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STORAGE TEMPERATURE AND ATMOSPHERE INFLUENCED POSTHARVEST QUALITY OF MARULA (*Sclerocarya birrea* subspecies *caffra*) FRUITS

A thesis submitted in partial fulfillment of the requirements for the award of the Master of Science Degree in Crop Science (Horticulture)

By

AGISANYANG TAUTSAGAE (200600011)

July, 2020

Supervisor : **PROF. V. E. EMONGOR**

Department of Crop and Soil Sciences

Botswana University of Agriculture and Natural Resources

Co-Supervisor: **DR. S. O TSHWENYANE**

Department of Crop and Soil Sciences

Botswana University of Agriculture and Natural Resources

CERTIFICATION

Main supervisor's name	Signature	Date
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Co-supervisor's name	Signature	Date
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Head of Department's name	Signature	Date
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APROVAL

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Co-supervisor's name	Signature	Date
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Head of Department's name	Signature	Date
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Dean of Faculty's name	Signature	Date
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STATEMENT OF ORIGINALITY

I, Agisanyang Tautsagae declare that this thesis, hereby submitted for the award of Master of Science Degree in Crop Science (Horticulture) at the Botswana University of Agriculture and Natural Resources, is my own work and has not been submitted to any other institution for any award. Where other sources have been used; they have been appropriately cited and acknowledged.

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(Authors Signature)

On _____day of _____20 _____in _____

DEDICATION

This work is dedicated to my husband and children for their support and encouragement during the challenges of graduate school life. To my parents who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve. I am very thankful for having you in my life.

ACKNOWLEDGEMENTS

I would like to thank my husband, daughters (Refilwe and Fatimah), father and mother, for giving me the strength to reach for the stars and chase my dream. Their prayers, love and support has kept me going. Many thanks and appreciation to my research supervisor Prof. V.E. Emongor for persevering with me throughout my research. His advices, insightful criticism, patience and encouragement aided me in writing of this thesis. My co-supervisor Dr.S.O. Tshwenyane who has generously given her time and expertise to better my work. I thank her for the contribution and support. I must as well acknowledge Marang Mosupiemang, Tebogo Stimela, Goitseone Malambane and Connie Gwatidzo for their technical assistance and advice. I am also grateful for the help offered by the casual labourers during field and laboratory work, my friends and siblings for their continued support and encouragement. The tissue culture laboratory members are a wonderful group of people whom I enjoyed working with and learning from.

To God be the glory.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ABA	Abscisic acid
ADP	Adenosine diphosphate
AOX	Alternative oxidase
AOAC	Association of Official Analytical Chemists
AVG	Aminoethoxyvinylglycine
AsA-GSH	Ascorbate/glutathione cycle
ATP	Adenosine triphosphate
Ca	Calcium
CA	Controlled atmosphere
CAT	Catalase
CM	Centimetres
CI	Chilling injury
CO_2	Carbondioxide
CRD	Completely randomized design
C_2H_4	Ethylene
DNA	Deoxyribonuvleic acid
FAD_2	Fatty acid desaturase
G	Grams
GABA	γ-Aminobutyric acid
HSP 19-11	Heat shock proteins genes
HOA	High O_2 atmosphere
Kg	Kilograms
KMUTT	King Mongkut's University of Technology Thonburi

LDPE	Low density polyethylene
LTC	Low temperature conditioning
LOX	Lipoxygenase
LTP	Lipid transfer protein
MAP	Modified atmosphere packaging
1-MCP	1-methylcyclopropene
MDA	Malaodialdehyde
MeJA	Methyl jasmonate
Ml	Millilitres
mRNA	Messenger ribonucleic acid
NADH	Nicotinamide adenine dinucleotide
NAD (P) H	Nicotinamide Adenine Dinucleotide Phosphate
NO	Nitric oxide
O_2	Oxygen
PAL	Phenylalanine ammonia lyase
PLD	D- phospholipase
POD	Peroxidase
PPO	Polyphenol oxidases
SA	Salicyclic acid
SAM	S-adenosylmethionine
SSC	Soluble solids content
SOD	Superoxide dismutase
TTA	Titratable acidity
UV-C	Ultraviolet C

ABSTRACT

Low temperature storage is the most effective technology for maintaining quality and extending the postharvest life of fresh horticultural produce. However, horticultural produce of tropical and subtropical in origin are susceptible to chilling injury (CI) when stored at temperatures below their critical minimum temperatures. Therefore, low temperature storage alone is not suitable for produce of tropical and subtropical in origin. This study was carried out to evaluate the influence of storage temperature and atmosphere on postharvest quality of marula fruits during and after storage. The treatments were storage temperature (6, 8, 10, 12 and 25°C) and storage atmosphere (MAP and Air). The results of the study showed that the steady state equilibrium in MAP was significantly ($P < 0.05$) influenced by storage temperature. The steady state equilibrium was reached after 4, 6, 8 and 9 days in MAP fruit stored at 12, 10, 8 and 6ºC. Storage temperature and atmosphere interactered significantly ($P < 0.05$) to lower the respiration rate of marula fruit compared to fruit stored in Air. Storage temperature below 12ºC significantly ($P < 0.05$) increased CI incidence, CI severity and proline content of marula fruit. As storage temperature decreased below 12°C, the incidence and severity of CI, and proline content increased. Marula fruit in MAP and stored in various temperatures had significantly ($P < 0.05$) lower electrolyte leakage than fruit stored in Air. Storage temperature significantly ($P < 0.05$) influenced fruit quality (fruit colour, titratable acidity (TTA), juice pH, soluble solids content (SSC), and vitamin C contnet). Temperatures below 12ºC delayed and retarded marula fruit colour development, reduced SSC, increased TTA and juice pH. Fruit stored at 6ºC had significantly ($P < 0.05$) higher vitamin C content of 92 mg/100 ml juice than fruit stored at 8, 10, 12 and 25ºC. Storage temperature and atmosphere significantly ($P < 0.05$) interacted to influence fruit weight loss. Marula fruit in MAP and stored at lower temperatures had significantly ($P < 0.05$) lower weight loss than fruit held in Air. The results further showed that marula fruit stored at 12˚C in MAP had significantly longer shelf-life of 21 days than fruits in Air stored at 6, 8, 10 and 12˚C which had a shelf-life of 12, 13, 15 and 19 days, respectively. It was concluded that in order to reduce marula fruit CI incidence and severity, maintain fruit quality, and extend shelf-life and the marketing period; the fruit should be stored in MAP and at 12°C. However, it was also recommended that this study be repeated with other MAP technologies such as waxing and low density polymeric films with different porosities.

Key words: Marula fruit, modified atmosphere packaging, chilling injury, electrolyte leakage, proline content, postharvest losses

CHAPTER 1

1.0 INTRODUCTION

Fruits, nuts and vegetables play a significant role in human nutrition, especially as sources of vitamins, minerals, dietary fibre and antioxidants. Increased consumption of a variety of them on a daily basis is highly recommended because of associated health benefits, which include reduced risk of some forms of cancer, heart disease, stroke and other chronic diseases (Kader and Rolle, 2004). There is an increasing global demand for traditional and rare fruits, which increase the gastronomic diversity as they provide new flavours, aromas, colours and an attractive appearance for consumers (Ortiz-Hernández and Carrillo-Salazar, 2012). Fruit commercialization, is affected by quality reduction due to inappropriate postharvest handling methods, which largely influences the economic outcome and marketing (Toivonen and Hodges, 2011). Postharvest losses vary greatly across commodity types, with production areas and the season of production. Losses of fresh fruits in both developed and developing countries are estimated to range from 2% for potatoes to 23% for strawberries, with an overall average of 12% losses between production and consumption sites (Kader and Rolle, 2004). These losses have resulted in about one third of horticultural crops produced never consumed by humans (Kader and Rolle, 2004).

1.1 Background

Marula *(Sclerocarya birrea* subspecie*s caffra*) belongs to the family Anacardiaceae. In Botswana it is commonly called morula, but in South Africa it is commonly known as marula (English), moroela (Afrikaans), umGanu (isiZulu), nkanyi (Xitsonga), morula (Sepedi) and mfula (Tshivenda) (Venter and Venter, 1996). According to Mateke (1995), in Kenya it is named mura (Meru) and didissa (Borana). The genus *Sclerocarya* is derived from the Greek word *skleros*, meaning hard and *karyon*, meaning a nut, which refers to the hard stone of the fruit. The genus *Sclerocarya* has three subspecies in Africa namely: *birrea*- North Africa, *caffra*-Southern Africa and *multifoliolata*- Tanzania. It bears a diploid chromosome number of 2n = 26. The most important parts of the marula tree are the fruits, nuts, bark, leaves and stem. The marula tree is highly sensitive to frost and grows best in frost-free areas under warm conditions. It is adapted to dry and hot weather conditions.

1.2 Distribution

The distribution of the marula in Africa has followed the Bantu migration and it occurs throughout Africa in warm frost-free climate as an important food in their diet (Venter and Venter, 1996). Occasionally it may grow in high-lying areas, which experience very short sub-zero spells in winter (Gous *et al*., 1988). It is native to South Africa, Malawi, Namibia, Niger, Botswana, Gambia, Zambia, Zimbabwe, Sudan, Eswatini, the Democratic Republic of Congo, Ethiopia, Kenya, Tanzania (including Zanzibar), Angola and Uganda (Venter and Venter, 1996). Marula is also present in Madagascar (possibly introduced) and has been introduced into Mauritius, Reunion, India and Australia (Venter and Venter, 1996). It has been grown as an experimental crop in Israel (Venter and Venter, 1996). In general, marula trees grow at altitudes of up to 4,500 m above sea level (Taylor *et al*., 1995; Hall *et al*., 2000).

In South Africa, the tree is widely distributed in many game parks and in the rural areas of Limpopo, KwaZulu-Natal, the Eastern Cape and Mpumalanga. It is more dominant in the Limpopo and Mpumalanga Provinces. The marula tree currently is distributed worldwide and is valuable to millions of people (Venter and Venter, 1996). It is now grown in parts of Asia, Europe, America and Australia.

Marula is indigenous in Botswana and is distributed countrywide mostly in bushveld, woodland mainly on sandy soils (Taylor and Moss, 1982; Taylor and Kwerepe, 1992; Taylor *et al*., 1995; Leakey, 2005). Marula tree occurs naturally and is found in arid and semi-arid areas with summer rainfall varying from 250 to 1,000 mm (Venter and Venter, 1996). It is an indigenous tree adapted to poor soils and it occurs naturally in various types of woodland, on sandy soil or occasionally on sandy loam. The marula tree can easily be propagated by seed, cuttings and grafting (Venter and Venter, 1996).

1.3 Plant Description

Marula is a medium to large tree, usually 9 m tall, but it can grow up to 18 m. It is a single-stemmed tree, with a dense, spreading crown and deciduous foliage. The tree is dioecious, though occasionally reported as having both sexes on the same tree (Taylor *et al*., 1995). The old stems are silver-grey in colour and fairly smooth. The bark peels off in disc-shaped flakes, giving the trunk a mottled appearance. The interior bark is red or pink with darker stripes. The leaf petioles occur in three to eight opposite pairs of leaflets and a terminal one, 30-100 × 15-40 mm toothed margins. The leaves have sharply pointed leaflets. The flowers are arranged in

a bunch, 5 to 8 cm long. Female and male flowers are separated, whether on the same tree or on different trees.

The fruit has been described by several researchers as plum-sized and thick, very juicy and aromatic (Leakey, 2005; Colin, 2007). When ripe, it has a light yellow skin, with white, succulent flesh and a strong, distinctive and turpentine flavour. The stone is walnut-sized and has a thick wall. The flesh clings onto its brown stone and is very fibrous and juicy. It has a characteristic turpentine flavour. Inside the woody stone are two to three oblong kernels and each kernel is protected by a small bony "lid", which becomes detached when the stone is cracked. One tree of marula can produce up to 500 kg of fruit per year (Venter and Venter, 1996). Average crop yields are about 30 kg per tree, although large trees can bear heavily, up to 70,000 fruit per tree (Venter and Venter, 1996).

1.4.0 Socio-economic Importance

Generally, the marula tree gives excellent shade in garden parks and streets. Almost every part of the marula fruit is utilised for example fresh ripe fruit is very popular and is usually consumed fresh by biting or cutting through the thick, leathery skin and sucking the juice or chewing the mucilaginous flesh, after removal of the skin (Mojeremane and Tshwenyane, 2004). Marula fruit is used to make a potent local fermented alcoholic beverage called marula beer or wine, which provides many rural brewers with an important source of seasonal income. South Africa produces a good-quality liqueur commercially and there are numerous small enterprises producing marula jam and jelly. Pasteurized juice has also been marketed, though problems have been experienced with it due to ''browning" and with the flocculation of certain enzymes. The latter problem has since been overcome (Taylor *et al*., 1995; Leakey, 2005). Jelly, juice, salad dressing and jam can also be made from the fruit and the skin can be processed into glue, soap, ointment, atchar and vinegar (Colin, 2007).

