern (Marriot, 1980; Jiang *et al.*, 2000).

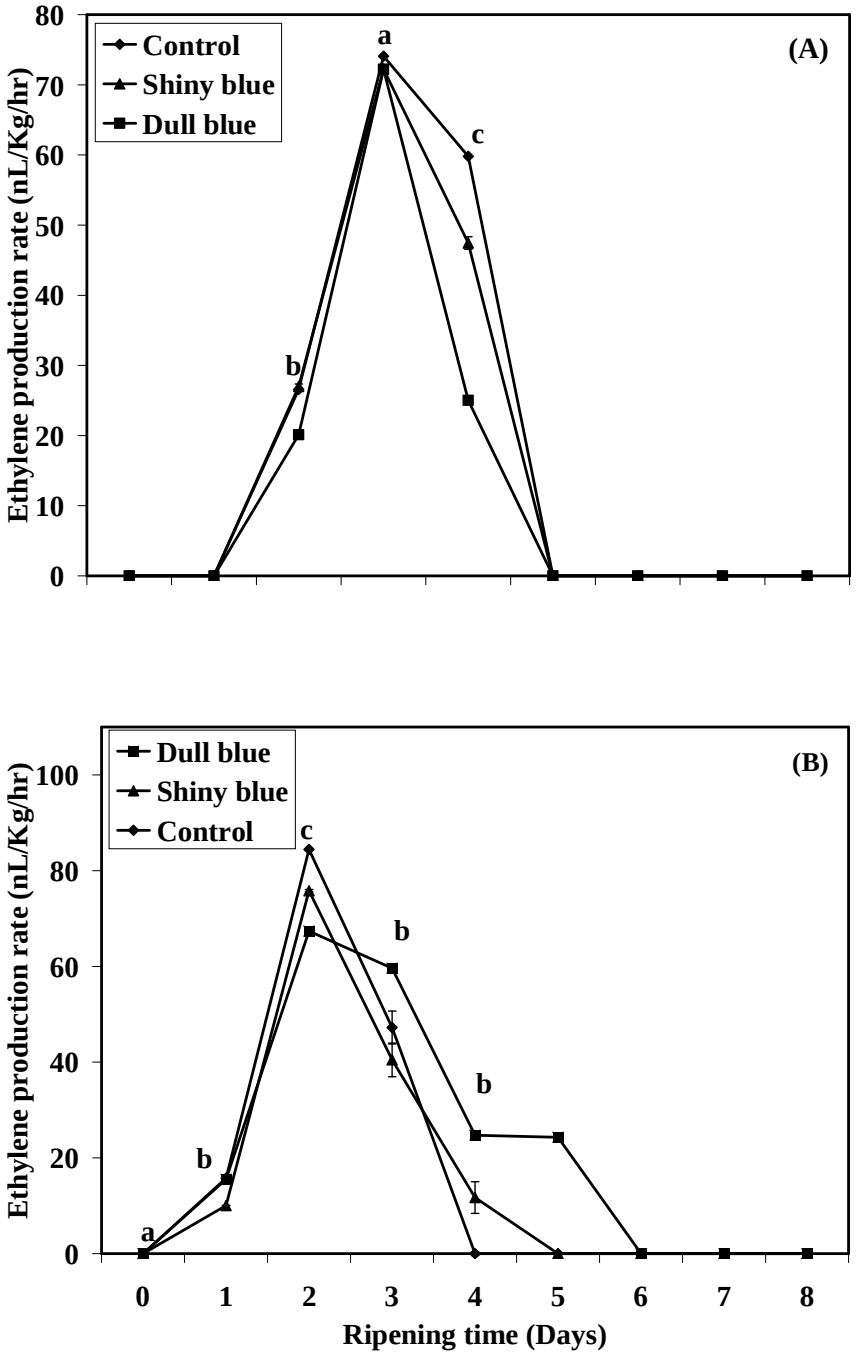

Figure 5.20: Ethylene production in fruits at harvest and during ripening of cultivar Grand Nain (A) and Williams (B) from bunches left uncovered and those covered with dull and shiny blue covers during growth. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

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The fruits from the control of banana cultivar Williams exhibited higher ethylene production compared to those grown under covers. The control fruit were grown in the open and could have been exposed more to such stresses as dust, fungal spores and blemishes among others, encountered during growth and may therefore produce slightly higher levels of ethylene. Bruising of the peel could have induced stress ethylene and hence the higher values of ethylene in the control fruits. Stress ethylene production has been associated with bruising in banana (Dominguez and Vendrell, 1993). Peel abrasion also increased ethylene production in bananas due to production of stress ethylene although not significantly (Llado and Dominguez, 1998). Various findings have been reported for effect of ethylene production due to bagging. In apples, both higher ethylene production (Fan and Mattheis, 1998) and no effect on production (Barden and Bramlage, 1994) have been reported which may reflect the type of bagging used. The different ethylene production patterns between the two banana cultivars in the current study may indicate a cultivar effect.

5.3.11 Respiration (carbon dioxide production) rate

Respiration rate was slightly affected by bagging after the climacteric in fruits of cultivar Grand Nain only in the fifth and the sixth day (Fig. 5.21 A). However, all the other days during ripening, bagging had no effect on respiration rate. Respiration rate for banana cultivar Williams was not significantly ($p>0.05$) affected by bunch covering (Fig. 5.21 B). This may indicate that there was no modification of fruit internal atmosphere by bagging enough to result in changes in skin permeability and large enough to suppress respiration (Amarante *et al.*, 2002). The increase in respiration rate exhibited a true climacteric pattern (Seymour *et al.*, 1993).

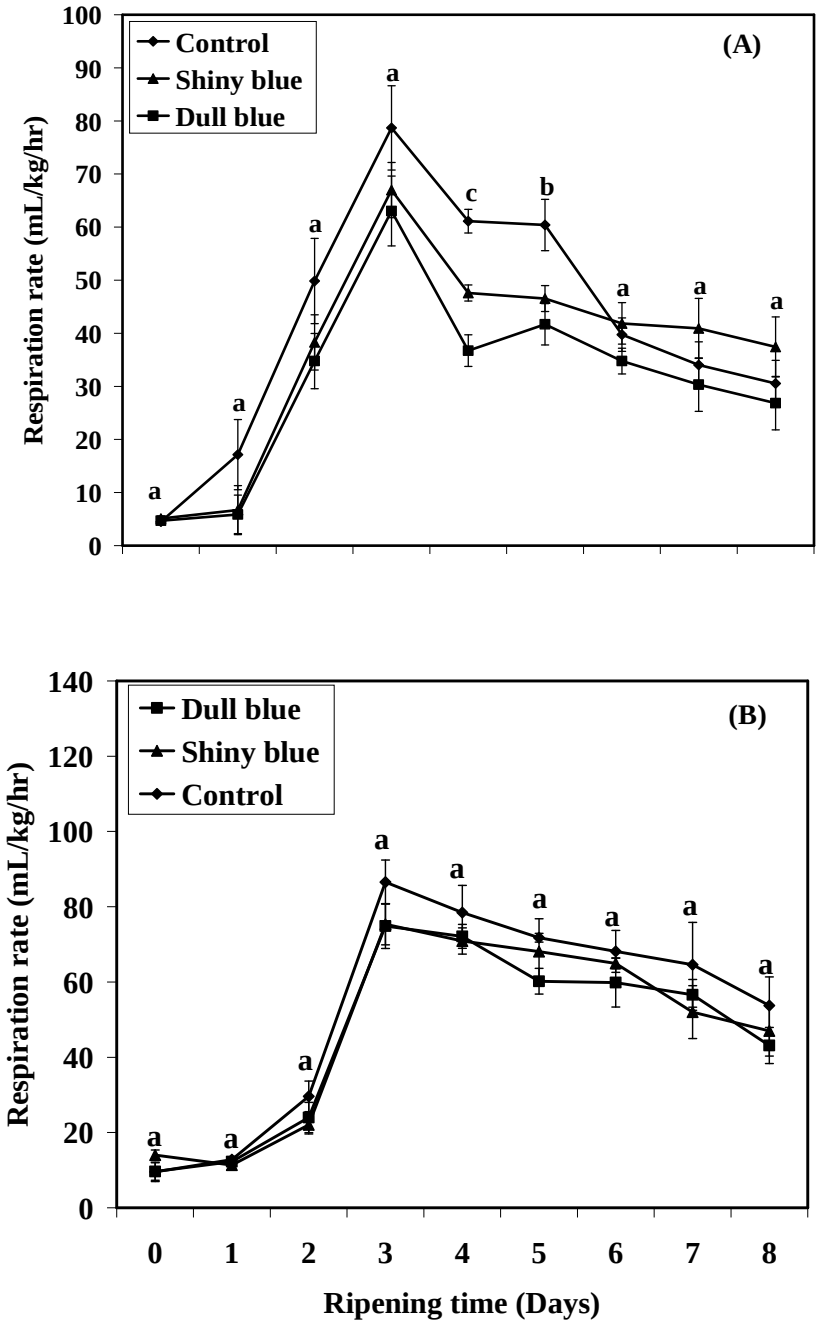

Figure 5.21: Respiration rates at harvest and during ripening of banana fruits of cultivar Grand Nain (A) and Williams (B) from bunches left uncovered and those covered with dull and shiny blue covers during growth. Vertical bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

In a typical climacteric fruit, a low respiration rate is observed before the onset of ripening, and then there occurs a surge in respiratory activity (Azzolini *et al*, 2005). Respiration in pears was also not affected by bagging (Amarante *et al*., 2002).

5.3.12 Visual appeal

Bagged banana fruits in the current experiment had minimal bruises (2-5%) and were significantly ($p \leq 0.05$) cleaner from dust, spider webs and bird drops at harvest compared to the unbagged fruits (>50%) for both banana cultivars Grand Nain and Williams (Table 5.5 and 5.6) based on the Merz 0-6 scale (Merz, 2000). In the current study, sunburn was not rated as a blemish.

Table 5.5: Effect of bunch covers on bunch area covered by blemishes, dust, spider webs and bird droppings of tissue-cultured banana cv. Grand Nain using Merz 0-6 scale (Merz, 2000), adopted for surface area covered by dirt instead of lesions where,0=0%, 1=0 to 2%, 2=2 to 5%, 3=5 to 10%, 4=10 to 25%, 5=25 to 50% and 6= >50% of the affected surface area.

Treatment	**Area covered by blemishes (Scale 0-6)**
Control	6[a]
Dull blue	2[b]
Shiny blue	2[b]
LSD	1.6

Values in the column followed by the same letter are not significantly different according to LSD test (α=0.05). Values are means of 3 replicates.

Table 5.6: Effect of bunch covers on bunch area covered by blemishes, dust, spider webs and bird droppings of tissue-cultured banana cv. Williams using Merz 0-6 scale (Merz, 2000), adopted for surface area covered by dirt instead of lesions where, 0=0%, 1=0 to 2%, 2=2 to 5%, 3=5 to 10%, 4=10 to 25%, 5=25 to 50% and 6= >50% of the affected surface area.