1.4.1 Nuts

The kernels are extracted from the nuts and are very popular amongst rural people and are highly delicious and nutritious (Leakey, 2005). They make good snacks and can be consumed raw or roasted and for the purpose of adding a unique flavour to the food. Also they are mixed with vegetables or meat or may be grounded by pounding and used in baking of traditional breads and/or formed into a cake before consumption. Oil extracted from the kernels is highly

valued, particularly by the cosmetic industry due to its slow oxidising properties (Mojeremane and Tshwenyane, 2004). The good- tasting oil is used for cooking and for products such as preservatives (Venter and Venter, 1996).

1.4.2 Leaves

Fox and Norwood (1982) reported that some South African tribes cook the leaves as relish. According to Palgrave (2002), cattle and wildlife eat the fruit and the leaves on the trees as well as on the ground. The leaves are nutritious and contribute to a healthy diet for livestock. During extended drought periods when there is no grass the marula leaves serve as fodder bank for livestock.

1.4.3 Bark

According to Mojeremane and Tshwenyane (2004), the bark contains 10-20% tannins as well as traces of alkaloids. It provides fibre and gum, which is mixed with soot and water to give ink or red dye. The bark is used for medicinal purposes and treats a variety of ailments, notably fever, boils, diarrhoea and blood circulation problems (Palgrave, 2002). When mixed with other medicinal plants, the bark treats various infections such as syphilis, leprosy, dysentery, hepatitis and rheumatism (Palgrave, 2002).

1.4.4 Wood

In spite of the tree being protected by legislation as well as by traditional culture in Botswana, its wood is occasionally used for making furniture and, to lesser extent paneling. It is also a popular wood for carvings and household articles such as pestle and mortar, chairs and sculptures (Taylor *et al.*, 1995).

1.5.0 Postharvest Losses

Fruits are living tissues and are diverse in morphology, structure, composition and general physiology. Due to high moisture content, active metabolisms, tender nature and richness in nutrients, fruits are vulnerable to dehydration, physiological disorders, environmental stress, mechanical injury and pathological breakdown; and are therefore considered to be highly perishable (Kader, 2005; Emongor, 2010). The shelf-life of the fruits is, therefore, short and significant deterioration following harvest can occur. Postharvest losses can occur at any point in the production and marketing chain. In a hungry and increasingly competitive world,

reducing postharvest food losses should be a major agricultural goal. So appropriate postharvest handling can play a major role in reducing losses. In order to reduce these losses, postharvest technologies which delay senescence and are able to maintain quality, must be applied. Such technology systems include temperature management, controlled atmosphere (CA) and modified atmosphere (MA) storages (Emongor, 2010).

1.5.1 Temperature

Among the various factors that affect postharvest losses, temperature management is one of the most important tools for extending the shelf-life of fruits, because it regulates the rate of all associated physiological and biochemical processes (Khorshidi *et al*., 2010). However, low temperatures may cause chilling injury and higher ones can reduce the storage life of the product (Pholoma, 2016). Many studies on the effect of storage temperature on quality and storage life of fruits have been undertaken and they indicated that temperature plays an important role on quality of fruits after harvest (Kader and Rolle, 2004; Emongor, 2010; Khorshidi *et al*., 2010; Mutari and Debbie, 2011; Pholoma, 2016; Ramagonono, 2018). Temperature and storage duration have been reported to affect the vitamin C content of fruits and the growth of postharvest rots (Khorshidi *et al.*, 2010; Punitha *et al*., 2010; Emongor and Ramagonono, 2019). Precooling, refrigeration, proper relative humidity and optimal atmospheric composition in storage facilities and packages are essential to reducing postharvest losses of commodities that are destined to reach the consumer in fresh conditions (Kader *et al*., 1989).

According to Hardenburg (1986), for every 10^{o}C increase in temperature, the rate of respiration is roughly doubled or even trebled. For example, an apple held at 10^{o}C ripens and respires about three times as fast as one held at 0^{o}C. This increase in respiration has a direct impact on the shelf-life of fresh produce. The storage life of commodities varies inversely with the rate of respiration. Products with a high rate of respiration generally have a shorter shelf-life than those with a lower rate of respiration. The lower the storage temperature the longer the shelf-life (Hardenburg *et al*., 1986; Emongor, 2010; Kader, 2013). There is a significant improvement in shelf-life by storing horticultural produce at 0^{o}C compared to 3 or 5^{o}C (Hardenburg *et al*., 1986; Kader, 2005). The storage life of products can be adversely affected by storing them at the wrong temperature. Asparagus is chilling sensitive and so the shelf-life is actually reduced by storing it at 0^{o}C as the optimum storage temperature is 2^{o}C. It is

important that chilling sensitive products are stored at the correct temperature as chilling injury will make them unsalable (Hardenburg *et al*., 1986; Kader and Rolle, 2004; Emongor, 2010; Emongor, 2015). It is often critical that fresh produce rapidly reach the optimal pulp temperature for short-term storage if it is to maintain its highest visual quality, flavor, texture and nutritional content (Kader, 2013). For most produce maintaining cool temperature will increase storage life by lowering respiration rate, decreasing sensitivity to ethylene and reducing water loss.

1.5.2 Chilling Injury

Chilling injury (CI) is a storage disorder that occurs at temperatures below the critical threshold but non-freezing temperature (Hardenburg *et al*., 1986; Mercer and Smittle, 1992; Sharom *et al*., 1994; Kader, 2004; Emongor, 2015). The problem limits the use of low storage temperature to manage postharvest ripening of fruits because the temperatures that are low enough to delay ripening, decay and senescence may also be damaging to the fruit. Most fruits that have originated from the tropical or subtropical regions are chilling sensitive (Gross *et al*., 2002). Some horticultural crops of temperate origin are also susceptible to low temperature injury. Those temperate crops have lower critical threshold temperatures (generally below 5 to 10°C). At these chilling temperatures, the tissues weaken because they are unable to carry on normal metabolic processes.

Low temperature storage is the most effective method of slowing deteriorative metabolic and pathological processes in harvested commodities. However, CI possesses a major limitation to cold storage in most tropical and subtropical fruits. These fruits are very sensitive to chilling stress, when exposed to temperatures lower than 10^{o}C, but above their freezing points. Various physiological and biochemical alterations and cellular dysfunctions occur in chilling-sensitive species in response to chilling stress (Wang, 1982; Raison and Orr, 1990; Saltveit and Morris, 1990; Zhao *et al*., 2006). These alterations include stimulation of ethylene production, increase in respiratory rate, interference in energy production, and increase in activation energy, slowing of protoplasmic streaming, increase in permeability, reduction in photosynthesis, enzyme inactivation, membrane dysfunction, and alteration of cellular structure (Wang, 1982; Raison and Orr, 1990; Saltveit and Morris, 1990; Zhao *et al*., 2006). If chilling stress is prolonged, these alterations and dysfunctions will lead to the development of a variety of CI symptoms such as surface lesions, internal discoloration, water-soaking of the tissue, off-flavor, decay

and failure to ripen normally (Wang, 1982; Raison and Orr, 1990; Saltveit and Morris, 1990; Zhao *et al*., 2006).

1.5.3 Modified Atmosphere Packaging

Quality optimization and loss reduction in the postharvest chain of fresh horticultural produce are the main objectives of postharvest technology. Temperature control and modification of atmosphere are two important factors in prolonging shelf-life. Modified atmosphere packaging (MAP) is an effective tool used in the horticultural industry to extend shelf-life of various horticultural produce (fruits, vegetables and cut-flowers). MAP of fresh produce relies on modification of the atmosphere inside the package, achieved by the natural interplay between two processes which are the respiration of the produce and the transfer of gases through the packaging, that leads to an atmosphere richer in CO_2 and poorer in O_2 (Al-Ati and Joseph 2002; Fonseca *et al*., 2002; Kays and Paull, 2004; Mahajan *et al*., 2007; Mangaraj and Goswami, 2009; Emongor, 2010; Kader, 2013). Depleted O_2 and/or enriched CO_2 levels in the package reduces respiration, ethylene sensitivity and production, decay and physiological changes, delay enzymatic reactions, alleviate physiological disorders and preserve the produce from quality losses (Day, 1994; Kader *et al*., 1989; Gorris and Tauscher, 1999; Fonseca *et al*., 2002; Kays and Paull, 2004; Emongor, 2010; Kader, 2013). The equilibrium gas concentration thus developed within the package may extend the product shelf-life (McDonald *et al*., 1990; Omary *et al*., 1993).

Modified atmosphere packages should be carefully designed, as a system incorrectly designed may ineffective or even shorten the shelf-life of the produce (Emongor, 2010; Kader, 2013). The design should take into consideration not only steady-state conditions, but also the dynamic process, because if the produce is exposed for a long time to unsuitable gas composition before reaching the adequate atmosphere, the package may have no benefit (Fonseca *et al*., 2002; Kays and Paull, 2004; Mahajan *et al*., 2007; Moleyar and Narasimham, 1994; Kader, 2013). The design MA packages depends on a number of variables: the characteristics of the produce, the recommended atmosphere composition, the permeability of the packaging material to gases and its dependence on temperature and the respiration rate of the produce as affected by different gas composition and temperature (Fonseca *et al*., 2002; Kays and Paull, 2004; Emongor, 2010; Kader, 2013).

Temperature control is vitally important in order for MAP system to work effectively. Film permeability also increases as temperature increases, with CO_2 permeability responding more than O_2 permeability. Minimally processed products should be refrigerated at (0-5°C) to prolong their quality and safety (Rivera-López *et al*., 2005). Duration of cold storage also has an impact on final product quality (overall sensory quality declines and microbial load increases) (Gorny *et al*., 1999). The optimum storage temperature and atmospheric composition need to be maintained during the whole chain, from harvesting and processing to consumption.

The packaging of fruits and vegetables with plastic films helps to maintain high relative humidity and modify the concentration of O_2 and CO_2 in the atmospheres surrounding the commodity. The reduction of water loss from the tissue apparently inhibits the collapse of epidermal and underlying cells and prevents pitting formation. Packaging with low density polythene film alleviated chilling injury in cucumber fruit (Wang and Qi, 1997). The effects of polythene film wraps on the postharvest life of fruits maybe related to moisture conservation around the fruit as well as the change in the CO_2 and O_2 contents. Wrapping fruit individually in heat-shrinkable film is reported to drastically reduce pitting and scald in chilled grapefruit (Miller, 1990). It is also reported that mango fruit benefited in O_2 level ranges of 3-5% and 5-10% CO_2 by prolonging the shelf-life without compromising the quality of the fruit (Wang, 2010). The MAP has also been shown to delay chilling injury in bananas, pineapples, lemons and Japanese apricot (Wang, 2010).

1.6 Justification of Study

One of the most important problems in the fruit industry is that in general, the period when the market demand is greatest is not usually the period when the production is the highest. In the latter period, surpluses far exceed the off-take capacity of the usual market. This is particularly so in marula fruit being a seasonal crop with a short postharvest shelf-life and susceptibility to low storage temperature limiting its long term storage and distant marketing (Nerd and Mizrahi, 1993; Malik and Singh, 2005; Emongor and Tautsagae, 2016).

Marula studies that has been done in Botswana include: domestication and development of appropriate agronomic techniques of marula (Mateke, 1995); studies on chemical properties, uses and marketing of marula products (Ntlogelang, 2011); identification and control of

diseases and pests in the hard veld of Botswana (Ntlogelang, 2011); and control of marula fruit set using benzyladenine (Moatshe, 2009; Moatshe *et al*., 2011). One study on effects of storage temperature on postharvest quality, ripening and marketability of marula fruits has also been undertaken in Botswana (Emongor and Tautsagae, 2016). Emongor and Tautsagae (2016) reported that marula fruit stored at temperatures less than 12°C suffered CI and the severity of CI depended on temperature. Nerd and Mizrahi (1993) in Israel reported that marula fruit stored at 4°C suffered CI, while those stored at 12 and 20°C did not suffer CI. The development of CI symptoms negatively affects marula fruit quality, shortens storage life and its marketing (Emongor and Tautsagae, 2016). The incidence and severity of CI as a physiological disorder limits the application of low temperature storage on tropical or subtropical fruits such as marula. The impact of CI on the agro-food industry has serious economic consequences (Sevillano *et al*., 2009; Aghdam, 2013; Peng *et al*., 2013). Thus, for crops that are sensitive to chilling temperatures, low temperature storage alone is not a suitable way for maintaining their quality (Wang, 1990; Peng *et al*., 2013; Emongor, 2015). No studies dealing with methods of alleviation of CI in marula fruit in Botswana or elsewhere has been reported. Therefore, how to manage marula fruit after harvest becomes an important task for the postharvest physiologists and the horticultural industry in Botswana and worldwide. Hence, methods other than refrigeration need to be explored for proper postharvest handling of chilling sensitive crops such as marula fruits. This study, therefore, intends to evaluate if MAP can alleviate CI in marula fruit stored at various temperatures. The results of this study could benefit Botswana's farming industry in terms of contributing to the economy by commercialization of marula production, processing and storage, job creation and improving food security in the country.

1.7.0 General Objective

To evaluate the influence of storage temperature and atmosphere surrounding the marula fruit on postharvest quality of marula fruit during and after storage with the goal of contributing to the commercialization of marula fruit production, processing and storage, therefore enhancing food security in Botswana.

1.7.1 Specific Objectives

1. To evaluate the influence of storage temperature on postharvest of marula fruit.
2. To evaluate the effect of storage atmosphere on the postharvest quality of marula fruit

1.8 Hypotheses

1. H_0: Storage temperature has no influence on the postharvest quality of marula fruit.

Ha: Storage temperature has influence on the postharvest shelf life of marula fruit.

2. $H_{0:}$ Storage atmosphere has no influence on postharvest quality of marula fruit.

Ha: Storage atmosphere has influence on postharvest quality of marula fruit.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 General Review

There is hardly any literature available on the storage temperature of marula fruit, except of the work of Emongor and Tautsagae (2016) and Nerd and Mizrahi (1993); which is general on effects of storage temperature on fruits and methods of alleviation of CI in tropical and subtropical fruits.

In recent years, quality has become one of the most important consumer decision factors in the selection among competing products and services (Emongor, 2010). Most consumers of horticultural goods have a variety of choices when they are about to purchase a product. It is therefore essential for a business to maintain a perfect quality of the product in order to sustain the competition. Improvement in product quality is often accompanied by reduction in cost and increase in the profitability of the business (Hardenburg *et al.*, 1986**).** The quality of the food products in conformity with consumer requirement and acceptance is determined by their sensory attributes, chemical composition, physical properties, and level of microbiological and toxicological contaminants, shelf-life, packaging and labelling (Kays and Paull, 2004; Emongor, 2010). Past studies have shown that, the most important criteria for selecting a fruit are the freshness, the taste and the appearance (Kays and Paull, 2004).

2.2 Product Quality

Commodities should be in excellent condition and have excellent quality if minimum storage life is desired. The commodities should be as free as possible from skin breaks, bruises, decay and other deterioration. Bruises and other mechanical damage not only change the appearance of the product, but are good avenues of entrance for decay organisms (Emongor, 2010). Decay has been shown to be greater in bruised areas of horticultural produce than unbruised areas (Emongor, 2010). Severely bruised prunes developed 25% decay, whereas unbruised prunes developed 1-3% decay during storage (Hadenburg *et al.*, 1986). Mechanical damage also increases moisture loss (Emongor, 2010). The rate of moisture loss may be increased by as much as 400% by a single bad bruise on an apple (Hadenburg *et al.*, 1986; Kays and Paull, 2004*).* Horticultural produce for storage should be harvested at optimum physiological maturity, because storage life may be reduced if they are immature or over mature.

2.3 Effects of Temperature on Storage of Fruits

Temperature is the single most important environmental factor that influences the deterioration of harvested horticultural commodities, most perishable commodities last longest at temperature near 0°C (Wang, 1990; Willes *et al*., 1998; Brecht and Celia, 2012; Kader, 2013). Temperature management is the most effective tool for maintaining quality and safety, and for extending the postharvest life of fresh horticultural commodities (Brecht and Celia, 2012, Kader, 2013; Emongor, 2015; Chaudhary *et al*., 2017). Harvesting of fruits cuts them off from their water source, but are still alive and will lose water, and, therefore, turgor through transpiration (Hardenburg *et al*., 1986; Kays and Paull, 2004; Emongor, 2010). Field heat can accelerate the rate of respiration and with it the rate of quality loss (Hardenburg *et al*., 1986; Kays and Paull, 2004, Kader, 2013). Proper cooling protects quality and extends both the sensory and nutritional shelf-life of produce. It is often critical that fresh produce rapidly reach the optimal pulp temperature for short-term storage if it is to maintain its highest visual quality, flavour, texture and nutritional content (Kader, 2013). For most produce maintaining cool temperature will increase storage life by lowering respiration rate, decreasing sensitivity to ethylene and reducing water loss (Willes *et al*., 1998; Sevillano *et al*., 2009; Mworia *et al*., 2012; Aghdam, 2013).

After harvest, most fruits including marula remain alive and temperature during storage plays an important role in their quality. Ripening of the fruit can be slowed to extend shelf-life by reducing the fruit temperature. Low temperature storage is a postharvest technology used widely to extend shelf-life of fruits and allows the preservation of fruit quality after harvest (Hardenburg *et al*., 1986; Sevillano *et al*., 2009; Mworia *et al*., 2012; Aghdam, 2013; Emongor, 2010; 2015). Low temperature slows down most cell metabolic activities and delays fruit ripening and plant senescence (McGlasson and Adato, 1979; Hardenburg *et al.*, 1986; Sevillano *et al.*, 2009; Mworia *et al*., 2012; Aghdam, 2013; Emongor, 2010; 2015). However, most tropical and subtropical fruits are susceptible to CI (Wang, 1993; Gross *et al.*, 2002; Peng *et al*., 2013; Emongor, 2015; Chaudhary *et al*., 2017). Critical chilling temperatures are usually below 15°C, but above freezing point. Such crops including marula are damaged when stored below its critical temperature (generally 10-13°C for most varieties). In some cases, damage symptoms may not become apparent for several days or until transfer to warmer temperature (Wang, 1993). The sensitivity of fruits to temperature below the critical threshold is dependent on the cultivar, duration and temperature of exposure, time of harvest, degree of fruit maturity, waxing, environmental conditions during and after low temperature storage (Lim *et al*., 2009;

Emongor, 2015; Chaudhary *et al*., 2017). Therefore, technologies that could help the improvement of fruits to resist low temperature storage and prolong their shelf-life even their trade over long distance would be valuable. Therefore, for fruits that are sensitive to chilling temperatures, low temperature storage alone is not a suitable method to maintain their quality and postharvest shelf-life.

2.4.0 Chilling Injury

2.4.1 Physiology of Chilling Injury

The CI in most tropical or subtropical fruits is a kind of physiological disorder resulting from low temperature stress (Gross *et al.*, 2002; Peng *et al*., 2013; Wang, 2013; Emongor, 2015; Chaudhary *et al*., 2017). The CI is known to significantly change the micro-structure of the tissue which in severe cases may lead to tissue breakdown due to failure to carry normal metabolic processes (Han *et al*., 2006). The CI is reported to cause membrane damage via oxidation of membrane lipids, leading to structural changes and increased membrane permeability (Sharom *et al*., 1994; Zhao *et al*., 2006). Various physiological, biochemical alteration and cellular dysfunction occur in chilling sensitive species in response to chilling stress (Wang, 1982; Raison and Orr, 1990; Saltveit and Morris, 1990). These alterations include increased membrane permeability and alteration of activities of membrane proteins, loss of membrane integrity, leakage of solutes, increase in the activation energy of membrane associated enzymes, disorganization of cellular and sub-cellular structure, dysfunction and imbalance of metabolism, accumulation of toxic substances, stimulation of ethylene production, uneven ripening, pitting, discolouration, water-soaking, internal browning, off-flavour, tissue breakdown, and invasion by postharvest pathogens (Wang, 1982; Raison and Orr, 1990; Saltveit and Morris, 1990). Membranes with highly unsaturated fatty acids are reported to tolerate lower storage temperatures than tissues with more saturated fatty acids (Markhart, 1986). Lipid peroxidation is also reported to influence CI (Simon, 1974; Thompson, 1984), but the correlation between lipid peroxidation and CI has not been established (Mercer and Smittle, 1992). Malondialdehyde content and electrolyte leakage are used to indicate lipid peroxidation of membrane lipids and membrane permeability which increase during low temperature storage. If chilling stress is prolonged, these alterations cascade to development of CI symptoms such as skin surface pitting, sunken or surface lesions, uneven ripening, pulp discoloration, greyish-scald discoloration of the skin, water-soaking of the tissue, off-flavour, susceptibility to fungal decay, reduced aroma, carotenoids (Saltveit and Morris, 1990; Ding *et*

al., 2007; Emongor, 2015; Pholoma, 2016) and become more serious when fruits are transferred to room temperature probably because they satisfactorily ripen at 21-24°C.

2.4.2 Chilling Injury in Marula

Marula is a climacteric fruit which ripen after reaching physiological maturity. Marula fruits abscise before ripening; at this stage the skin colour is still green and the fruit is firm (Nerd and Mizrahi, 1993; Emongor and Tautsagae, 2016). Once harvested the fruits can be handled at various temperatures during storage, transportation and marketing. Marula is subtropical and tropical in origin (Emongor, 2015; Emongor and Tautsagae, 2016). However, most fruits that are of tropical or subtropical in origin are reported to be chilling sensitive (Tasneem, 2004, Lim *et al*., 2009; Emongor, 2015). Understanding the physiological mechanisms responsible for the activation and development of CI would allow the design of strategies to avoid or delay CI appearance.

Currently, the recommended storage temperature of marula fruit is 12°C (Nerd and Mizrahi, 1993; Emongor and Tautsagae, 2016). Nerd and Mizrahi (1993) in Israel stored marula fruit at 4, 12 and 20°C for 14 days. They reported that fruit stored at 12 and 20°C ripened as evidenced by the yellow peel colour and juice could be squeezed. Marula fruit stored at 20°C developed a deeper yellow colour, had higher juice content and lower acidity than fruit stored at 12°C (Nerd and Mizrahi, 1993). Marula fruit stored at 12°C did not develop CI (Emongor and Tautsagae, 2016). Marula fruit stored at 4°C for 14 days suffered from CI as evidenced by brown spot development on the peel which significantly influenced the development of CI incidence and severity of CI in marula fruit. As storage temperature decreased below 12°C, the incidence and severity of CI increased (Emongor and Tautsagae, 2016). The CI symptoms of skin pitting, poor colour development, poor aroma and surface lesions developed were observed in marula fruit stored at temperatures of less than 12°C, though severity varied with storage temperature and duration of storage from 8-16 days (Emongor and Tautsagae, 2016). Marula fruit stored at 0, 4 and 8°C developed chilling injury symptom after seven and nine days, respectively, but severity of chilling was high in fruit stored at 0 and 4°C (Emongor and Tautsagae, 2016).

2.4.3 Factors Affecting Chilling Injury Susceptibility

Factors affecting the susceptibility to CI include the origin of the crop, genetic makeup of the commodity, stage of development or maturity, metabolic status of the tissue, and a number of environmental factors such as temperature, light, relative humidity, and atmospheric composition (Patterson and Reid, 1990; Lim *et al*., 2009; Emongor, 2015; Chaudhary *et al*., 2017). The origin of a crop or the genetic makeup of a plant determines whether the species is sensitive or resistant to chilling (Patterson and Reid, 1990). In chilling-sensitive species, the critical threshold temperature may vary with the stage of development or maturity. For example, avocados, honeydew melons, mangoes, papayas, and tomatoes are more sensitive to chilling when they are less mature (Paull, 1990; Kays and Paull, 2004; Sevillano *et al*., 2009; Aghdam; 2013; Chaudhary *et al*., 2017). The metabolic status and the chemical composition of the tissue at the time of chilling can affect the resistance of tissue to chilling. Chilling-resistant tissues tend to have a higher degree of unsaturation of fatty acids in the membrane lipids than chilling sensitive tissues (Tabacchi *et al*., 1979; Mirdehghan *et al*., 2007; Promyou *et al*., 2008; Cao *et al*., 2009; Aghdam, 2013). This has been reported in banana, pomegranate and loquat fruit (Mirdehghan *et al*., 2007; Promyou *et al*., 2008; Cao *et al*., 2009). Maintenance of membrane integrity at low temperature has been reported to be important in the resistance to chilling temperature (Lyons, 1973; Rui *et al*., 2010). As indicators of membrane damage, electrolyte leakage and malaodialdehyde (MDA) content are generally considered to be indirect measurements of membrane integrity and can reflect the occurrence of CI and loss of membrane integrity (Shewfelt and Purvis, 1995; Zhao *et al*., 2009; Junmatong *et a*l., 2012; Pholoma, 2016). Lipolytic cascade in memebrane lipids deterioration during senescence and CI was achieved by the concerted activities of a variety of membranous lipolytic enzymes such as phospholipase D (PLD) and lipoxygenase (LOX) (Pinhero *et al*., 1998; Wang, 2001; Mao *et al*., 2007; Rui *et a*l., 2010). Mao *et al*. (2007) showed that the development of CI in cucumber fruit was accompanied by increase in PLD and LOX activities when exposed to chilling stress, and that the enhanced tolerance to CI by heat treatment was related to the reduction in activities of both enzymes. Rui *et al*. (2010) reported that heat treatment decreased LOX and PLD activity in response to chilling stress in loquat fruit and the reduction of internal browning, main symptom of CI in loquat fruit. The reduction in internal browning of loquat fruit treated by heat treatment and exposed to chilling stress was associated to the reduction of PLD and LOX activities (Rui *et al*., 2010). The results of Mao *et al*. (2007) and Rui *et al*. (2010) suggests

that these two enzymes (PLD and LOX) might be associated with the initiation of CI by being involved in membrane deterioration and signalling pathway in response to chilling stress.