Treatment	**Area covered by blemishes (Scale 0-6)**
Control	6[a]
Dull blue	2[b]
Shiny blue	2[b]
LSD	1.2

Values in the column followed by the same letter are not significantly different according to LSD test (α=0.05). Values are means of 3 replicates.

The covered fruits were more visually appealing and cleaner compared to the unbagged fruits (Plate 5.1). This agrees with the findings of Weerasinghe and Ruwapathirana (2002) that banana fruits grown under covers had no blemishes at all and were attractive to consumers at a glance while unbagged fruits had black spots and blemishes caused by thrips and freckle fungi attacks. Similarly, postharvest fungal attack on lychee fruit was also reduced by bagging (Kooariyakul and Sardsud, 1997).

(A) **(B)**

PLATE 5.1: Visual appearance of banana cultivar Grand Nain fruits grown unbagged (A) and bagged (B) at harvest

A few of the covered fruits suffered sunburn which adversely affected fruit quality (Plate 5.2) especially in the hot season. This affected the bunches which were not well covered by leaves during growth. Top hand was mainly affected especially for bunches covered with dull blue covers probably due to more heat absorbed inside the cover compared to the shiny blue covers which may have reflected some heat away. Elsewhere, bagging of bananas resulted in sun scorching of the fruits irrespective of the colour of the bunch covers (Weerasinghe and Ruwapathirana, 2002). However, this can be overcome by maintaining enough leaves on the plant to shade the plant and also by using reflective blue covers (Anon, 2003). Pulling leaves over the covered bunches may also reduce/prevent sunburn. Also, inserting a newspaper on the inside of the bunch cover to cover top hands to prevent them from sun scorch has been found to work (Linbing *et al.*, 2004). The blue polyethylene covers have been shown to absorb more blue-green and ultraviolet lights which may cause sunburn to banana fruits (ShihChao *et al.*, 2004).

PLATE 5.2: Sunburn of fruits of top hands caused by shiny blue covers of banana cultivar Grand Nain at harvest

5.4 Conclusion and way forward

The study has shown that perforated dull and shiny blue bunch covers may be used in commercial banana orchards in Kenya to produce high quality fruits. The physical and biochemical properties of the banana fruits were not adversely affected by the bunch covers. Also, the fruits grown covered were more visually appealing as they were clean and had minimal bruises compared to those grown uncovered which implies reduced water usage during postharvest preparation of the fruits. Washing the fruits grown uncovered would only wash away the dirt but not the blemishes that are permanent. However, the bunch covers caused sun scald of a few top hands during the hot months. In the current study, sun scald was not rated as affecting visual appeal and only the unscalded fruits were rated for visual appeal. Bunch covers may therefore be useful mainly in the cooler months of the year and also in cooler climates where sunburn may not be a major concern. However, the use of bunch covers should be coupled with proper postharvest handling procedures to ensure that the clean, visually appealing fruits are not bruised during the postharvest

period. Such fruits could also be targeted for the export market where they may fetch better prices as the consumer clientele appreciates the visually appealing fruits and are willing to pay more for such fruits. A cost benefit analysis also needs to be done to find out whether banana bagging is profitable in Kenya. This work should also be carried out in other agro-ecological zones in the country such as in Upper midland zones 1 and 2 (UM1 and UM3), especially the cooler banana growing areas in the Meru region in Eastern Province. Other banana varieties may also be tested as they may exhibit differences in the way they are affected by the sun.

CHAPTER SIX

6.0 EFFECT OF COMBINING BUNCH COVERS AND 1-METHYLCYCLOPROPENE ON POSTHARVEST CHARACTERISTICS OF TISSUE-CULTURED BANANA (*Musa* spp.)

6.1 Introduction

High postharvest losses incurred during marketing of bananas could be attributed to rapid softening due to ripening and even rotting. In Kenya, these losses have been estimated at 40% and have been attributed to such causes as over-ripening among others (Chege *et al*., 1995). Fruit softening occurs very rapidly and is one of the main causes of quality deterioration during postharvest handling. Bananas are classified as climacteric fruits and hence show marked physiological changes during ripening. This poses a big challenge during transportation and distribution, and results in considerable postharvest loss.

Further, the handling, transportation, ripening and marketing of bananas require sophisticated technology and facilities for temperature control at 13°C to 14°C, modification of storage atmosphere to lower O_2, higher CO_2 levels and higher relative humidity (Thompson and Burden, 1995). These are not yet available in the banana producing areas of Kenya. Therefore, for developing countries like Kenya, there is need for alternative non-sophisticated technologies for extension of postharvest shelflife at ambient temperature and humidity conditions. This would allow subsistence producers, middlemen and retailers to use such technologies for long distance transport of fruits because of simplicity and low cost without refrigeration and atmosphere modification. Reduction of postharvest losses increases

food availability to the growing human population, decreases area needed for production and conserves natural resources (Kader, 2003).

Bananas being climacteric fruits are highly sensitive to endogenous and exogenous ethylene causing them to deteriorate fast after harvest (Seymour *et al.*, 1993; Jiang *et al*, 1999). Most of the qualitative changes that take place in climacteric fruits are mediated by ethylene, the ripening hormone which is the simplest organic compound with biological activity and plays a critical role in climacteric fruit ripening (Abeles *et al*., 1992). Therefore, regulating ethylene concentrations around climacteric fruits is an important commercial tool to achieve ripening at targeted times after harvest and to reduce the variability in ripening within fruit lots.

Ethylene responses can be controlled by regulation of its production and/or action (Mathooko, 1996). Inhibitors of ethylene action are considered favourable for use in agriculture since they provide protection against both exogenous and endogenous ethylene. 1-Methylcyclopropene (1-MCP) a synthetic cyclic olefin (Sisler and Blankenship, 1996; Sisler and Serek, 1997), is the one compound that seems to provide a new approach to manipulating ethylene action and has been identified as one of the potential inhibitors of ethylene (Blankenship and Dole, 2003). 1-Methylcyclopropene is a gas under ambient temperature and normal pressure conditions and has a formula of C_4H_6 and a molecular mass of 54 (Qiuping, *et al*, 2006). It acts by binding permanently and irreversibly to ethylene receptor sites in plant tissue (Sisler and Serek, 1997; Ciardi and Klee, 2001) thereby preventing ethylene from eliciting signal transduction and translation. This prevents ethylene-

induced effects in plant tissues for extended periods of time (Serek *et al*., 1994). This causes fruits to ripen and soften slowly, thereby, facilitating distribution and maintaining their high edible quality conditions for longer periods of time and has commercial significance for distribution centers and supermarkets. Plant tissues eventually recover sensitivity to ethylene apparently by synthesis of new ethylene receptors (Blankenship and Dole, 2003; Adkins *et al*., 2005) and unlike other inhibitors of ethylene action, the fruits do not need continuous exposure to 1-MCP or need to use sophisticated equipment.

Despite the wide use of 1-MCP in USA and Europe (Blakenship and Dole, 2003), few studies in developing countries have utilized this tool in extending the postharvest storage life of tropical commodities despite its simplicity and promising potential. Application of 1-MCP treatment has been shown to extend avocado fruit shelflife by 40% (Hoffman *et al*., 2000; Pesis *et al*., 2002) while it has also been shown that 1-MCP can enhance apple storability for as long as 9 months at ambient conditions of temperature and humidity (Watkins *et al*, 2000) while they store for only 61 days at ambient conditions without 1-MCP treatment (Varela *et al*., 2008). The storage life of other fruits such as peach (Mathooko *et al*., 2001), pear (Ekman *et al*., 2004), tomato (Wills and Ku, 2002, Mostofi *et al*., 2003) and banana (Jiang *et al*., 1999; Harris *et al*., 2000; Pelayo *et al*., 2003) has been enhanced by use of 1-MCP treatment. Indeed, 1-MCP is used internationally in the apple industry to improve retention of taste and textural quality attributes (Vallejo and Beaudry, 2006). However, it has also been shown that in apple fruit, the response to 1-MCP is cultivar dependent (Blankenship and Dole, 2003) and such response has, however,

not been tested in tissue-cultured banana fruits. It has also been shown that in banana fruit, the results of the use of 1-MCP treatment and the ability of the fruit to eventually ripen, is dependent on fruit maturity stage (Harris *et al*., 2000), cultivar, prior exposure to ethylene and growing conditions (Blankenship and Dole, 2003). No protocol has yet been developed for banana in the tropics. This shows that the use of 1-MCP treatment on banana still needs to be researched on in order to be able to devise suitable protocols that may be applicable in the banana industry for commercial use by all stakeholders.

The use of polyethylene bunch covers to mainly improve fruit quality during winter in the subtropical areas and to reduce physical blemishes tropical areas has become universal (Robinson, 1996; Amarante *et al*., 2002). However, although in some cases it has been shown to affect fruit structure and peel thickness (Amarante *et al*., 2002) hence susceptibility to mechanical damage, the impact of this practice on postharvest behaviour and response to postharvest treatments has not been adequately investigated for tissue-cultured banana in the tropics. Bunch covers probably have an effect on the physiological and biochemical characteristics of tissue-cultured bananas and their response to postharvest treatments. Further investigation on their effect on postharvest storage life in combination with postharvest treatments needs to be established. A lot of research has been done with respect to yield and crop performance of tissue-cultured banana, yet very little information is available on postharvest behaviour, quality and response to postharvest treatments. Moreover, although 1-MCP has been shown to delay the ripening of conventional banana, its effect has been shown to be variable and

dependent on the fruit maturity (Harris *et al.*, 2000) and growing conditions among others (Blakenship and Dole, 2003) but no such work has been reported using tissue-cultured banana in the tropics singly or in combination with bunch covers.

It is against this background that the efficacy of 1-MCP in combination with bunch covers on tissue-cultured banana fruits which has not hitherto been tested under tropical conditions was carried out. The objective of the study was to determine the physiological, biochemical and qualitative responses of tissue-cultured banana fruits to 1-MCP treatment alone or in combination with bunch covers, with a view of regulating ripening and extending postharvest storage life at ambient conditions of temperature and humidity.