Apart from direct effect of low temperature on the molecular organization of membrane lipids, the loss of integrity of the membrane itself is boosted by oxidative processes, because low temperature stress increases the levels of reactive oxygen species (ROS) (Sevillano *et al*., 2009; Aghdam, 2013). Defence against oxidative stress consists of ROS scavenging genes and ROS avoidance genes (Møller, 2001, Kocsy *et al*., 2001; Sato *et al*., 2001; Aghdam, 2013). Reactive oxygen species scavenging genes includes superoxide dismutase (SOD), catalase (CAT), the ascorbate/glutathione (AsA-GSH) cycle, the glutathione peroxidase and thioredoxin system (Møller, 2001). Superoxide dismutase catalyses the dismutation of O_2 to H_2O_2 and CAT scavenges H_2O_2 to form H_2O and O_2. The AsA-GSH cycle is also an important mechanism in the removal of ROS in plants (Aghdam, 2013). Its activation seems to produce a positive effect by inhibiting the development of CI (Sato *et a*l., 2001; Kocsy *et al*., 2001; Aghdam, 2013). The ROS avoidance genes include alternative oxidase (AOX). The plant AOX pathway branches from the main respiratory electron transport chain, bypasses the final steps of the cytochrome respiratory pathway and catalyses the oxidation of ubiquinone (Purvis, 1995; Møller, 2001; Aghdam, 2013). It has been suggested that by maintaining the flow of mitochondrial electrons, AOX maintains activation of NAD(P)H dehydrogenase and proton-pumping NADH dehydrogenase (Møller, 2001) and helps in generation of sufficient ATP required for the rapid adaptation and the maintenance of plant tissue homeostasis (Purvis, 2001; Moore *et al*., 2002; Hansen *et al*., 2002). In this process, AOX is involved in the reduction of ROS by preventing electrons from reducing O_2 and thus reduces the level of O_2 in the mitochondria (Wagner and Krab, 1995; Møller, 2001; Mittler, 2002). High levels of reducing sugars and proline have also been reported to correlate positively with the resistance to CI (Purvis, 1981; Purvis and Grierson, 1982; Pholoma, 2016).

2.4.4 Alleviation of Chilling Injury in Fruits

The ultimate goal of CI research is to find effective techniques to alleviate injury induced by chilling. The reduction of CI can be achieved either by increasing the tolerance of commodities to chilling stress or by retarding the development of CI symptoms in the chilling-sensitive fruits. With the increased focus on reducing CI development, various techniques including physical and chemical methods, have been used in postharvest treatments to control CI (Wang,

1990; Peng *et al*., 2013; Pholoma, 2016). Some of the methods already reported to reduce CI include: high or low temperature conditioning, intermittent warming, CA storage, MA packaging, applications of growth regulators or other chemicals, waxing and other coatings (Wang, 1993; Tasneem, 2004; Kader, 2013, Peng *et al*., 2013; Pholoma, 2016; Chaudhary *et al*., 2017). The first three approaches manipulate and modify the storage environment while the others involve direct treatment to the commodities. Some techniques are more effective in alleviating CI in certain crops than others, and the optimum treatment conditions vary with different commodities.

2.4.4.1 Temperature Conditioning

2.4.4.1.1 Low Temperature Conditioning

Low temperature conditioning (LTC) is an optional method for increasing tolerance of horticultural crops to low temperature. Exposure of chilling-sensitive tropical and subtropical fruits and vegetables to temperatures slightly above the critical chilling range increases the tolerance of these commodities to chilling during subsequent low temperature storage and delays the development of CI symptoms (Wang, 1993; 1994; 2013; Peng *et al*., 2013). The crucial factors of this technique are the temperature differences between the conditioning and storage temperature, and the duration of the conditioning treatment. For example, loquat can be conditioned at 5°C for six days before storage at 0°C to alleviate internal browning (Cai *et al*., 2006). Carambola fruit preconditioned at 10°C for seven days have less CI during subsequent storage at 5°C (Ali *et al*., 2004). A seven-day exposure of grapefruit to 16°C reduces CI at 5°C storage (Maul *et al*., 2011). Low temperature conditioning combined with other techniques such as treatment of fruits with methyl jasmonate, heat treatments and MAP have a synergistic effect on CI in peach, avocado, carambola, mango and grapefruit (Hoffman *et al*., 2003; Woolf e*t al*., 2003; Ali *et al*., 2004; Tasneem, 2004; Jin *et al*., 2009a).

The beneficial effects of LTC have been attributed to enhancing antioxidant content and antioxidant enzyme activities, augmented s-adenosylmethionine (SAM) decarboxylase activity, increased levels of polyamines (spermine and spermidine) and reducing electrolyte leakage in zucchini squash, peach and sweet potatoes (Kramer and Wang, 1990; Wang, 1995; Padda and Picha, 2007; Jin *et al*., 2009a). Low temperature conditioning treatment also increases the content of adenosine triphosphate (ATP) and enhances activities of energy metabolism enzymes in peach (Chen and Yang, 2012). Expression of lipid membrane

modification enzymes, including fatty acid desaturase (FAD_2) and lipid transfer protein (LTP) are induced by LTC treatment in grapefruit (Sapitnitskaya *et al*., 2006). This suggests that chilling tolerance is enhanced by LTC treatment through activating various molecular mechanisms (Maul *et al*., 2011). Low temperature conditioning induces an adaptive response in fruits to chilling stress. This adaptation to lower temperatures may also be the result of various physiological and biochemical modifications induced by the conditioning treatment. Some of the modifications include reducing the loss of membrane phospholipids; increasing sugar, starch and proline content; maintaining high levels of polyamines, squalene, and long-chain aldehydes; and increasing the ratio of unsaturated to saturated fatty acids (Hatton, 1990; Kramer and Wang, 1990; Wang, 1995; 2013).

2.4.4.1.2 High Temperature Conditioning

Heat treatment, including hot air vapour, forced hot air and hot water dipping have been widely used in controlling CI in a number of fruits (Klein and Lurie, 1991). There are several effective combinations of temperature and duration of heat treatments, which range from 43 to 55°C and from a few minutes up to two hours, depending on treatment method, species and fruit size (Lurie, 1998; Peng *et al*., 2013; Wang, 2013; Pholoma, 2016). A heat shock treatment of 48-52°C for 10 minutes followed by storage at 2-5°C for 40 days was effective in controlling CI in loquat fruit (Wu *et al*., 2004). Hot water treatment (dipping) of mango fruits cultivar 'Keitt' at 55°C for 10 minutes followed by storage at 7°C for nine weeks was effective in alleviating CI (Pholama, 2016). Hot water treatment (dipping) of persimmon and orange in a temperature range of 40-54°C for 2-120 minutes had positive results in preventing CI (Zhang *et al*., 2005; Ghasemnezhad *et al*., 2008). Hot air treatment at 38-39°C for 12 hours has been recommended for reducing CI in grapes, peach, loquat and tomato (Zhang *et al*., 2005; Jin *et al*., 2009b; Lu *et al*., 2010; Rui *et al*., 2010). Heat treatment has been reported to inhibit ethylene biosynthesis and delays softening of plums (Serrano *et al*., 2004). Heat treatment is further reported to express heat shock proteins genes (HSP 19-11) and antioxidant defensive genes in grapefruit (Sapitnitskaya *et al*., 2006). Sun *et al*. (2010) reported that there was accumulation of Ps-CII sHSP1 mRNA transcripts in plum fruit due to heat treatment. Heat treatment is also reported to induce increase in transcripts for a low-molecular weight (KD 17) and a 70 KD heat-shock protein, reduced chromatin condensation and DNA breakdown, and suppressed oxidative activity in apple fruit (Wang *et al*., 2001). These results suggest that heat treatment induces chilling tolerance by activating different stress-related genes.

However, heat treatment can also cause flesh mealiness in peach and nectarine fruits after harvest (Fallik, 2004). In addition, heat treatment combined with MAP effectively reduced internal breakdown, but promoted flesh reddening in heat-treated peach fruit (Malakou and Nanos, 2005). Heat treatment combined with methyl jasmonate alleviated CI and counteracted the side effect of mealiness and flesh reddening in peach fruit (Jin *et al*., 2009b). Bassal and El-Hamahmy (2011) reported that hot water dipping at 40°C for 20 minutes combined with pre-storage conditioning at 16-18°C for six days effectively reduced CI and maintained fruit quality of oranges.

2.4.4.1.3 Intermittent Warming

Intermittent warming is the interruption of low temperature storage with one or more short periods of warm temperature. This brief warm temperature treatment can increase the storage life of some chilling-sensitive commodities, but must be applied before chilling injury becomes irreversible. When it is still at the reversible stage, raising the temperature usually induces higher metabolic activities and allows the tissue to metabolize excess intermediates accumulated during chilling or to replenish any substances which were depleted during chilling (Wang, 2013). Shifting of temperatures from cold to warm and then from warm to cold probably induces a rapid readjustment of metabolism such as increased biosynthesis of polyunsaturated fatty acids (Emongor, 2010; Wang, 2013). Warming of chilled tissues for short periods may help to repair damaged membranes, organelles, or metabolic pathways (Lyons and Breidenbach, 1987). Intermittent warming has been reported to alleviate CI injury in cucumbers, grapefruit, lemons, nectarines, peaches, cucumber, sweet peppers, tomatoes, and zucchini squash (Forney and Lipton, 1990; Wang and Wang, 1992; Wang, 1993; Wang, 2013). When CI has progressed to the irreversible stage, exposure of chilled fruits to warmer temperatures enhances the degradative processes and accelerates the development of CI symptoms (Emongor, 2010; Wang, 2013). Therefore, it is important to detect CI early. Early detection of chilling injury can be achieved by measuring the stimulation of ethylene production (Wang and Adams, 1982; Field, 1990), the changes in Fourier transform infrared spectra (Buta and Wang, 1993), or the increase in nuclear magnetic resonance imaging signals (Wang and Wang, 1992).

2.4.4.2 Controlled Atmospheres

Controlled atmosphere (CA) storage is very effective in maintaining quality, reducing CI, and extending storage period or shelf-life in a number of fruits. Wang *et al*. (2005) reported that

CA storage (5% O_2 + 5% CO_2) reduced CI of peach fruit stored at 0°C. Loquat fruit stored in CA storage of 10% O_2 + 1% CO_2 and 1°C for more than 50 days had normal flavour and a low CI index (Ding *et al*., 2002). However, CO_2 concentration higher than 8-10% causes the development of internal browning and brown surface spotting in several fruits (Wang, 1993; Peng *et al*., 2013).

High O_2 atmosphere (HOA) overcomes the disadvantage of low O_2 atmosphere, and is effective in inhibiting enzymatic discolouration, preventing anaerobic fermentation and undesirable water and odour losses (Peng *et al*., 2013). Exposure of loquat fruit to high O_2 (> 90%) significantly reduced the incidence of internal browning and inhibited polyphenol oxidase (PPO) activity during storage at 1°C (Zheng and Xi, 2000). Wang *et al*., (2005) reported that 70% O_2 atmosphere storage inhibited CI in peach fruit by increasing the activities of superoxide dismutase (SOD) and catalase (CAT), and reduced the content of malondialdehyde (MDA). Under HOA storage (70-100%), litchi and logan fruits showed low internal browning (Tian *et al*., 2005). This was attributed to inhibition of the activity of PPO and maintenance of high levels of ATP, ADP and energy charge of litchi fruit by HOA storage (Duan *et al*., 2004).

2.4.4.3 Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is a low-cost and effective method of maintaining fresh quality and extend shelf-life of fruits and vegetables. Fruits are sealed in polyethylene (PE) film bags with a relatively low permeability to gases, which regulates the atmospheric conditions. The major effects of MAP are the maintenance of high humidity and modification of atmospheric composition (Saltveit, 1997; Wang, 1993; 2013; Peng *et al*., 2013). Several researchers have reported that MAP could be useful method in controlling CI development in various kinds of fruits (Kader *et al*., 1989; Saltveit, 1997; Fonseca *et al*., 2002; Kader, 2013; Peng *et al*., 2013; Bodbodak and Moshfeghifar, 2016). Cucumber fruit packaged in perforated or sealed 31.75 µm low density polyethylene (LDPE) bags had lower CI than non-wrapped fruit in storage at 5°C (Wang and Qi, 1997). Loquat fruit kept in LDPE bags (0.02-0.05 mm thickness) and stored at 1 and 5°C for 30 days had higher fruit quality and lower CI than unwrapped fruit (Zheng and Xi, 2000; Ding *et al*., 2002a). Litchi fruit packaged in LDPE and stored at 13°C for nine days had higher sugar and organic acids contents and lower browning of pericarps induced by CI than control fruit (Somoboonkaew and Terry, 2010). Pulp browning, softness, and flavour of MAP-banana fruits were better than control fruits, which was

associated with lower activities of phenylalanine ammonia lyase (PAL) and PPO induced by chilling temperatures (Nguyen *et al*., 2004).