6.2 Materials and Methods

6.2.1 Study area, plant material and parameters evaluated

Banana bunches of cultivar Williams were tagged in an already existing banana orchard 2008-2009 in Maragua District, Central Kenya, Agro-Ecological Zone, upper midland 3 (AEZ UM3) (Jaetzold and Schmidt, 1983). The farm is located at latitude 00º 49' 14"S and longitude 037º 08' 35" E as marked by a Global Positioning Satellite (GPS) instrument (Magellan, Triton, China). The bananas had been grown using the recommended agronomic practices (Anon, 2002). Perforated shiny blue bunch covers were applied to the bunches when the flower bracts had hardened and the hands had started to curl upwards. The bunch covers had perforations measuring 8 mm spaced at 10.5X9 cm and a thickness of 5µm and were left to hang for about 150 mm below the distal hands and were securely attached to

the bunch stalk above the proximal hand using a rubber band. Some of the tagged bunches were not covered and they served as control. The treatments were applied randomly in a completely randomized design and were replicated three times. The bunches were allowed to grow to full maturity and were then harvested. During transportation all precautions were taken to avoid damage to the fruit. Equatorial region hands of the bunches were selected in order to maintain uniformity of the samples. The position of the hand has an effect on the size of the fruit (Stover and Simmonds, 1987). The fruits were then washed with tap water to remove latex and dirt and were then subjected to fungicidal treatments by dipping for 1 min in 100 ppm sodium hypochlorite (Jik, Reckitt Benckiser-East Africa Limited, Kenya) in order to control spoilage during postharvest storage due to common fungal diseases such as anthracnose (*Colletotrichum musae*) and crown rot. The fruits were allowed to air-dry on the benches.

The fruits from both the control and covered treatments were divided into two lots and each was placed in 12 litre air tight plastic containers fitted with self-sealing rubber septum. 1-Methylcyclopropene gas was generated from Smartfresh^{TM} powder (Rohm and Haas Co., Japan) with a 3.3% w/w 1-MCP according to manufacturer's instructions. An air tight syringe was used to inject 20 ppm 1-MCP gas into one lot and other lot left untreated to serve as control. The fruits were kept in the containers for 24 h. Fruits were arranged into completely randomized design (CRD). The fruits were then ripened at ambient conditions of temperature (24±1˚C) and relative humidity (60±5%). Fifteen fingers per replicate were placed on the bench for green life evaluation. Fruits were then analysed for changes in physical, physiological and

chemical parameters during storage at ambient conditions. Parameters measured during storage were: total soluble solids (TSS), starch content, TTA, chlorophyll content, subjective and objective colour and firmness. Ethylene production and respiratory rates were also measured and the fruits were also evaluated for green life.

6.2.2 Analyses and Determinations

6.2.2.1 Starch content

Starch content was determined as earlier indicated in section 3.2.3.3. However starch content was determined from stage 1 through to senescence for fruits from the uncovered and covered control, while for the fruits grown covered or uncovered and treated with 1-MCP, the content was determined until the experiment was terminated when the fruits started to rot.

6.2.2.2 Firmness

Hand fruit firmness assessment was determined as described earlier in section 3.2.3.6 although the measurements were carried out at ripeness stage one to senescence for the control bagged and unbagged and until the end of the experiment for the fruits grown covered and uncovered and treated with 1-MCP. Firmness measured objectively was determined as indicated earlier in section 4.2.1.6 with the above mentioned modifications.

6.2.2.3 Colour

Colour measured both subjectively and objectively was determined at harvest and during storage until senescence for fruits from covered and uncovered control and until the termination of the experiment for the fruits grown covered and uncovered and treated with 1-MCP. The assessment methods are as described earlier in section 3.2.3.17 with the above mentioned modifications.

6.2.2.4 Total soluble solids content

Total soluble solids content was measured as described earlier in section 3.2.3.7. The analysis was carried out at harvest and during ripening until senescence for fruits from covered and uncovered control and until the termination of the experiment for the fruits grown covered and uncovered and treated with 1-MCP.

6.2.2.5 Total titratable acidity

Total titratable acidity (TTA) was determined by titration with 0.1N NaOH in the presence of phenolphthalein indicator as indicated earlier in section 3.2.3.8 with some modifications. The acidity was determined at harvest and during ripening until senescence for fruits from covered and uncovered control and until the termination of the experiment for the fruits grown covered and uncovered and treated with 1-MCP.

6.2.2.6 Chlorophyll content

Chlorophyll content was determined using the method of Arnon, (1949) as indicated earlier in section 3.2.3.4 with a few modifications. Chlorophyll determination was carried out at harvest and during storage until the end of the experiment for all treatments.

6.2.2.7 Determination of ethylene production and respiration (carbon dioxide production) rates

Ethylene production and respiration rates were determined as described earlier in section 4.2.1.12 with slight modifications. The rates were determined at harvest and during ripening for the fruits grown covered and not covered and not treated with 1-MCP and until the end of the experiment (when they started to rot) for those grown covered and not covered and treated with 1-MCP.

6.2.2.8 Green life

Green life was determined as indicated earlier in section 3.2.3.12.

6.2.3 Statistical analysis

Data were examined for normality using R software and outliers by scatter plot using Ms Excel software. Data were then subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS statistical programme (SAS, 2001). The means were compared according to Student Newman Keul's (SNK) test ($\alpha = 0.05$) and LSD ($\alpha = 0.05$) to test for significant effects.

6.3 Results and Discussion

6.3.1 Ethylene production rate

Ethylene production was significantly affected ($p \leq 0.05$) by both the growing conditions and application of 1-MCP (Fig. 6.1). Fruits grown uncovered and not treated with 1-MCP produced the ethylene peak first at about 5 days followed by fruits grown covered but not treated with 1-MCP at about 6 days, while fruits grown covered and uncovered treated with 1-MCP produced the peaks at about 18 and 19 days, respectively. In all cases, ethylene production decreased rapidly after the climacteric a phenomenon found in banana fruits (Zhang *et al.*, 2006).

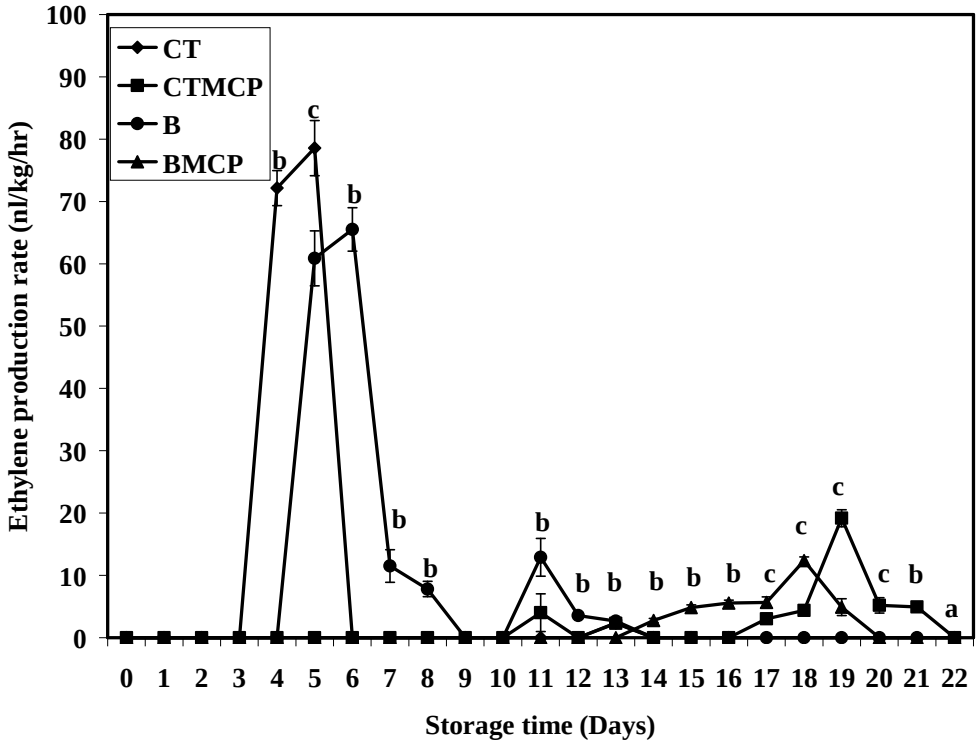

Figure 6.1: Effect of bunch covers and 1-MCP treatment on ethylene production during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP, respectively. Bars show standard SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Bagging significantly ($p \leq 0.05$) reduced the peak of ethylene production for the fruits grown covered compared to those grown uncovered and not treated with1-MCP. This could be due to growing stresses caused by dust, bird drops and blemishes among others encountered by the bunches grown uncovered which may lead to high ethylene production. Stress ethylene production has been associated with bruising in banana (Dominguez and Vendrell, 1993). Also, it could have been due to the

uncovered fruits intercepting high photosynthetically-active radiation (PAR) which may have affected the postharvest behaviour including ethylene production. Blue polythene bunch covers have been shown to reduce PAR compared to no cover and hence may affect postharvest behaviour (Harvey, 2006).

I-Methylcyclopropene treatment significantly ($p \leq 0.05$) delayed the peak of ethylene production and also reduced the amount of ethylene produced. In the banana fruit, ethylene is produced through a sequence of biochemical events. The most thoroughly investigated portion of the pathway involves the steps linking methionine, its conversion to S-adenosyl methionine (SAM) by methionine adenosyl transferase enzyme, its conversion to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase enzyme, and the subsequent production of ethylene from ACC by the action of ACC oxidase enzyme ((Seymour *et al*, 1993). Preclimacteric bananas produce very little ethylene and contain little amounts of ACC (Seymour *et al*, 1993). At the onset of ripening, ACC content, ethylene production and the capacity to produce ethylene are greatly enhanced which indicates an increase in the amount or the activity of ACC synthase which converts SAM to ACC and ACC oxidase which converts ACC to ethylene (Seymour *et al*, 1993; Zhang *et al*., 2006). In the present study, ethylene production may have been delayed and reduced due to reduction or blockage of ethylene producing precursors and enzymes such as 1-aminocyclopropane-1-carboxylic acid (ACC), ACC synthase and ACC oxidase (Seymour *et al*, 1993; Zhang *et al*., 2006) while in the control fruits the processes were uninterrupted and hence the early production of high amounts of ethylene. This agrees with the findings of Pathak *et al*. (2003) and Zhang *et al*. (2006) who found

that ACC concentration in control fruit increased uninterrupted while the increase was delayed significantly by 1-MCP treatment. Ethylene production and rate was also delayed and reduced in apple and grapefruit (Blankenship and Dole, 2003) and strawberries (Aguayo *et al.*, 2006). However, some studies (Golding *et al.*, 1998; Inaba *et al.*, 2007) have reported that 1-MCP application to banana fruits elevated ethylene production**.** This differs with the findings of the current study where ethylene production was significantly reduced. This is probably due to the fact that in the other studies, 1-MCP was applied after the onset of ripening, a case that has been shown to increase ethylene production (Inaba, 2007). The increased ethylene production by application of 1-MCP after the climacteric suggest that its biosynthesis in banana fruit is regulated in a positive feedback manner at least during the pre-climacteric period, but is either independent from ethylene or under negative feedback regulation mechanism at the ripening stage (Inaba, 2007). In the current study, 1-MCP was applied at the pre-climacteric stage. The failure of the banana fruits to ripen in this study could be due to limited formation of new ethylene receptor binding sites (Jiang *et al.*, 1999b). It may also be due to full occupation of existing binding sites or persistence of 1MCP in the tissues (Bagnato *et al.*, 2003).