2.4.4.4 Waxing and Coating

Waxing and coating are widely-used in various kinds of fruits during postharvest treatment (Kader *et al*., 1989; Wang, 1993; Peng *et al*., 2013). Waxing not only improves the appearance of fruits and vegetables but also restricts gas exchange and retards transpiration of fresh produce (Kader, 2013). Edible coating combined with MAP is beneficial in reducing respiration, delay oxidative reaction and therefore, improved the CI tolerance of apple and banana (Pranoto *et al*., 2005; Vilas-Boas and Kader, 2006). The CI was reduced by waxing grapefruit and limes (Wang, 2013). Rojas-Graü *et al*. (2007) reported that alginate and gellan edible coatings containing N-acetylcysteine prevented browning and extended the shelf-life of fresh-cut 'Fuji' apples.

2.4.4.5 Ultraviolet-C Irradiation

Ultraviolet-C (UV-C) light has been applied to many fruits to control postharvest diseases and CI development (Kays and Paull, 2004; Vicente *et al*., 2005; Pongprasert *et al*., 2011). Bell peppers treated with UV-C light (7 kJ m^{-2}) exhibited lower CI and higher quality variables than control Bell peppers (Vicente *et al*., 2005). The UV-C treatment at 0.03 kJ m^{-2} significantly reduced the incidence and severity of CI (membrane damage, browning and chlorophyll degradation) in banana fruit stored under low temperature (Pongprasert *et al*., 2011). There was a lower activity of LOX, PPO, and lower MDA content in UV-C treated banana fruits compared to control fruit (Pongprasert *et al.*, 2011).

2.4.4.6 Chemical Treatments

2.4.4.6.1 Growth Regulators and Natural Products

Growth regulators influence a wide range of biochemical and physiological processes in plant tissues. The modifications of these processes may in turn alter the chilling tolerance (Wang, 1994). The level and balance of certain growth regulators can also affect the susceptibility of plant tissues to CI (Ismail and Grierson, 1977). Abscisic acid (ABA) was reported to stabilize the micro-tubular network, suppresses ion leakage and prevents loss of reduced glutathione and membrane phospholipids (Rikin *et al*., 1979). ABA applications reduced CI in grapefruit (Kawada *et al*., 1979) and zucchini squash (Wang, 1991). Aminoethoxyvinylglycine (AVG)

and 1-methylcyclopropene (1-MCP), ethylene synthesis inhibitors (Emongor, 2010; Lurie, 2010) have been reported to influence CI in fruits (Dong *et al.*, 2001; Zhou *et al*., 2001; McGlasson *et al*., 2005). Ethylene treatments reduced CI in 'Honey Dew' melons (Lipton and Aharoni, 1979) and, peaches and nectarines (Dong *et al*., 2001; Palou and Crisosto, 2003), but increased CI in avocadoes (Chaplin *et al*., 1983; Pesis *et al*., 2002) and citrus (Yuen *et al*., 1995; Porat *et al*., 1999). Ethylene is the fruit ripening hormone, therefore, its effects on CI may be mediated through its effects on maturity and ripening (Wang, 1994; Lurie, 2010; Emongor, 2010). McGlasson *et al*. (2005) examined AVG treated 'Artic Snow' nectarines during storage and found that CI (internal bleeding and wooliness) developed sooner in AVG treated fruits than untreated. While Ju *et al*. (1999) reported that a combined treatment of AVG with gibberellic acid (GA_3) prevented CI in 'Fleiching' peaches. Treatment of peaches and nectarines with 1-MCP increased the development of CI (Dong *et al*. 2001; Zhou *et al*., 2001).

Polyamines are a group of polycationic organic compounds and are ubiquitous in cells (Wang, 1994). Postharvest treatments with exogenous polyamines increase internal polyamine levels and reduce CI (Kramer and Wang, 1989). The reduction of CI by polyamines may be related to their antioxidant activity and stabilizing effects on membrane (Wang, 1994). Methyl jasmonate (MeJA) occurs naturally in a wide range of higher plants and has been shown to affect a number of biological processes (Peng *et al*., 2013). Numerous research reports support MeJA as an endogenous plant growth regulator that plays key roles in plant growth, development and responses to environmental stresses (Cao *et al*., 2012; Zhang *et al*., 2012; Peng *et al*., 2013; Wang, 2013;). Application of MeJA to tomato, papaya, zucchini squash and guava fruit reduced CI (Wang and Buta, 1994; Ding *et al*., 2002b; González-Aguilar *et al*., 2003; 2004). Combination of MeJA and hot air or low temperature conditioning was effective in reducing CI in peach fruit (Jin *et al*., 2009a, b). Cao *et al*. (2009; 2010) reported that treatment of loquat fruit with 10 µmol/L MeJA alleviated CI symptoms and maintained overall fruit quality in cold storage. Chilling tolerance was enhanced in peach or loquat fruits by MeJA treatment through inducing antioxidant system and regulate proline and γ-aminobutyric acid content (Cai *et al.,* 2011; Cao *et al.*, 2012). Treatment of chilling-sensitive crops with MeJA induces the synthesis of some stress proteins, such as HSP, pathogenesis-related proteins and alternative oxidase (Ding *et al*., 2001, 2002; Fung *et al*., 2004). Zhang *et al*. (2012) claimed that MeJA induces gene transcription and enzyme expression related to arginine catabolism in cherry tomatoes, which helps to improve chilling tolerance.

Salicyclic acid (SA) belongs to a group of phenolic compounds widely-distributed in plants, and involved in regulation of many processes in plant growth and development, including fruit ripening and senescence (Peng *et al*., 2013). It is also known for its induction of plant defence against biotic and abiotic stress and is reported to increase chilling tolerance in several fruits including tomato, banana and kiwi fruit (Kang *et al*., 2003; Zhang *et al*., 2003). Application of SA on plum fruits reduced CI by suppressing the activities of PPO and peroxidase (POD), and promoting the accumulation of polyamine (Luo *et al*., 2012). It also reported that SA induces expression of HSP and enhances antioxidant systems in peach and sweet pepper, which increases the resistance to CI (Fung *et al*., 2004; Wang *et al*., 2006).

γ-Aminobutyric acid (GABA), as a natural signal molecule, is highly effective in regulating cold stress in fruits and other plants (Shelp *et al*., 1999). γ-Aminobutyric acid content increases in response to environmental stresses, including high CO_2, heat, and CI (Kinnersley and Turano, 2000; Deewatthanawong *et al*., 2010). It has been reported that exogenous GABA alleviates CI in cold-stored peach fruit by inducing antioxidant activity and increasing the level of ATP and energy charge (Shang *et al*., 2011; Yang *et al*., 2011a). Accumulation or application of endogenous GABA has been associated with the reduction of CI in cold-stored loquat fruit (Cao *et al*., 2012).

2.4.4.6.2 Calcium

Good correlations between calcium (Ca) content in tissues and the susceptibility of fruits or vegetables to CI have been reported (Hewajulige *et al*., 2003; Wang, 2010; Aghdam, 2013; Patel *et al*., 2016; Koushesh-Saba *et al*., 2016). High Ca content in pineapples significantly lowered the incidence and severity of CI (Hewajulige *et al*., 2003). Exogenous Ca applications significantly increase fruit tissue Ca content and affects some of the changes associated with fruit ripening, disorders such as CI and senescence (Pooviah, 1986; García *et al*., 1996; Manganaris *et al*., 2007; Koushesh-Saba *et al*., 2016). Postharvest Ca treatments to peach, strawberry, apricots and tomatoes (García *et al*., 1996; Antunes *et al*., 2003; Arzani *et al*., 2005; Manganaris *et al*., 2007; Aghdam, 2013; Koushesh-Saba *et al*., 2016). Calcium plays a very important role in the structure of the cell wall in which cross-link free carboxyl groups on adjacent polygalacturonate chains present in the middle lamella of the plant cell wall contribute to cell to cell adhesion and cohesion, thus leading to the higher firmness of the fruit tissues (Burns and Pressey, 1987; Saure, 2005; Koushesh-Saba *et al*., 2016). Calcium strengthens cell

walls and cell membranes and help tissues withstand chilling stress (Wang, 2010; Koushesh-Saba *et al*., 2016; Patel *et al*., 2016).

2.4.4.6.3 Nitric Oxide Treatment

Nitric oxide (NO), is a highly reactive free radical gas, acts as a multifunctional signaling molecule in various plants' tissue (Bessom-Bard *et al*., 2007). Nitric oxide also modulates plant hormones and defence against biotic and abiotic stress (Xu *et al*., 2005). Preharvest treatment has been reported to alleviate CI in cold stored Japanese plums (Singh *et al*., 2009), mangoes (Zaharah and Singh, 2011) and cucumber (Yang *et al*. 2011b). Zhu *et al*. (2008) reported that NO combined with intermittent warming treatment could counteract the side effect of intermittent warming alone in preventing CI development in peach fruit. Exogenously applied NO protects kiwifruit against oxidative damage caused by ROS during storage (Zhu *et al*., 2008). Accumulation of endogenous NO in response to chilling-induced oxidative stress in loquat fruit results from enhancement of antioxidant enzymes in the fruit during cold storage (Xu *et al*., 2012). The alleviation of CI by NO might be related to suppression of ethylene production and respiration (Singh *et al*., 2009; Zaharah and Singh, 2011). It is also suggested that NO enhances chilling tolerance in fruits by triggering antioxidant defence systems and regulating activities of cell wall metabolism-associated enzymes (Xu *et al*., 2012).

2.4.4.7 Genetic Modification

Great genetic variation in plants provides opportunity for modifying chilling sensitivity by transferring resistant genes to sensitive species (Wang, 2010). Species from high altitudes or latitudes such as wild tomatoes and potatoes which grow naturally in Ecuador and Peru are usually less sensitive to CI than those from low altitudes or latitudes (Patterson and Reid, 1990). Therefore, these wild species are likely sources of chilling resistant genes for possible use in conventional breeding or genetic engineering. Genetic engineering is a potential technique to be used to improve the resistance to CI in various fruits. Identifying and isolating the genes responsible for chilling resistance and the subsequent transfer of these genes into susceptible species provide a viable avenue for alleviating CI. Structural information for cDNA clones for mRNA coding of chilling resistant genes or the use of classical genetic mapping is required to narrow down the genome and the region on the chromosome which carries the resistant trait (Wang, 2010). It has been reported that ethylene could enhance the activity and protein amount of PAL, and mRNA transcripts of $MaPAL_1$ and $MaPAL_2$ of banana fruit during cold storage,

which suggests that PAL was positively related with tolerance of banana fruit to CI (Wang *et al*., 2007). The gene of ER-type *LeFAD*$_3$ was cloned, isolated and characterized from tomato (Yu *et al*., 2009). They suggested that the overexpression of *LeFAD*$_3$ leads to an increased level of 18:3 fatty acids and alleviates the injuries under chilling stress. Immunological screening or antisense RNA technology may be applied for characterization of the genes. The success of these molecular biological techniques would also depend upon proper transfer of the genes and the successful expression of the transferred genes.

2.5 Modified Atmosphere Packaging on Storage of Fruits

The primary factors in maintaining quality and extending the postharvest life of fresh fruits and vegetables are harvesting at optimum maturity, minimizing mechanical injuries, using proper sanitation procedures, and providing the optimum temperature and relative humidity during all marketing steps (Kader *et al.*, 1985; Hardenburg *et al*., 1986; Shewfelt, 1986; Kader *et al*., 1989; Mangaraj and Goswami, 2009; Emongor, 2010; Kader, 2013). Secondary factors include modification of O_2, CO_2, and/or C_2H_4 concentrations in the atmosphere surrounding the commodity to levels different from those in air. This is referred to as CA or MA. The CA implies a greater degree of precision than MA in maintaining specific levels of O_2, CO_2, and other gases. In MAP the modification of the atmosphere inside the package is achieved by the natural interplay between two processes, the respiration of the products and the permeation of gases through the packaging (Smith *et al*., 1987; Mahajan *et al*., 2007; Mangaraj and Goswami, 2009; Peng *et al*., 2013; Wang, 2013). The MAP involves packaging actively respiring produce in polymeric film packages to modify the O_2 and CO_2 levels within the package atmosphere. The modified atmosphere affects physicochemical and physiological processes in fruits positively or negatively (Fonseca *et al*., 2002; Mahajan *et al*., 2007; Valero and Serrano, 2010). Some of the beneficial effects of MAP include delayed ripening by inhibiting the production of ethylene and sensitivity, reduced transpiration, reduced softening and compositional changes, reduced water loss, reduced decay and physiological changes such as oxidation and reduced respiration (Kader *et al*., 1989; Pary, 1993; Prusky and Kee, 1993; Kader, 1995; Saltveit, 1997; Gorris and Tauscher, 1999; Kader, 2013). Use of MAP in climacteric fruits such as mango, apart from reducing respiration rate, it also delays climacteric respiration (Singh and Rao, 2005; Yahia, 2006). In addition, conditions created in MAP (low O_2 and high CO_2) interfere with ethylene biosynthesis by hindering the activity of 1-Aminocyclopropane-1-carboxylic acid (ACC) oxidase, the enzyme that catalysis the conversion of ACC to ethylene. It has also been shown that CO_2 is an antagonist to ethylene action and impedes its autocatalytic

synthesis (Yang and Hoffman, 1984). Matching commodities' physiological characteristics with those of the package is critical in attaining the beneficial effects of MAP. Recent advances in MAP have led to the design and manufacture of polymeric films with a wide range of gas permeability characteristics to cater for different commodities with varied physiological attributes (Fonseca *et al*., 2002; Jayas and Jeyamkondan, 2002; Mahajan *et al*., 2007). Efficacy of such polymeric films is further enhanced through impregnation with ethylene, oxygen and carbon dioxide absorbers and anti-microbial compounds (Mangaraj and Goswami, 2009; Peng *et al*., 2013; Wang, 2013). One such package is the 'Activebag®', a flexible polymeric package that utilizes unique antimicrobial materials that preserves freshness and prevents spoilage, substantially increasing the storage and shelf-life of perishable commodities (Omry, 2011).