6.3.2 Respiration (carbon dioxide production) rates

Respiration rates were significantly affected ($p \leq 0.05$) by both the growing conditions and application of 1-MCP (Fig. 6.2). Bagging significantly delayed and reduced respiratory peak for fruits not treated with 1-MCP. Fruits grown uncovered and not treated with 1-MCP produced the respiratory peak first at about 6 days followed by fruits grown covered but not treated with 1-MCP at about 7 days, and

fruits grown covered and uncovered treated with 1-MCP produced the peaks at about 19 and 20 days respectively. Control unbagged fruits may have had an early and enhanced respiratory peak 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).

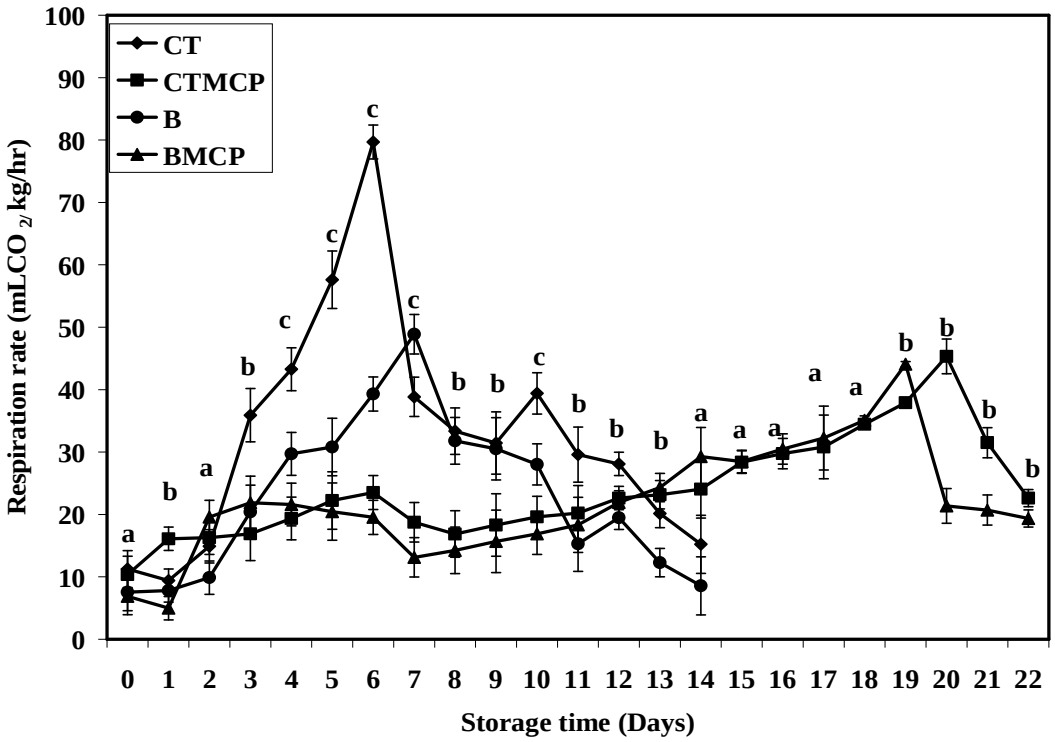

Figure 6.2: Effect of bunch covers and 1-MCP treatment on carbon dioxide production during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP respectively. Bars show SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Similar findings to the current study were also reported for mango (Runkua, 2009). Bagged banana fruits not treated with 1-MCP had reduced respiration probably due to lower O_2 and higher CO_2 internal partial pressure as reported in bagged pears (Amarante *et al*., 2002), although respiration was not affected in the latter case. Delayed respiration peaks and reduced respiration rates by 1-MCP treatment have been observed in various studies of bananas (Golding *et al*., 1998; Blakenship and Dole, 2003; Lohani *et al*., 2004; Zhang *et al*., 2006; Inaba *et al*., 2007) and other fruits like Sapodilla (Quiping *et al*., 2006). Climacteric respiration has been found to be either totally ethylene dependent or ethylene-independent (Pech *et al*., 2008). The findings of the current study indicate that it was ethylene-dependent which would explain the delayed and reduced respiratory peak due to delayed and reduced ethylene peak (Fig. 6.1). In strawberry, 1-MCP inhibited the ethylene-induced respiratory increase in early-harvested fruit, but not in late harvested fruit.

6.3.3 Starch content

Starch content decrease was faster in the fruits grown uncovered (CT), followed by those grown covered (B), and those grown covered and treated with 1-MCP (BMCP) and those grown uncovered and treated with 1-MCP (CTMCP) in that order. By the 15^{th} day, according to Cornell starch chart (Appendix II) fruits from the CT, B, BMCP and CTMCP treatment had about 30%, 50%, 70% and 80% starch content left, respectively (Fig. 6.3).

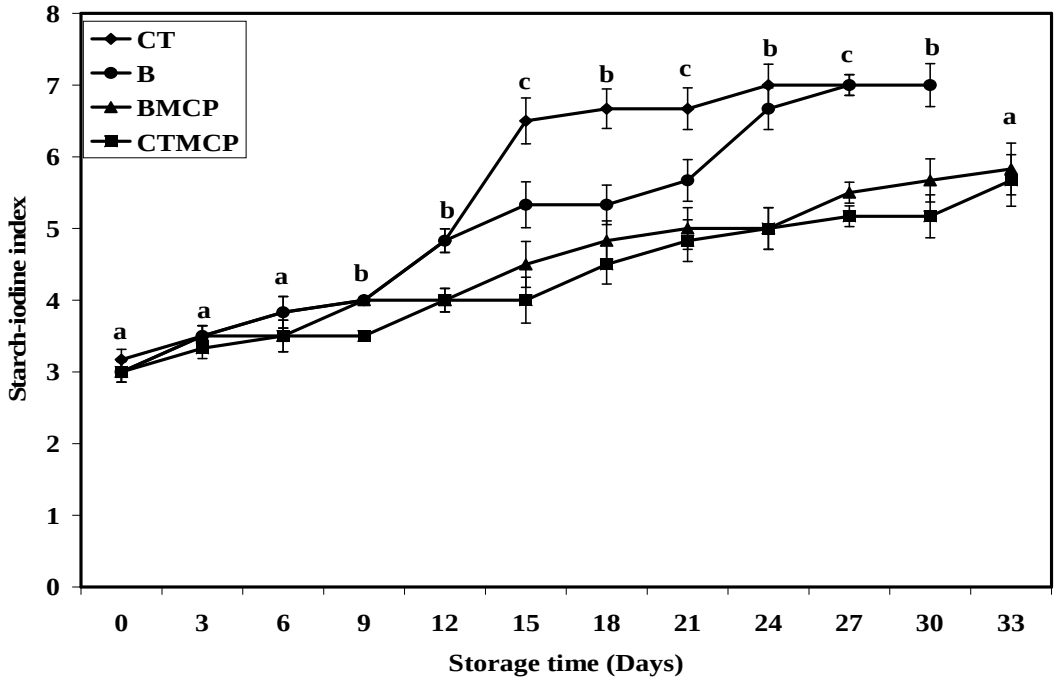

Figure 6.3: Effect of bunch covers and 1-MCP treatment on rate of starch degradation in the pulp during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP respectively. Bars show SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Starch degradation in control fruits grown covered and uncovered proceeded normally although the fruits grown uncovered had a faster rate of degradation possibly due to their exposure to a lot stresses like dust, chaffing, bird drops among others, during growth compared to the fruits grown covered. Starch degradation in fruits grown covered and uncovered and treated with 1-MCP was delayed and did not attain the rate and final content of the fruits which were not treated with 1-MCP (Fig. 6.3). There was however no significant difference ($p>0.05$) between the fruits treated with 1-MCP and grown covered or uncovered which may imply that bagging

had no effect on starch degradation of these fruits. In the present study, respiration rates and peak were delayed and reduced, respectively (Fig. 6.2). This may have adversely affected starch degradation in 1-MCP treated fruits grown covered and uncovered since ATP which is necessary for starch hydrolysis is produced during climacteric respiration (Seymour *et al.*, 1993). Ethylene production and peak were also delayed and reduced (Fig. 6.1) which may also have negatively affected starch degradation. 1-Methylcyclopropene slowed starch hydrolysis in bananas (Golding *et al.*, 1998), apples (Blakenship and Dole, 2003; Thammawong and Arakwa, 2007) and Tommy Atkins mango fruit (Plotto *et al*., 2003). Starch degradation to sugars in bananas occurs rapidly during the ripening process which allows the fruits to attain desired sweet taste for consumption. Enzymes for both hydrolytic and phosphorolytic breakdown of starch have been identified in the banana (Seymour *et al.*, 1993).

The primary product of starch breakdown is sucrose via sucrose phosphate synthase. The hexose sugars then arise from sucrose hydrolysis perhaps by the action of acid invertase in the vacuole whose activity has been shown to increase during banana ripening. The conversion of starch to sucrose and sucrose turnover creates a high demand for ATP, and sugar accumulation and respired carbon dioxide were highly correlated (Seymour *et al*., 1993).

6.3.4 Total soluble solids (TSS) content

Unbagged control (CT) fruit began to increase in TSS on day 6, while for bagged control (B) TSS began to rise on day 9. Total soluble solids for uncovered fruits

treated with 1-MCP (CTMCP) began to increase on day 24 while that of covered fruits treated with 1-MCP (BMCP) rose from day 21 (Fig. 6.4).

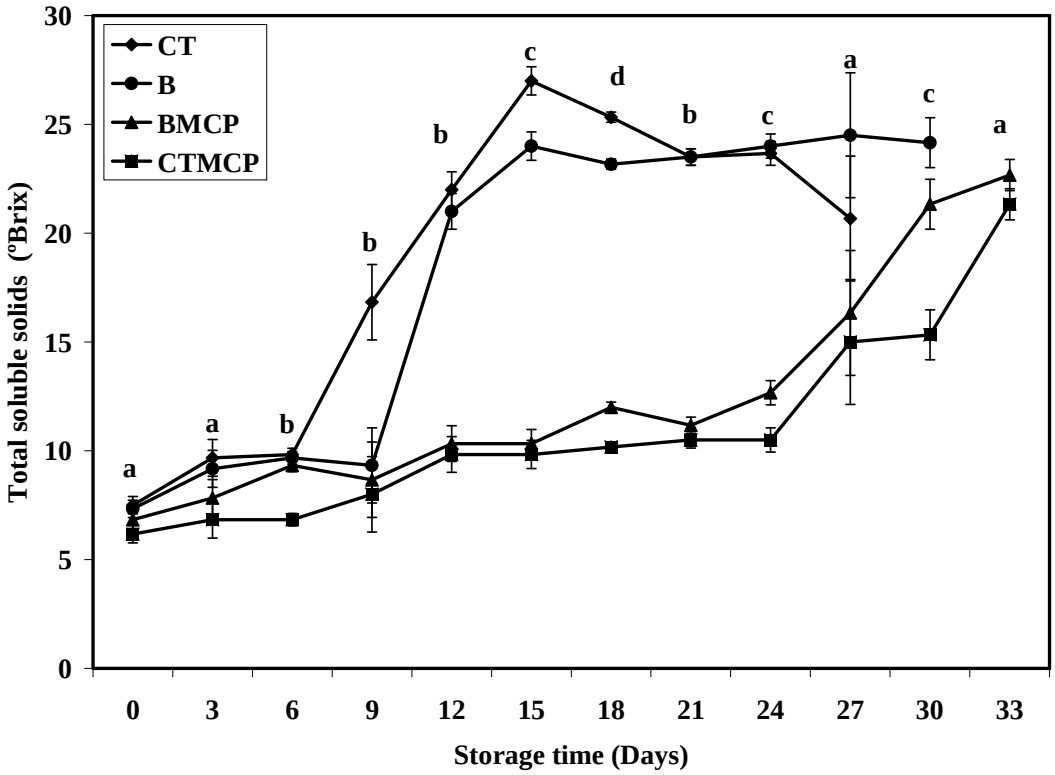

Figure 6.4: The effect of bunch covers and 1-MCP on total soluble solids of tissue cultured banana cultivar Williams during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP respectively. Bars show SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Total soluble solids content increase was delayed in both treatments of 1-MCP irrespective of the growing conditions. These results agree with those of Bagnato *et al.*, (2003) who found that TSS formation in banana fruits was delayed when treated with 1-MCP at 30ppm. Generally, TSS increases due to the alteration in cell wall

structure and breakdown of complex carbohydrates into simple sugars during storage (Rathore *et al*., 2007). Total soluble solids have also been shown to increase due to partial breakdown of pectins and celluloses (Roe and Bruemmer, 1981). In the present study, TSS levels in 1-MCP treated fruits grown covered and uncovered were delayed but rose to high levels at the end of the experiment, which may indicate the synthesis of some ethylene binding sites. They however did not attain TSS content levels of the control fruits not treated with 1-MCP. Ethylene production in this study was delayed and reduced (Fig. 6.1) and this may have affected sugar metabolism negatively. Respiration in this study was also significantly delayed and reduced by 1-MCP (Fig. 6.2) and hence the reduction of carbohydrate metabolism which provides a large proportion of the TSS in bananas. Carbohydrate metabolism has been shown to be negatively affected by delayed production and reduced respiration rates (Golding *et al*., 1998). Total soluble solids were measured from the beginning of the experiment to the termination of the experiment in this study which for the fruits not treated with 1-MCP was at ripeness stage 7, while for the fruits treated with 1-MCP was at the stage where the fruits had started rotting and did not necessarily coincide with the fully ripe stage and was in most cases at low levels of ripeness, hence the low TSS content.

The increase in TSS at the end of the experiment may indicate the presence of some respiratory substrates in the 1-MCP treated fruits. Sugar formation in bananas has been shown to be delayed and reduced by 1-MCP treatment due to reduced respiration even when ethylene production was enhanced (Golding *et al*., 1998). This is possibly due to the fact that sugar formation from starch which forms a large

proportion of TSS in the banana may not be a fully ethylene-dependent process (Seymour *et al.*, 1993) but highly depends on respiration (Golding *et al.*, 1998). Similar findings were reported for banana by DeMartino *et al.* (2010).

6.3.5 Total titratable acidity (TTA) content

Generally, TTA content increased at a faster rate for the untreated fruits whether grown under cover or not while its increase was delayed significantly by 1-MCP application (Fig. 6.5). Indeed, TTA increase was delayed by 1-MCP treatment although the fruits eventually attained high TTA levels at the end of the experiment. Changes in acids in the banana may be ethylene-dependent hence the delay of TTA increase by the 1-MCP treated fruits since peak ethylene production in this study was found to be delayed and reduced considerably (Fig. 6.1). Respiration production and peak were also delayed and lowered significantly ($p \leq 0.05$) by 1-MCP in this study (Fig. 6.2) and this may also have affected TTA increase. Loss of acidity in plums was shown to be slowed down by 1-MCP application due to a reduction in the respiratory process (Salvador *et al*., 2003). The effect of 1-MCP on TTA has been found to be variable with some crops such as tomatoes and sapodilla fruits, having their ethylene-dependent acidity loss inhibited (Quiping *et al*., 2006), while others such as apricots, 'Shamouti' oranges and plums having the acidity loss unaffected (Dong *et al*., 2002; Blakenship and Dole, 2003; Menniti *et al.*, 2004). This may be due to whether the TTA loss or increase is ethylene-dependent or independent. In melons, TTA loss is ethylene-independent and is therefore not affected by application of 1-MCP (Pech *et al*., 2008).

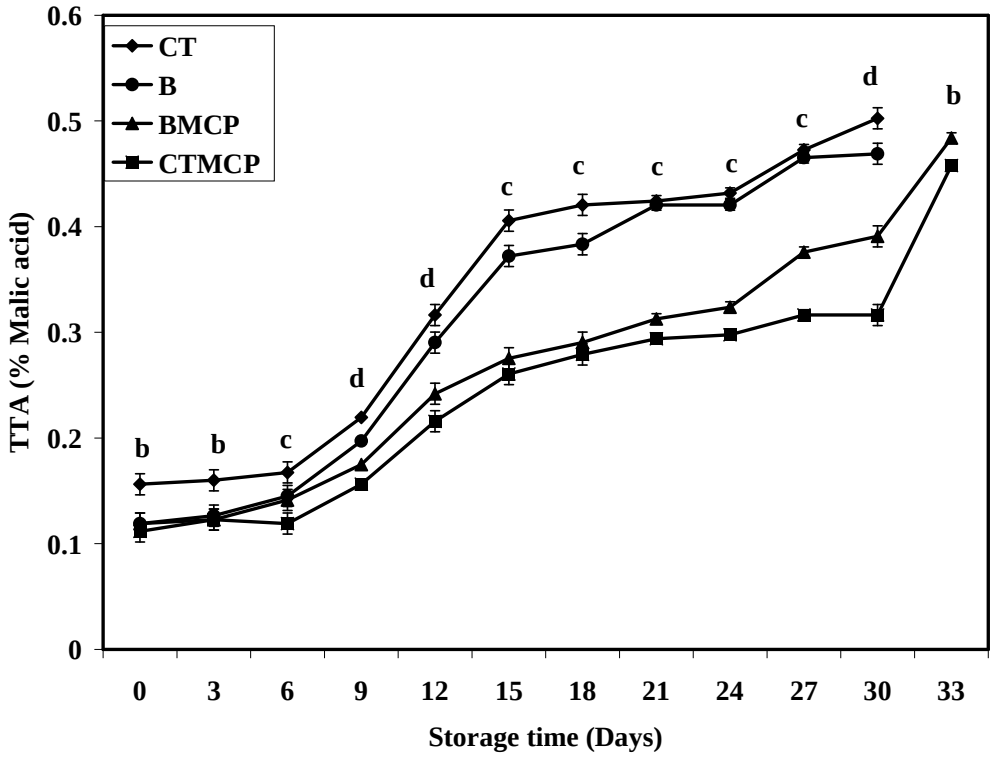

Figure 6.5: The effect of bunch covers and 1-MCP on total titratable acidity (% Malic acid) during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP respectively. Bars show SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Total titratable acidity loss or increase during ripening may also depend on plant species and storage conditions (Blakenship and Dole, 2003). The increase at the end of the experiment in TTA content of the 1-MCP treated fruits in this study may have been due to synthesis of some ethylene binding sites which may have made the fruit to become sensitive to ethylene.

6.3.6 Firmness

Fruit firmness loss was delayed and disrupted by 1-MCP application (Figs. 6.6, 6.7A and 6.7B). The fruits from the control treatments became soft although those grown under cover took a little longer to soften. Bunch covers significantly ($p \leq 0.05$) affected the softening rate especially for fruits not treated with 1-MCP. This could be due to the fact that the uncovered fruits were exposed to a lot stresses like dust, chaffing, bird drops among others, during growth compared to the fruits grown covered which may have hastened ethylene production and respiratory climacteric (Figs. 6.1 and 6.2), thus enhancing pectin and starch degradation hence earlier softening compared to those grown covered. Also, stresses during the growing period due to leaf scaring, dust accumulation and bird droppings experienced by the control fruits grown uncovered compared to the fruits grown covered could have initiated production of stress-ethylene.

In 'Towanese' fruits, some stress has been shown to function as a primary signal that triggers stress-ethylene production in the tissue of the calyx, and this diffuses into the pulp tissue of the fruit where ripening-ethylene production is activated, which, in turn causes rapid softening (Inaba, 2007). Fruits treated with 1-MCP had their softening delayed and disrupted irrespective of whether grown under cover or not. Banana softening has been attributed to starch conversion to sugars, cell wall degradation and loss of water through transpiration (Seymour *et al.*, 1993; Ahmad *et al.*, 2001). Effect of 1-MCP on inhibition of banana softening during ripening has been repeatedly observed (Blankenship and Dole, 2003; Jiang *et al.*, 2004). However, further research on effect of 1-MCP on banana ripening is necessary in

order to try and optimize application rates and conditions that will allow normal banana ripening after 1-MCP treatment. This is because 1-MCP has been shown to be effective in other climacteric fruits without causing undesirable effects (Blankenship and Dole, 2003).