From the above literature review, it is evident that, there is no information in the alleviation of CI of marula fruit. There were only two researches that dealt with storage of marula fruit. Therefore, this study will contribute to the knowledge gap on marula fruit storage and methods of alleviating CI.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Experimental Site

Laboratory experiment was conducted at the Botswana University of Agriculture and Natural Resources (BUAN), Department of Crop and Soil Sciences (CSS). BUAN is located at Sebele (latitude 24 34 °S and longitude 25 57 °E, altitude of 994 m above sea level), 10 km from city of Gaborone. The experiment took place between January and May 2020.

3.2 Experimental Design

A 5 x 2 factorial experiment laid down in a completely randomized design (CRD) was used with three replications. The treatments were storage temperature (6, 8, 10, 12 and 25 ± 1ºC) and storage atmosphere (modified atmosphere packaging-MAP and Air-20.9% O_2 + 0.03% CO_2). The fruits were stored at 6, 8, 10, 12 and 25 ± 1 °C (room temperature as control) and either in MAP or Air. Due to the seasonality of the fruits, the experiment was done once between January-May 2020. Fresh marula fruits were collected randomly at the green mature stage (physiological maturity) from 10 different marula trees around Sebele to make a representative sample. The fresh fruits were firm, green, uniform and free from bruises and defects judged subjectively based on epidermal colour. The fruits were washed with distilled water to remove soils and other external material. Three kilograms (kg) of fruits from a composite sample were packaged in plastic bowls (Air-open) and low density polymeric film or plastics (MAP-3000 ml) and placed in different temperatures stated above.

3.3 Dependent Variables

Dependent variables analysed were: chilling injury incidence, chilling injury severity; shelf-life, fruit weight loss, fruit peel colour, respiration rate (carbon dioxide and oxygen), soluble solids content, titratable acidity, pH, vitamin C, proline content and electrolyte leakage. Since this was a destructive measurement, the above variables were determined prior to storage, during storage and immediately after removal from cold storage and after storage at room temperature (25± 1 °C).

3.3.1 Chilling Injury (CI) Severity

The CI was evaluated daily for incidence and severity of chilling in storage. The CI incidence was determined from a sample of 10 fruit/replicate/treatment. Fruit showing symptoms of CI were counted and expressed as a percentage. Chilling severity was evaluated on a predetermined scale form: 0 being no injury; 1 being slight injury (where 1/3 of the fruit showed some injury symptoms); 2 moderate injuries (where 2/3 of the fruit showed some injury symptoms) and 3 was severe injury (determined by more than 67% of the fruit showing injury depending on the peel damage (Zhao *et al*., 2006).

Chilling injury index was calculated by the following equation:

CII = [(no of fruits with no injury x 0) + (no of fruits with slight injury x 1) + (no of fruits with moderate injury x 2) + (no of fruits with severe injury x 3)] ÷ no of fruits sampled.

3.3.2 Incidence of Chilling Injury

This was evaluated on the number of fruits that developed chilling injury symptoms out of the total fruits used per treatment irrespective of the degree of chilling injury expressed as a percentage.

3.3.3 Shelf-life During Storage

This was the time which fruits was in storage in various storage temperatures and treatments until any of the physiological disorders become unacceptable.

3.3.4 Fruit Weight Loss

Marula fruits were weighed before and after storage to calculate the percent of the fresh weight loss. This was determined by subtracting the actual average weights of the fruits in each replication. The formula below was used to calculate percent weight loss.

$$\% \text{ weight loss} = \frac{\text{Initial average weight} - \text{Actual average weight after storage}}{\text{Initial average weight}} \times 100$$

3.3.5 Titratable Acidity

The fruit pulp and skin was cut and 100 g of the sample weighed, then 100 ml of distilled water was added to the sample. The mixture was then blended for five minutes. The sample was homogenized and filtered with five layers of cheese cloth to extract the juice. Twenty milliliters of the filtrate were then put in 50 ml conical flask and two drops of 1% phenolphthalein indicator was added. The sample was titrated with 0.1 N NaOH to end point and this was done in triplicates. The results were expressed as total titratable acidity equivalents as follows:

$$\text{g total titratable acidity/100 ml juice} = \frac{(\text{ml base} \times 1 \times 100 \times 2 \times \text{normality of base})}{\text{ml sample}}$$

3.3.6 pH

The extracted fruit juice was used to determine the fruit juice pH using a pH meter. The pH meter was immersed into the juice after being calibrated using the buffer.

3.3.7 Soluble Solids Content (SSC)

A sample of juice was expressed from marula fruits (twenty fruits per replication/ treatment was used) and used to determine the total soluble solids using a hand refractometer (Atago Model N1, American Optical, Buffalo, New York). Three drops of juice were directly placed on the prism surface of the refractometer and then the average sugar content was determined in Brix.

3.3.8 Fruit Peel Colour

Peel (skin) colour was measured using a Minolta chromameter (Minolta CR 200, Japan) equipped with 5 mm diameter measuring area. Measurements were reported in terms of CIE (Commission International de I' Eclaireage) L*a* b* color space and expressed as L* (whiteness/darkness, ranged from 0 to 100, while 100 being the lightest), a* (redness for positive value and greenness for the negative value) and b* (yellowness for positive and blueness for negative value) (McGuire, 1992). Calibration was performed against a standardized white calibration plate according to the manufacturer specifications. These were further used to calculate chroma (C*) using the equation below:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

3.3.9 Vitamin C Content

The skin and fruit pulp from twenty fruits per replicate were cut and mixed to form a composite sample and 20 g of the composite sample was weighed and put in a blender. Then 100 ml of metaphosphoric acid-acetic solution was added to the sample. The mixture was blended, homogenized and filtered with five layer of cheese cloth. Then 20 ml of the filtrate was placed in 50 ml conical flask and then three drops of the thymol blue indicator were added. The sample was titrated with 2, 6-dichloroindophenol Na salt solution to end point (AOAC, 1996). The titration was done in triplicate. Also, titration with 2, 6-dichloroindophenol three sample aliquots containing the standard ascorbic acid solution (20 ml) with metaphosphoric acid-acetic acid solution (for correction or blank) was done (AOAC, 1996). The results were expressed as mg ascorbic acid per 100 g as follows:

mg ascorbic acid/g Fresh weight = (average ml for sample titration – average ml for blank titration) × (ml of standard ascorbic acid solution /weight of sample ground) × (volume initial sample solution / volume sample aliquot titrated)

3.3.10 Electrolyte Leakage

Electrolyte leakage was determined according to the method of Chan *et al*. (1985), with slight modifications. Ten discs were taken with a 10 mm diameter cork borer from the peel and pulp tissue, then sample tissues were rinsed with distilled water to eliminate the electrolyte at the cut surface. The samples were placed in a flask containing 25 ml of 0.4 M mannitol. Incubation for 30 minutes at 25˚C was done where the electrical conductivity (EC) was measured in a suspending solution with an EC meter as an initial reading. The samples in a flask were re-heated at 98˚C for 15 minutes and the electrical conductivity re-measured after cooling. Membrane permeability was calculated using the formula given below:

$$\% \text{ Electrolyte leakage} = \frac{\text{initial ion leakage reading at initial temperature}}{\text{Final ion leakage reading at final temperature}} \times 100$$

3.3.11 Proline Content

The skin and pulp of marula fruits was cut and mixed from 20 fruits per replicate to form a composite sample. Zero point five (0.5 g) grams of composite fruit samples were weighed and homogenized in 10 ml of 3% aqueous sulfosalicylic acid. The homogenate was filtered through

Whatman filter paper (grade 1). Then 2 ml of the filtrate was reacted with 2 ml acid-nihydrin and 2 ml glacial acetic acid in a test tube for an hour at 100°C in water bath to develop the colours. Soon after removal from the water bath, the test tube was cooled in an ice bath and proline extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance was read in a UV 160 IPC spectrophotometer at 520 nm using toluene as a blank. Proline content in marula fruit pulp was determined using the formula given below:

µmole proline/g of fresh weight = (µg proline/ml × ml toluene /115.5 µg/mole) / (g sample) (Bates *et al*., 1973).

3.3.12 Respiration Rate

The respiration rate of marula fruits were expressed as O_2 consumption rate and or CO_2 production rate. The closed or static system was used for determining the respiration rate. One kilogram of marula fruits were placed in 2.3 litre jars and sealed with air tight lids fitted with rubber septum for sampling purposes. The fruits were incubated in closed jars for one hour. After one hour, the gas in the jars was analyzed by inserting a needle attached to the portable gas analyzer with a thermal conductivity detector (ADC Bio Scientific Limited, England). The analyzer displayed the concentration of CO_2 and O_2 in the sealed jar. This was done every two days until the end of study.

3.4 Data Analysis

The data collected was subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS). Treatment means were separated using the Least Significant Difference (LSD) at P = 0.05.

CHAPTER 4

4.0 RESULTS

4.1 Modified Atmosphere Packaging

In the current study storage atmosphere means air storage (fruit not packaged) and MAP. Fruit stored in Air means the atmosphere surrounding the fruit was 20.9% O_2 + 0.03% CO_2. The MAP was defined as the enclosure of marula fruit in low density polymeric film and the atmosphere was generated passively by the respiration of the fruit within the package as influenced by the characteristics and permeability of the polymeric film. The steady state equilibrium is reached when O_2 consumption equals O_2 diffusion into the package and CO_2 production equals CO_2 diffusion out of the package; and O_2 consumption equals CO_2 produced. The steady state equilibrium in the MAP was observed in different days depending on the storage temperature (Figure 1, 2). The steady state equilibrium was reached after 4, 6, 8 and 9 days in MAP fruit stored at 12, 10, 8 and 6ºC (Figure 1, 2). The MAP atmospheres at the steady state equilibrium were 10.2% O_2 + 10.2% CO_2, 14.0% O_2 +14.0% CO_2, 15.6% O_2 + 15.6% CO_2 and 16.1% O_2 + 16.1% CO_2 for fruit stored at 12, 10, 8 and 6ºC after 4, 6, 8 and 9 days, respectively (Figure 1, 2). The steady state equilibrium in MAP fruit stored at 25ºC occurred in less than 24 hours, because after 24 hours the gas composition in the package was 5.6% O_2 + 9.4% CO_2 (Figure 1, 2)

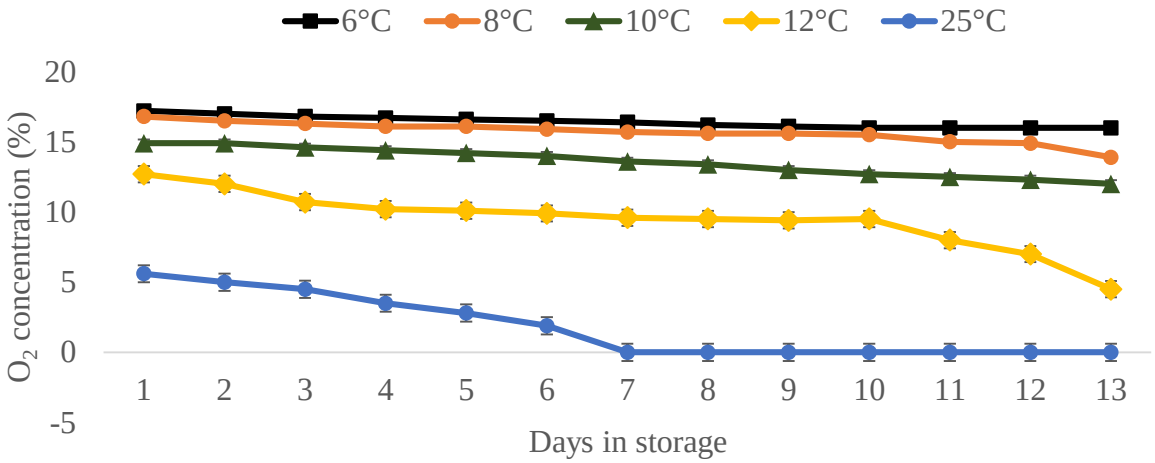

Figure 1: The relationship of storage temperature and O_2 concentration within MAP, bars are standard error