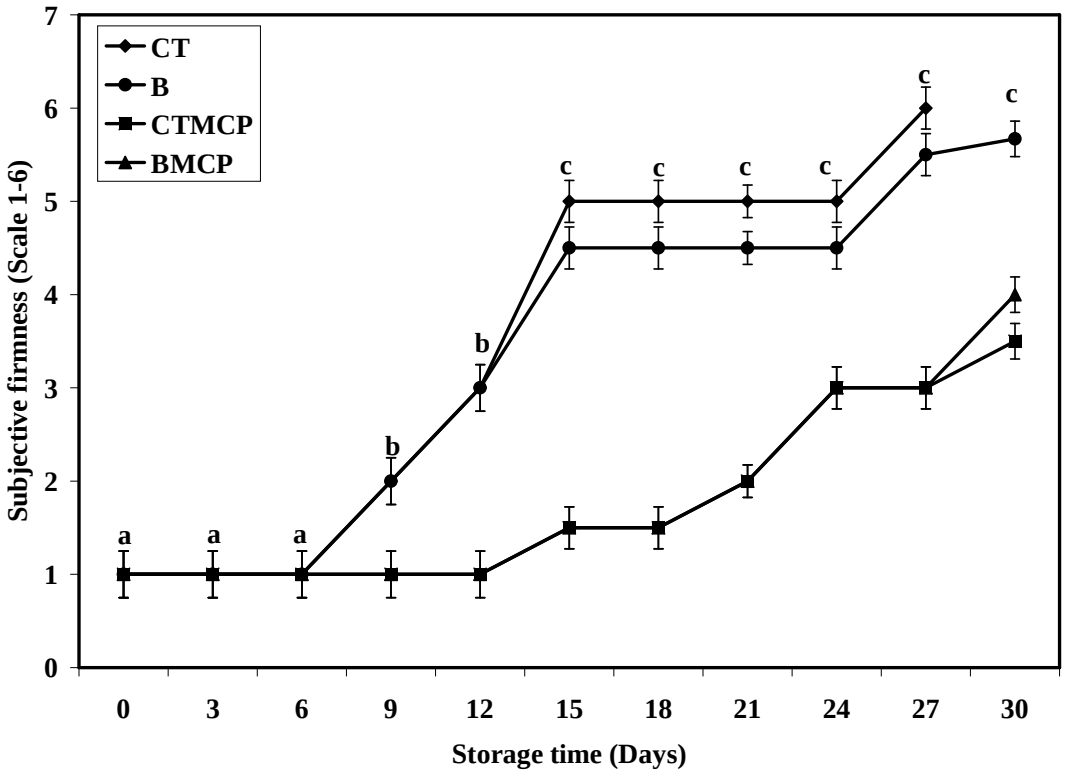

Figure 6.6: The effect of bunch covers and 1-MCP on subjective firmness of tissue cultured banana cultivar Williams during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP respectively. Bars show SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

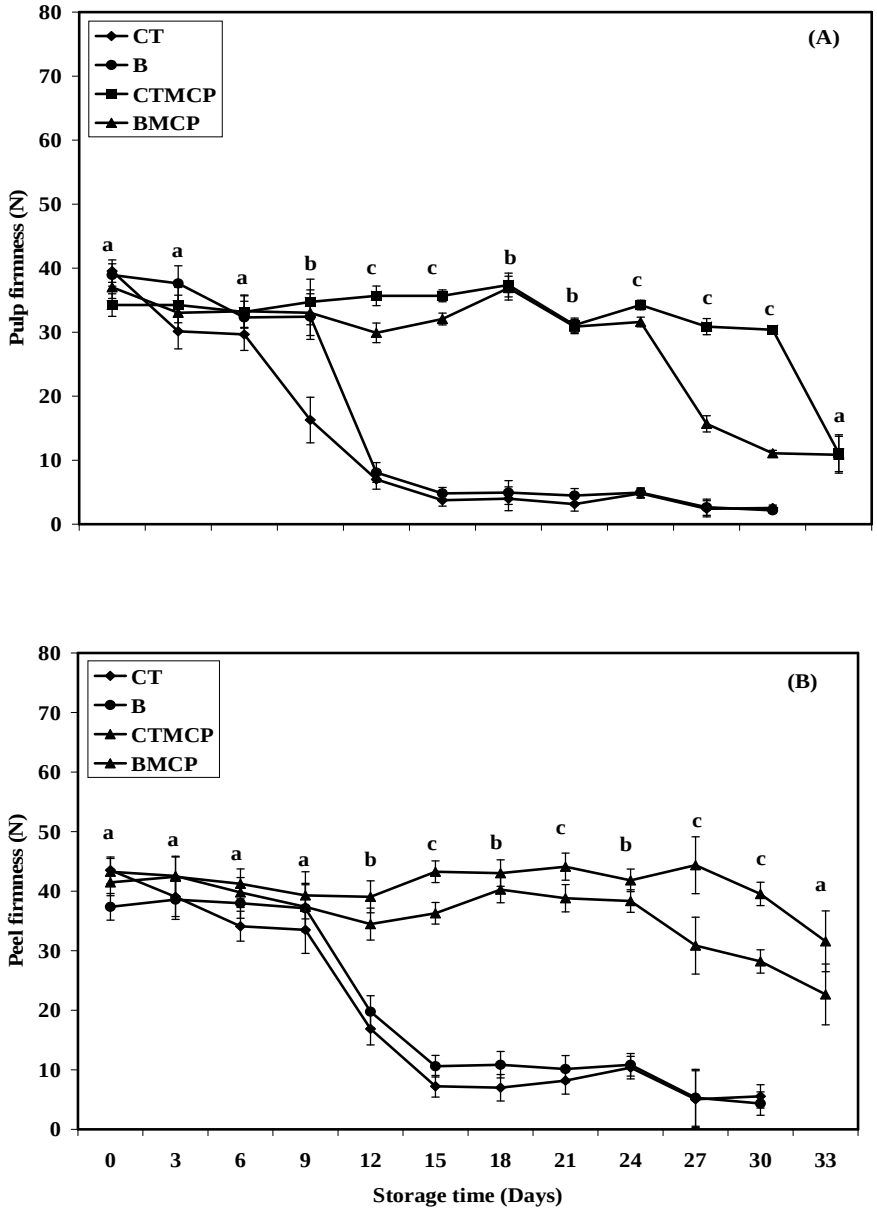

Figure 6.7: The effect of bunch covers and 1-MCP on objective firmness (N) of the pulp (A) and peel (B) of tissue cultured banana cultivar Williams during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP respectively. Bars show SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

Reports on effect of 1-MCP treatment on softening have been variable. Some crop species had their softening delayed while others were unaffected. Bananas treated with 30ppm had their firmness loss delayed and disrupted (Bagnato *et al*., 2003) while softening of fruits such as avocado, custard mango and papaya was only delayed but was later completed (Blakenship and Dole, 2003; Manenoi *et al*., 2007). However, firmness was maintained in nectarines and apricots by 1-MCP treatment (Blankenship and Dole, 2003).

During ripening, cell wall components are degraded and this brings about softening of the fruit. The cell wall of fruit are generally composed of cellular microfibrils tethered with xylogycans embedded pectin mesh and glycoproteins (Lohani *et al*., 2004). Pectin solubilization has been correlated with softening as softening appears to be mainly associated with changes in pectin fraction of the cell wall (Lohani *et al*., 2004). The delay in fruit softening in the present study may be due to lowering of activities of polygalacturonase (PG), pectin methyl esterase (PME), pectate lyase (PE) and cellulose by 1-MCP (Blakenship and Dole, 2003; Lohani *et al*., 2004). Infact, PME activity has been shown to be inhibited by 1-MCP treatment prior to ethylene treatment showing that 1-MCP strongly restricts the induction of PME by ethylene (Lohani *et al*., 2004). Similar observations were made on PG, PE and cellulose with banana fruits treated with 1-MCP prior to ethylene treatment whereas no inhibition occurred on the cell wall hydrolyses with banana fruits treated with ethylene (Lohani *et al*., 2004). This clearly suggests that 1-MCP inhibits softening of banana fruit as reported earlier (Pelayo *et al*., 2003). The changes in fruit firmness during ripening by ethylene and the effect of 1-MCP are therefore related to changes

in cell wall degrading enzymes (Lohani *et al*., 2004). Banana fruit softening is an ethylene-mediated change (Jiang *et al*., 1999) and ethylene production has been shown to be delayed and reduced by 1-MCP treatment (Jiang *et al*., 1999; Zhang *et al*., 2006). The increased loss of firmness for the 1-MCP treated fruits grown covered and uncovered towards the end of the experiment, although it did not reach that of untreated fruit, may have been due to the formation of new ethylene binding sites which then allowed for some softening to take place. This agrees with the findings of Jiang *et al*. (1999). Some workers (Pech *et al*., 2008), have found that there may exist some ethylene-independent softening in climacteric fruits which may also explain some of the slight softening in the 1-MCP treated fruits. However, the fruits never attained a soft texture adequate for consumption.

6.3.7 Peel colour and chlorophyll degradation

Peel colour measured subjectively was significantly ($p \leq 0.05$) affected by bunch covers and 1-MCP treatment (Fig 6.8 A). Control fruits grown uncovered lost their green colour slightly faster than those grown covered. However, fruits treated with 1-MCP whether grown covered or uncovered had the colour change from green to yellow delayed and disrupted. Hue angle (H°) value of the peel was significantly ($p \leq 0.05$) affected by pre-harvest bunch covers and 1-MCP treatments (Fig. 6.8 B). The Hue angle reduced faster in the fruits not treated with 1-MCP irrespective of the growing conditions while those treated with 1-MCP whether grown covered or uncovered had the slower hue angle reduction. Visually, degreening progressed faster in the control fruits grown uncovered followed by those grown under cover. Fruits grown under cover and not covered and treated with 1-MCP degreened at a

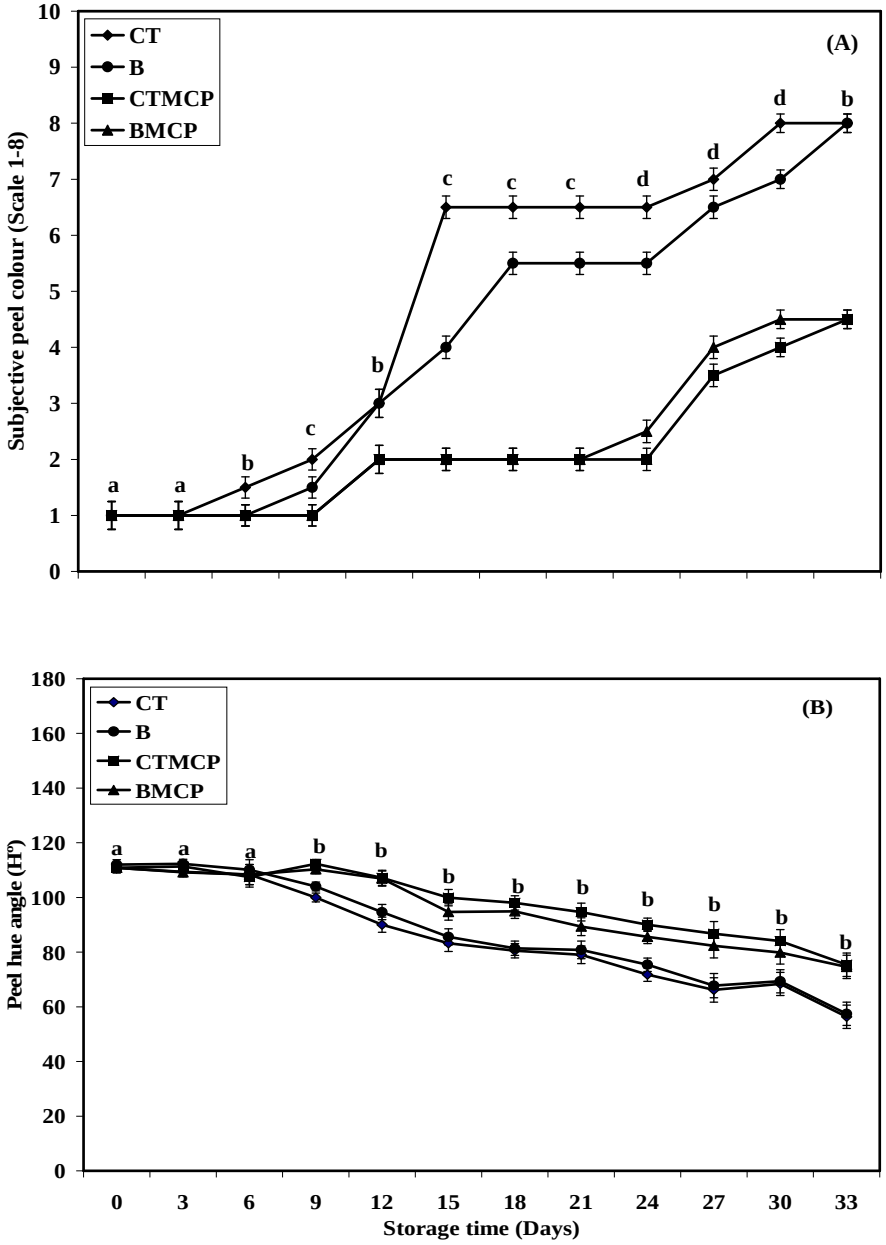

Figure 6.8: The effect of bunch covers and 1-MCP on subjective colour (A) and hue angle (B) of the peel of tissue-cultured banana cultivar Williams during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP respectively. Bars show SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