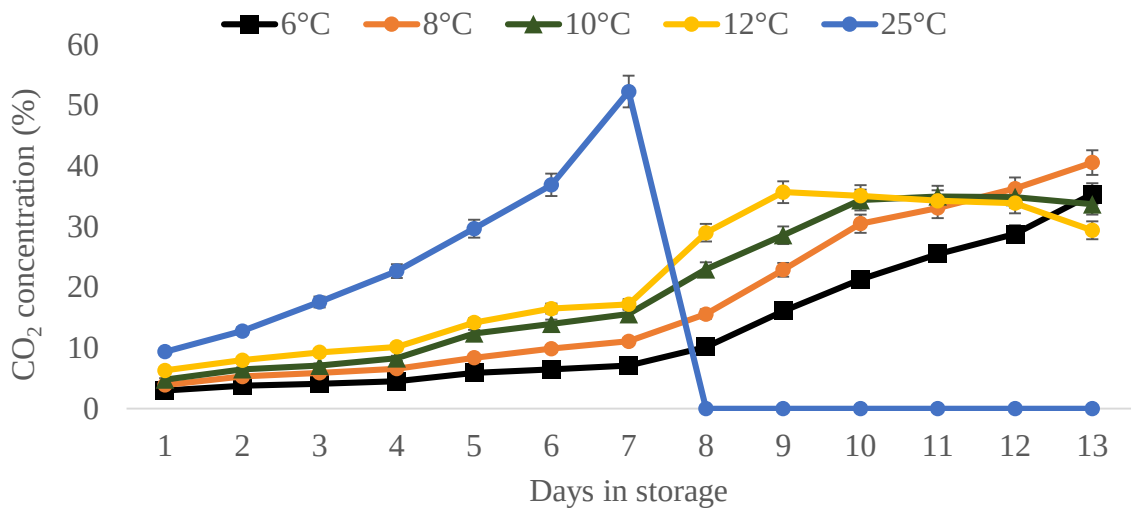

Figure 2: The relationship of storage temperature and CO_2 concentration within MAP, bars are standard error

4.1.1 Respiration

Storage temperature interactered significantly ($P < 0.05$) with storage atmosphere to lower the respiration rate of marula fruit compared to fruit in Air (Figure 2, 3). In each storage temperature, fruit stored in MAP had significantly ($P < 0.05$) lower respiration rate than fruit stored in Air at the same temperature (Figure 2, 3). Fruit stored in lower temperatures either in MAP or Air had significantly ($P < 0.05$) lower respiration rates than fruit stored at 25ºC (Figure 2, 3). The respiration rate (CO_2 production) increased with increase in days of storage, reaching a temporary peak (climacterick peak) on different days depending on storage atmosphere and temperature, with exception of fruit in MAP at 6 and 8ºC (Figure 2, 3). The fruit in MAP stored at 25, 12, 10, 8 and 6ºC reached the climacteric peak after 7 (52.3% CO_2), 9 (35.7% CO_2), 10 (34.4% CO_2), 13 (40.6% CO_2) and 13 (35.4% CO_2) days of storage (Figure 2). Marula fruit stored in Air at 25, 12, 10, 8 and 6ºC reached the climacteric peak after 5 (169.97 ml CO_2/kh/hr), 7 (87.18 ml CO_2/kg/hr), 11 (85.09 ml CO_2/kg/hr), 11 (84.21 ml CO_2/kg/hr) and 15 (68.32 ml CO_2/kg/hr) days of storage (Figure 3).

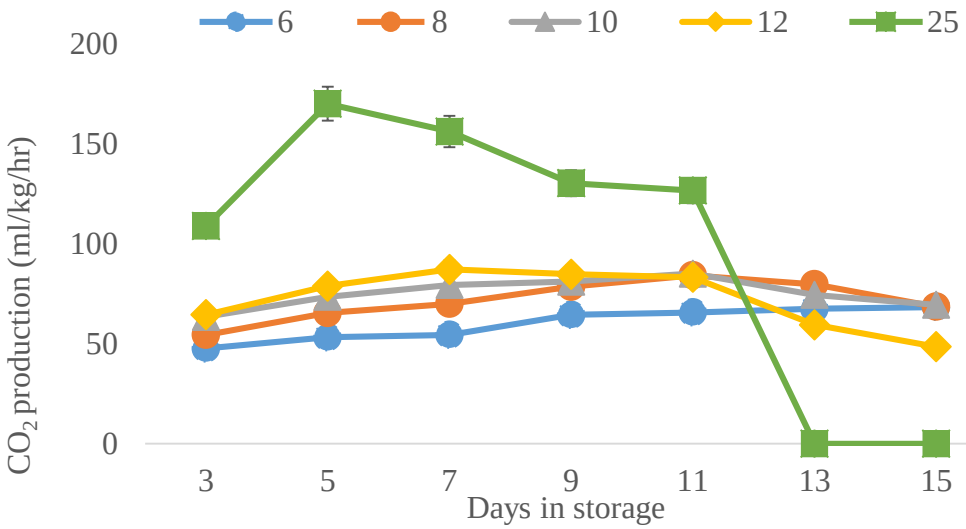

Figure 3: The relationship of storage temperature and CO_2 concentrations in marula fruit in Air, bars are standard error

4.2 Chilling Injury and Incidence

The results of this study showed that there was a significant ($P < 0.05$) interaction between storage temperature and atmosphere on CI incidence and severity (Figure 4, 5). The CI incidence and severity increased with decrease in storage temperature, irrespective of storage atmosphere and duration of storage (Figure 4, 5). At temperatures less than 12°C, the incidence and severity of CI significantly ($P < 0.05$) increased (Figure 4, 5). Fruit in MAP stored at 6, 8 and 10°C had significantly ($P < 0.05$) lower incidence and severity of CI than fruit stored at the same temperatures but in Air (Figure 4, 5). The CI symptoms such as water soaked areas, sunken depressions, uneven ripening, and poor colour development were observed on marula fruit stored at 6, 8 and 10°C (Figure 6, 7, 8a, 8b, 9a, 9b, 10a,10b, 11a, 11b, 12a, 12b, 13a, 13b). The CI symptoms were noticeable on fruits after 10 and 15 days of storage at 6, 8 and 10°C, respectively (Figure 6,7,8a, 8b, 9a, 9b, 10a,10b, 11a, 11b, 12a, 12b, 13a, 13b). Marula fruits stored at 12°C both in Air and MAP did not develop CI (Figure 14).

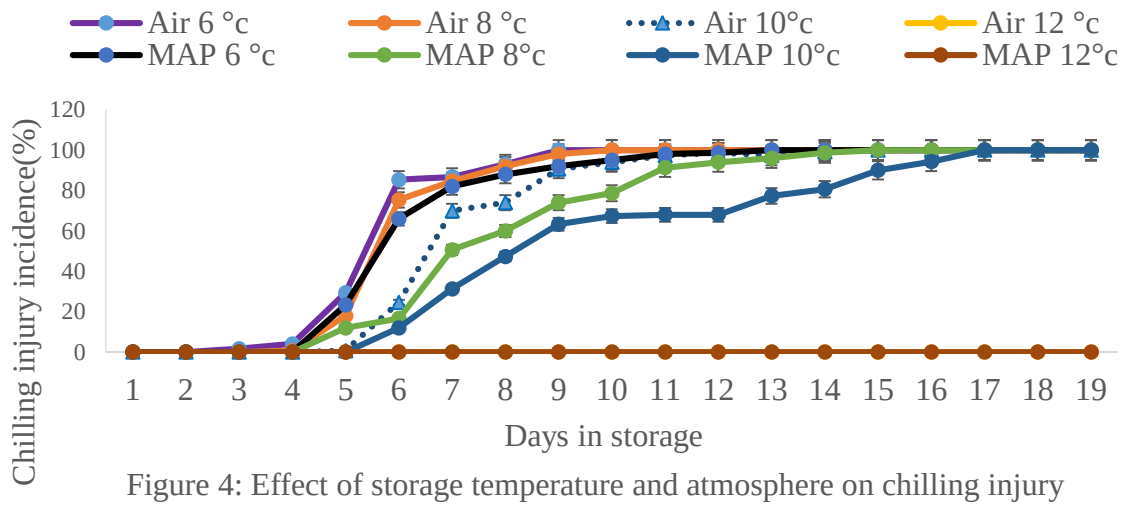

Figure 4: Effect of storage temperature and atmosphere on chilling injury incidence, bars are standard errors

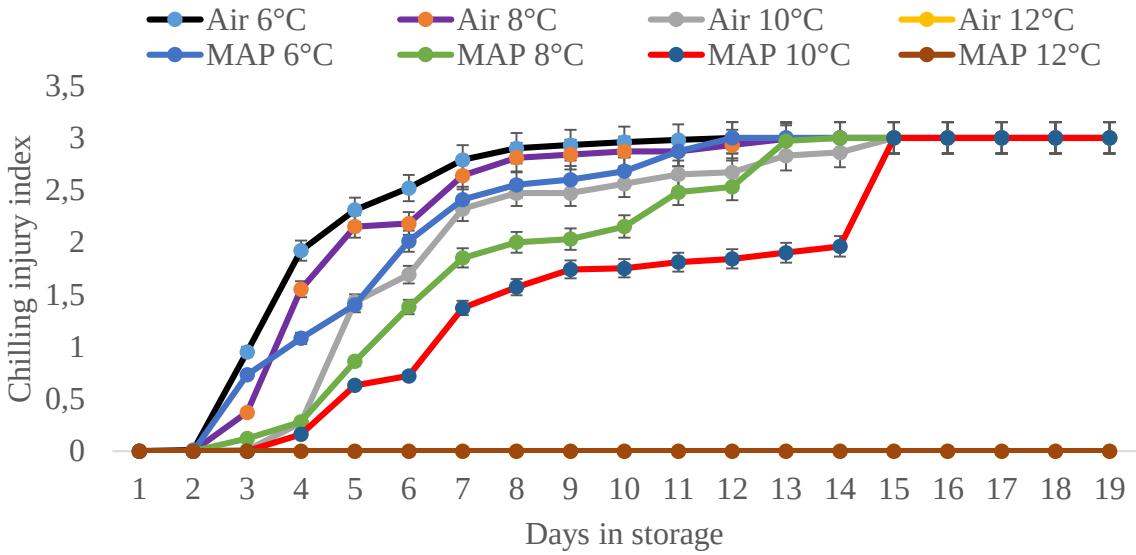

Figure 5: Interaction effect of storage temperature and atmosphere on chilling injury index, bars are standard errors.

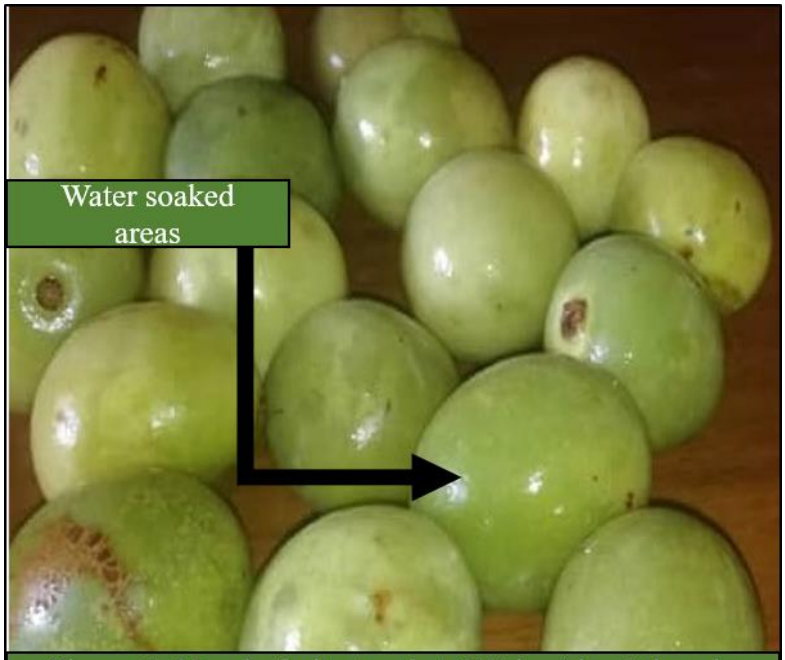

Figure 6: Marula fruit stored at 6°C in Air , 2 days in storage showing water soaked areas due to CI

Figure 7: Marula fruit stored at 6 ° C in Air , 5 days in storage showing poor colour development due to CI

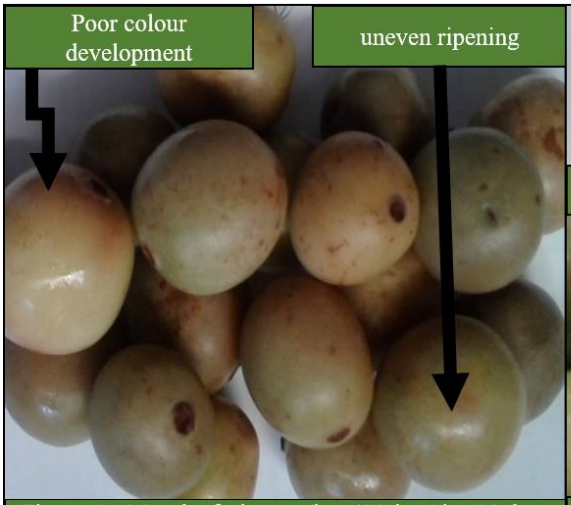

Figure 8a: Marula fruit stored at 6°C in Air , 10 days in storage showing poor colour development and uneven ripening due to CI