lower rate and did not degreen completely. Chlorophyll degradation was faster in the fruits from the uncovered control followed by the covered control. Fruits treated with 1-MCP irrespective of whether they were grown under covers or not had their chlorophyll degrading delayed and disrupted (Fig. 6.9). This effect of 1-MCP has been reported earlier (Bagnato *et al.*, 2003). Blotchy ripening of banana fruits where they attain uneven colour has also been reported by Blakenship and Dole (2003). The blotchy ripening may be due to positional differences in the rate of new synthesis of binding sites of ethylene (Jiang *et al.*, 1999).

1-Methylcyclopropene has been shown to delay or prevent chlorophyll degradation in fruits such as citrus (Blakenship and Dole, 2003), bananas (Jiang *et al*., 1999) and vegetables such as coriander (Blankenship and Dole, 2003). Colour changes and chlorophyll degradation are ethylene dependent (Golding *et al.*, 1998) hence the reduced chlorophyll degradation and delayed and uneven green colour loss since ethylene production was delayed and reduced by 1-MCP treatment in the current study (Fig. 6.1). Degreening is thought to be mediated by a multi-enzyme system in which chlorophyllase unmasks stable carotenoids present in mature peel (Golding *et al.*, 1998). Degreening has also been delayed and disrupted by 1-MCP treatment followed by propylene treatment showing that although yellowing is initiated by ethylene, completion of the process involves enzymes whose biosynthesis may be irreversibly disrupted by 1-MCP (Golding *et al*., 1998). However, 1-MCP has also been shown to have no effect on degreening of pummelo-grape fruit hybrid (Blakenship and Dole, 2003). This shows that 1-MCP has variable effects on

chlorophyll degradation and colour changes in different crop species. This may be affected by whether the crop is climacteric or non-climacteric (Blankenship and Dole, 2003).

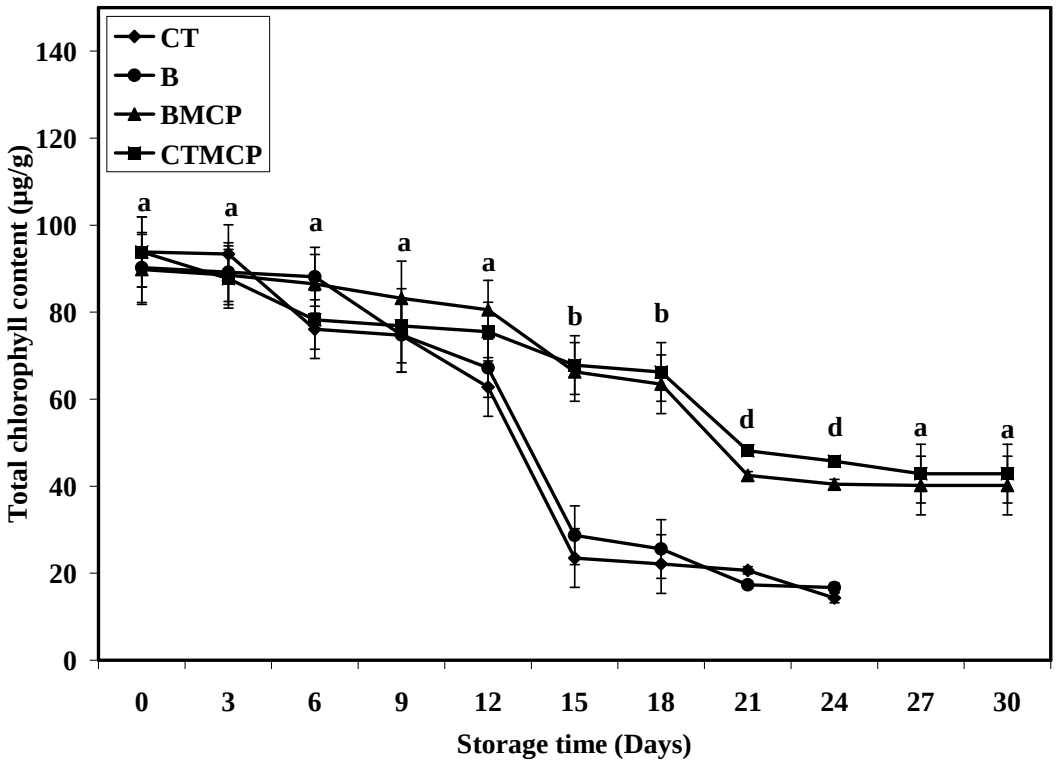

Figure 6.9: Effect of bunch covers and 1-MCP on total chlorophyll content of cultivar Williams banana fruits during storage. CT represents uncovered fruits not treated with 1-MCP, B represents fruits grown under covers and not treated with 1-MCP while CTMCP and BMCP represent fruits grown uncovered and treated with 1-MCP and fruits grown covered and treated with 1-MCP respectively. Bars show SE of the means of three replicates and where absent, bars fall within the dimensions of the symbol. Same letters at different periods indicate no significant difference according to Student Newman Keul's (SNK) test (α=0.05).

6.3.8 Green life

Green life was significantly ($p \leq 0.05$) affected by both the growing conditions and application of 1-MCP (Table 6.1).

Table 6.1: The effect of bunch covers and 1-MCP on green life of tissue-cultured banana cultivar Williams during storage

Treatment	(Days)
CT	9.00[a]
B	11.00[b]
BMCP	21.00[c]
CTMCP	23.00[d]
LSD	1.35

Values in the column followed by the same letter are not significantly different according to LSD test (α=0.05). Values are means of three replicates.

The fruits from the uncovered bunches had the shortest green life of 9 days followed those from covered bunches with a green life of 11 days, while fruits grown under covers and treated with 1-MCP and those not covered and treated with 1-MCP had a green life of 21 and 23 days respectively. Green life in this study was based on the occurrence of colour changes from green to yellow and since colour changes were affected by the1-MCP treatment, green life was also affected. Peel colour change is used as an indicator of ripening in banana (Turner, 1997).

6.4 Conclusion and recommendations

This study has shown that 1-MCP successfully delayed banana fruit ripening irrespective of whether grown covered or uncovered. 1-Methylcyclopropene is therefore a promising postharvest treatment for the extension of banana green life at ambient conditions of temperature and humidity. However, 1-MCP disrupted the ripening processes. There is therefore the need to optimize 1-MCP concentration

that will allow normal banana ripening. Bunch covers briefly delayed ripening and associated processes of banana fruits grown covered and ripened at ambient conditions and not treated with 1-MCP compared to those grown uncovered and not treated with 1-MCP. Fruits grown uncovered and not treated with 1-MCP ripened faster than those grown covered and not treated with 1-MCP probably due to exposure to growing stresses that may have accelerated the production of ethylene. However, at the end of the trial all the ripening parameters were similar in fruits of both treatments. Application of 1-MCP to fruits grown under covers or not covered, delayed ripening and associated processes. However, fruits grown under covers and treated with 1-MCP had the ripening processes beginning earlier than those grown uncovered and treated with 1-MCP. This could have been due to larger and possibly more physiologically mature fruit which were less sensitive to 1-MCP. 1-MCP effects have been shown to decrease with increased maturity in bananas. However, 1-MCP also disrupted these ripening processes. Degreening and softening were delayed and not completed in 1-MCP treated fruits irrespective of the growing conditions. Respiration rates, starch degradation, total soluble solids and TTA formation were also reduced and disrupted in 1-MCP treated fruits, irrespective of whether grown covered or uncovered. Ethylene production was also delayed and reduced in 1-MCP treated fruits compared to the control.

Bagging of banana bunches did not seem to influence the final postharvest quality of the bananas. However, 1-MCP treatment delayed and disrupted the ripening processes. It may be concluded that application of 1-MCP at the rate of 20ppm at ambient conditions to fully mature fruits caused the fruits to be unacceptable for

human consumption. The failure of the banana fruits to ripen in this study could be due to limited formation of new ethylene receptor binding sites since in the banana it has been shown that they can even take up to 30 days to recover for sensitivity to ethylene. It may also be due to full occupation of existing binding sites or persistence of 1MCP in the tissues. In bananas, factors such as maturity, growing conditions cultivar and prior exposure to ethylene have been shown to influence effects of 1-MCP application. In fact, the Williams cultivar has been shown to have fruits of mixed maturities in the same bunch which may limit commercial application of 1-MCP as it may produce mixed ripe fruits which is undesirable. However, application of exogenous ethylene before and/or after treatment with low rates of 1-MCP may possibly bring the fruits to ripen and further research on this should be conducted.

Research on high storage temperatures which have been shown to enhance ripening of 1-MCP treated fruits should also be conducted. Fruits of other banana cultivars could also be used to test 1-MCP efficacy.

CHAPTER SEVEN

7.0 GENERAL CONCLUSION AND RECOMMENDATIONS

Studies on the influence of inorganic fertilizers and micronutrients on postharvest quality of tissue-cultured bananas revealed that there is indeed an effect of these nutrients on the postharvest quality of the fruits. This shows the need to establish the soil status during the establishment and development of banana orchards. Banana nutrition is very important for commercial enterprises. There is need for commercial banana farmers to carry out soil analysis in order to determine the nutrient status of the soils so as to correct any deficiencies. The soil analysis results will also guide farmers on nutrients that are adequate in the soil and hence reduce overuse of fertilizers thereby cutting on cost of production This would help grow bananas that have high yields and acceptable postharvest quality. There is also the need to carry out a cost/benefit analysis to establish the profitability of the use of inorganic fertilizers.

Establishment of maturity indices for bananas is very important. This is especially useful for the newly introduced cultivars that the farmers may not have any idea as how long they take to mature and what features to look for to know when they are mature. However, there is need to establish the indices in various agro ecological zones since there is expected to be an effect of environment on the growth and development of bananas which may affect days to maturity among other maturity indices.

Bunch covers in this study have been shown to improve the banana visual appearance by reducing both permanent and other blemishes such as dirt to minimum while not

adversely affecting other internal qualities. However, the covers especially the dull blue covers caused a few fruits of top hands to be sun-scalded. This may indicate the need to conduct further research on bunch covers of other colours both shiny and dull. There is need to carry out research in other agro-ecological zones in order to determine the effect of environment on the covers. Application of agronomic practices such as pulling leaves over the growing bunches and also inserting newspapers inside the bunch covers may help control/reduce sunburn and should be explored. A cost/benefit analysis of the bunch cover technology should also be performed in order to find out if it is profitable.

In the current study, 1-MCP clearly delayed banana ripening, while the combination with the bunch covers did not have a significant effect. However, the fruits did not ripen to the desired state of firmness, colour and taste. Studies on ethylene treatment before and after 1-MCP treatment should be carried out in an attempt to produce ripe fruits that are acceptable to consumers. Other concentrations of 1-MCP treatment should be used in an attempt to extend the green life of banana fruits and still get the desired eating quality.

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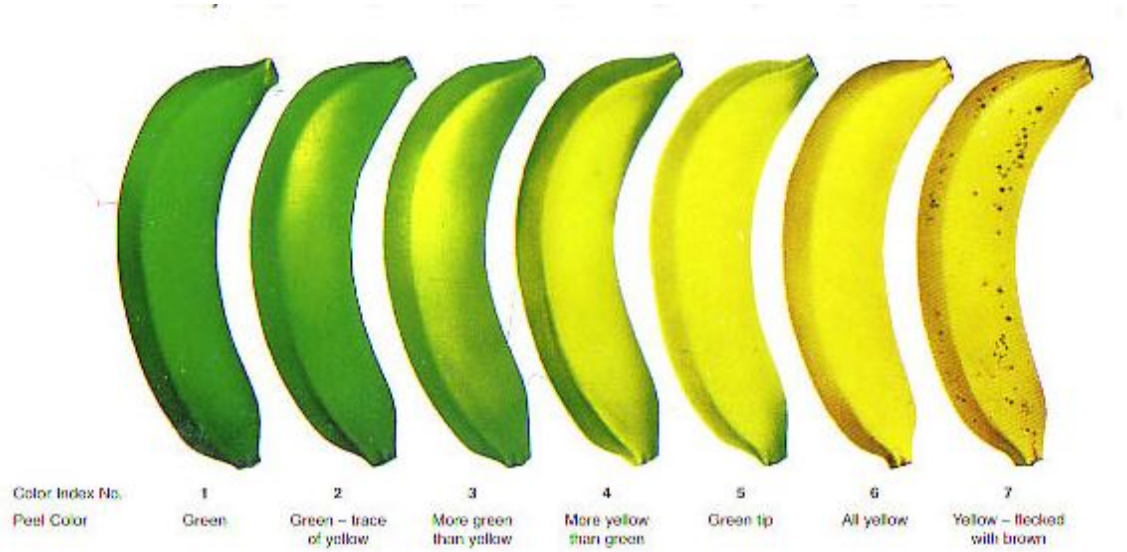
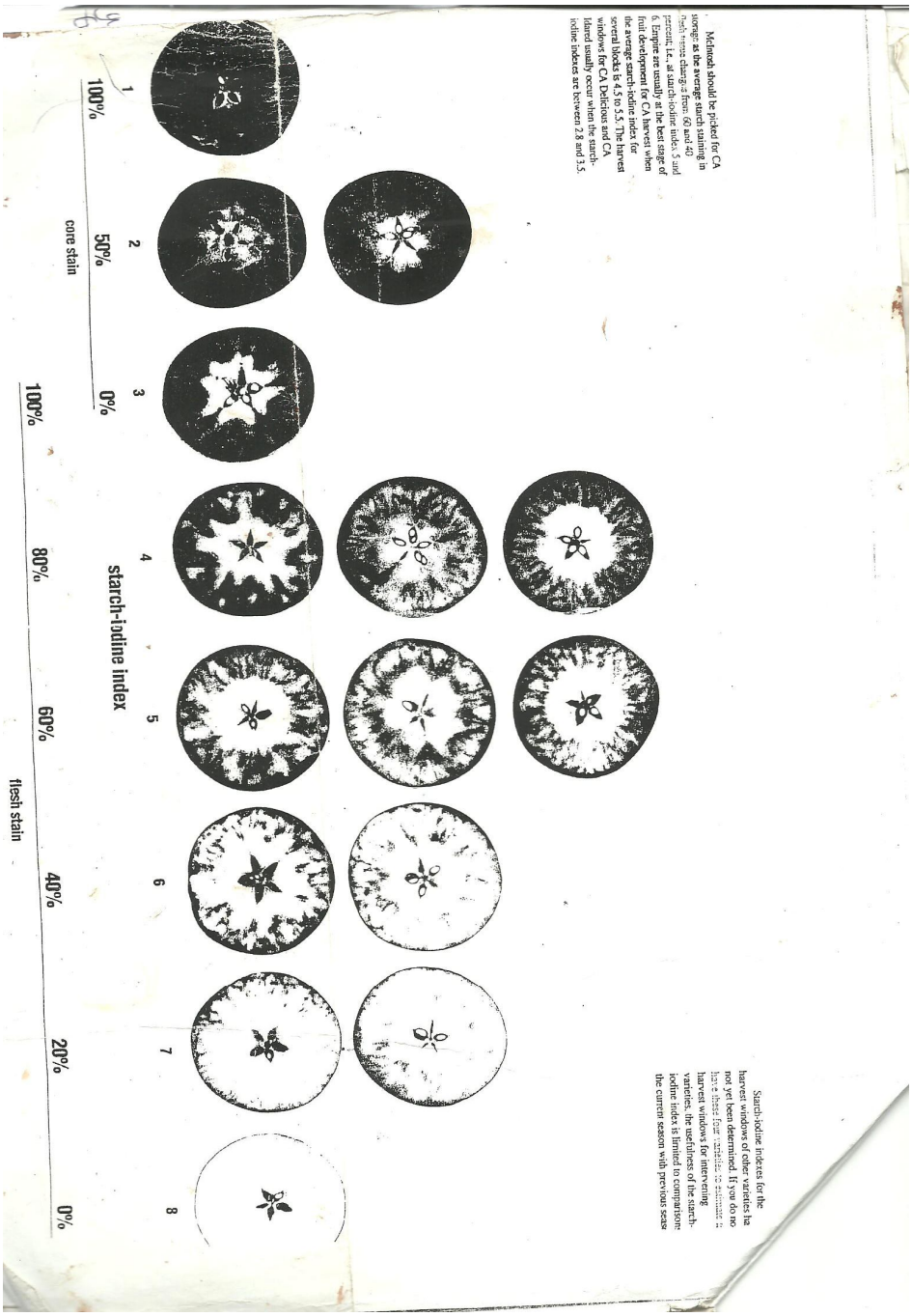
APPENDICES

Appendix I: Banana ripening chart



Source: CSIRO, 1972

Appendix II: Cornell Starch Chart



Source: Watkins, 1981

Appendix III: Sensory evaluation questionnaire for bananas

BANANA SENSORY ANALYSIS
Name:_____
Date:_____
e-mail
address_____
___

N.b. Please read through the questionnaire before undertaking the sensory evaluation
1. Please taste the six banana samples provided in sequence and indicate how much you like or dislike each one especially in relation to taste. Tick one option for each sample in the space provided and also fill in the number of the sample at the top of each column for all six samples

Sample no._____

9_____like extremely
8_____like very much

7_____like moderately
6_____like slightly
5_____neither like nor dislike
4_____dislike slightly
3_____dislike moderately
2_____dislike very much
1_____dislike extremely

Sample no._____

9_____like extremely
8_____like very much

7_____like moderately
6_____like slightly
5_____neither like nor dislike
4_____dislike slightly
3_____dislike moderately
2_____dislike very much
1_____dislike extremely

Sample no._____

9_____like extremely
8_____like very much

7_____like moderately
6_____like slightly

Sample no._____

9_____like extremely
8_____like very much

7_____like moderately
6_____like slightly
5_____neither like nor dislike
4_____dislike slightly
3_____dislike moderately
2_____dislike very much
1_____dislike extremely

Sample no._____

9_____like extremely
8_____like very much

7_____like moderately
6_____like slightly

5_____neither like nor dislike
4_____dislike slightly
3_____dislike moderately
2_____dislike very much
1_____dislike extremely

5_____neither like nor dislike
4_____dislike slightly
3_____dislike moderately
2_____dislike very much
1_____dislike extremely

2. Please sniff the six samples and evaluate for aroma and rate them in the scale below. Tick one option for each sample in the space provided and also fill in the number of the sample at the top of each column for all six samples

Sample no.	_____	_____	_____	_____	_____
5. Extremely good aroma	_____	_____	_____	_____	_____
4. Good aroma	_____	_____	_____	_____	_____
3. Moderate aroma	_____	_____	_____	_____	_____
2.Low aroma	_____	_____	_____	_____	_____
1. No banana aroma	_____	_____	_____	_____	_____

3. Evaluate the six samples for sweetness and indicate the amount of sweetness in the scale below. Tick one option for each sample in the space provided and also fill in the number of the sample at the top of each column for all six samples

Sample no.	_____	_____	_____	_____	_____
6. Extremely sweet	_____	_____	_____	_____	_____
5. Very sweet	_____	_____	_____	_____	_____
4. Sweet	_____	_____	_____	_____	_____
3. Slightly sweet	_____	_____	_____	_____	_____
2. Trace of sweetness	_____	_____	_____	_____	_____
1. Not sweet	_____	_____	_____	_____	_____

4. Evaluate the six samples for texture and rate them in the scale below. Tick one option for each sample in the space provided and also fill in the number of the sample at the top of each column for all six samples

Sample no.	_____	_____	_____	_____	_____
6. Very rough	_____	_____	_____	_____	_____
5. Rough	_____	_____	_____	_____	_____
4. Slightly rough	_____	_____	_____	_____	_____
3.Neither rough nor smooth	_____	_____	_____	_____	_____
2. Smooth	_____	_____	_____	_____	_____
1. Very smooth	_____	_____	_____	_____	_____

General comments_____
_____
_____
_____

Thank you very much for participating in this sensory evaluation.