Figure 8b: Marula fruit stored at 6°C in MAP, 10 days in storage showing water soaked areas due to CI

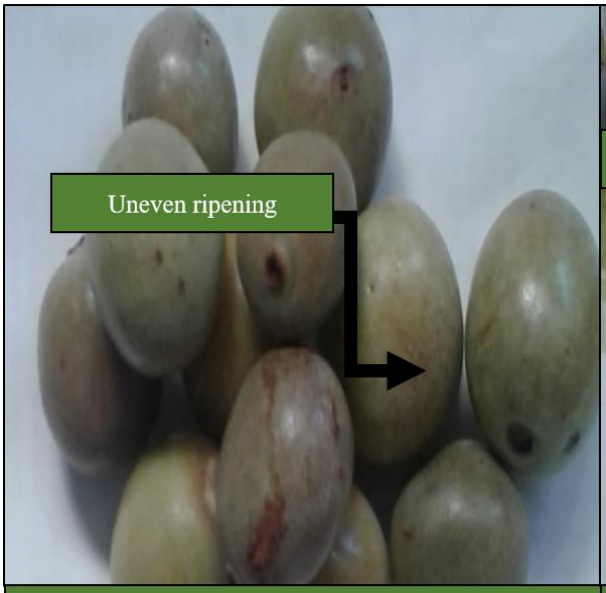

Figure 9a: Marula fruit stored at 8°C in Air, 10 days in storage showing uneven ripening due to CI

Figure 9b: Marula fruit stored at 8°C in MAP, 10 days in storage showing water soaked areas due to CI

Figure 10a: Marula fruit stored at 10°C in Air, 10 days in storage showing water soaked areas and poor colour development due to CI

Figure 10b: Marula fruit stored at 10°C in MAP, 10 days in storage showing water soaked areas due to CI

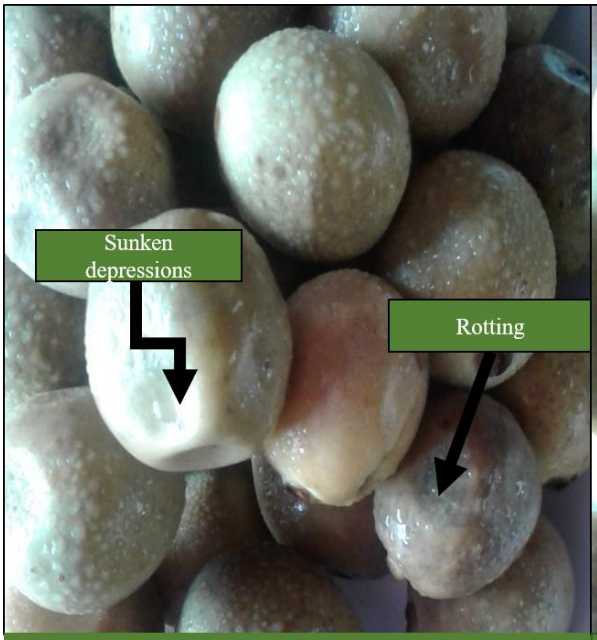

Figure 11a: Marula fruit stored at 6°C in Air, 15 days in storage showing sunken depressions and rotting due to CI

Figure 11b: Marula fruit stored at 6°C in MAP, 15 days in storage showing poor colour development due to CI

Figure 12a: Marula fruit stored at 8°C in Air, 15 days in storage showing poor colour development and sunken depressions due to CI

Figure 12b: Marula fruit stored at 8°C in MAP, 15 days in storage showing water soaked areas due to CI

Figure 13a: Marula fruit stored at 10°C in Air, 15 days in storage showing onset of ripening

Figure 13b: Marula fruit stored at 10°C in MAP ,15 days in storage.

Figure 14: Marula fruit stored at 12°C , 15 days in storage without CI

4.2.1 Proline Content

There were no significant ($P > 0.05$) interactions of storage temperature and atmosphere on fruit proline content (Figure 15). Marula fruits in MAP tended to have non-significantly ($P > 0.05$) lower proline content than fruit in Air, irrespective of the storage temperature (Figure 15). Storage atmosphere alone had no influence on fruit proline content. However, storage temperature significantly ($P < 0.05$) affected proline content of marula fruit (Figure 16). As storage temperature decreased, the fruit proline content significantly ($P < 0.05$) increased (Figure 15). Fruits stored at 6°C had the highest proline content of 0.04 µmoles/g, but fruit stored at 8, 10, 12 and 25°C did not significantly ($P > 0.05$) differ in their proline contents (Figure 16). Fruit stored at 6ºC (had severe CI) had significantly ($P < 0.05$) higher proline content than fruit stored at 12 and 25ºC which did not develop CI (Figure 16). The MAP alone had no significant ($P < 0.05$) effect on fruit proline content.

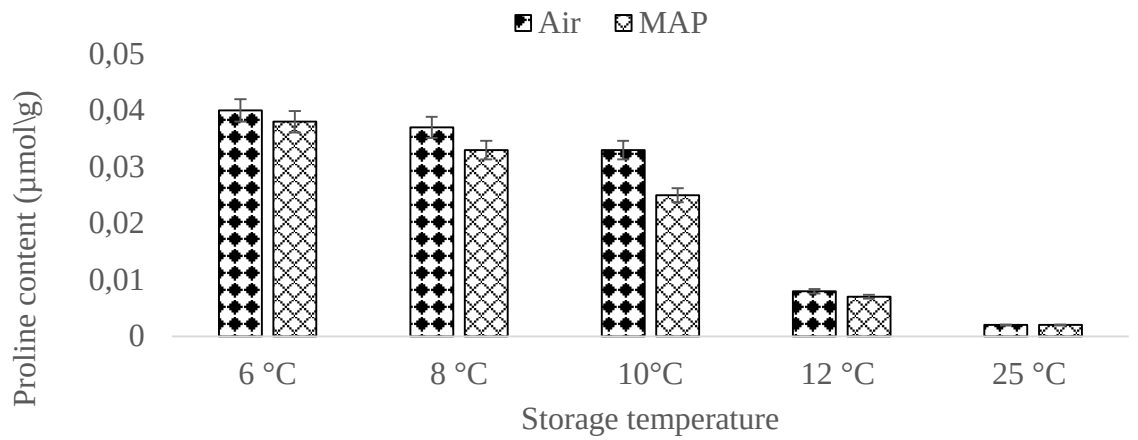

Figure 15: The interaction of storage temperature and atmosphere on proline content of marula fruit, bars are standard error

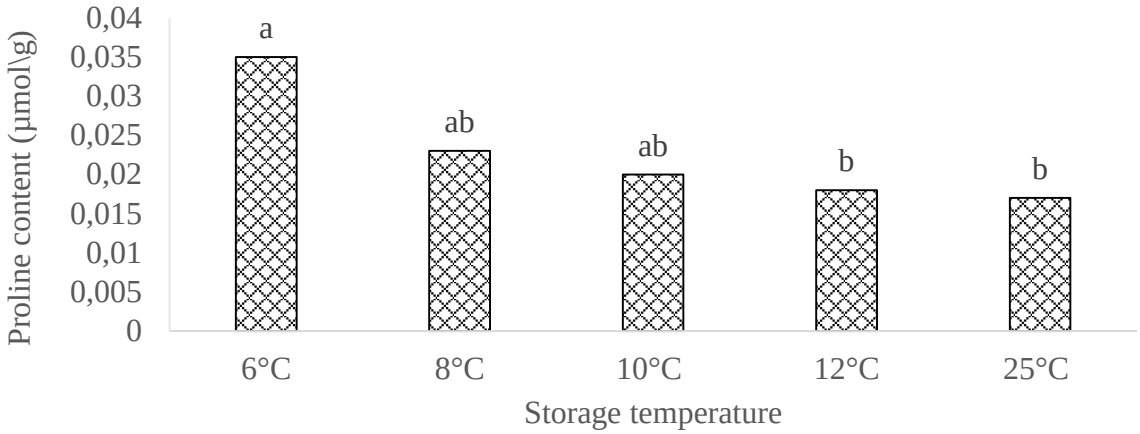

Figure 16: Effect of storage temperature on proline content of marula fruit. LSD = 0.0098

4.2.2 Electrolyte Leakage

In this study, there was a significant ($P < 0.05$) interaction between storage temperature and atmosphere on fruit electrolyte leakage (Figure 17). Marula fruit in MAP and stored in various temperatures had significantly lower electrolyte leakage than fruit stored in Air, except in fruit stored at 25ºC (Figure 17). Fruit stored in Air and held at 6ºC had the highest electrolyte leakage of 67.7% (Figure 17). The lowest electrolyte leakage was on fruit in MAP but stored at 25ºC (Figure 17).

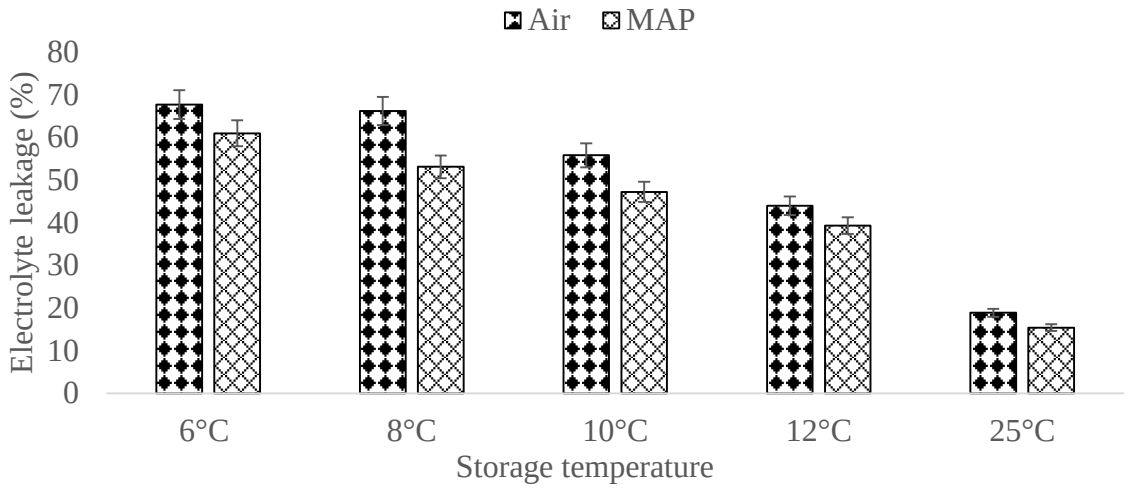

Figure 17: The interaction of storage temperature and atmosphere on the electrolyte leakage of marula fruit, bars are standard error

4.3 Fruit Colour Changes

There was no significant ($P > 0.05$) interaction between storage temperature and atmosphere on the colour development of marula fruit (Figure 18). Storage atmosphere had also no significant ($P > 0.05$) effect on marula fruit colour development during ripening. However, storage temperature significantly ($P < 0.05$) influenced fruit colour development. Fruit stored at 25ºC had significantly ($P < 0.05$) better colour development during ripening than fruit stored at 6, 8, 10 and 12ºC (Figure 19). Fruit stored at 6, 8, 10 and 12ºC did not significantly ($P > 0.05$) differ in their chroma values (Figure 19). Figure 20a, b and c shows the marula fruit colour at harvest, during onset of ripening and fully ripe fruits during ripening.

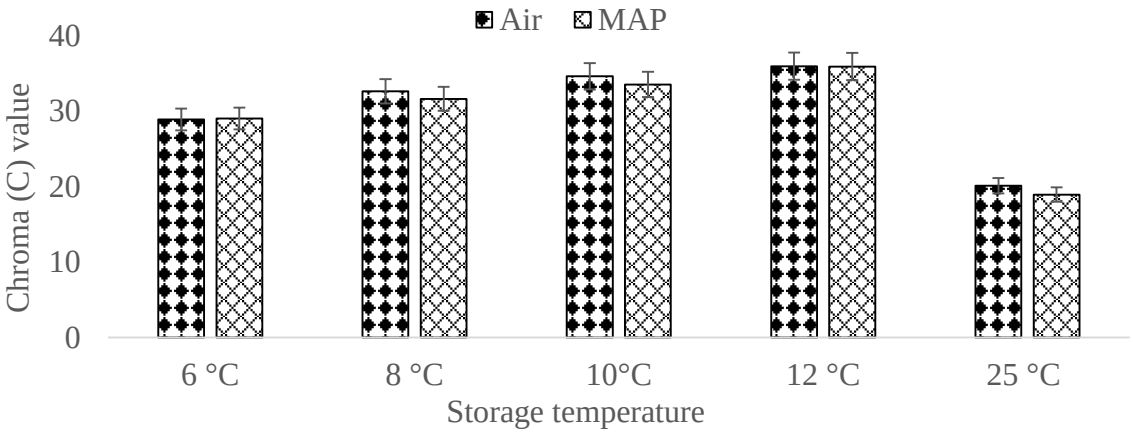

Figure 18: The interaction of storage temperature and atmosphere on the chroma of marula fruit, bars are standard error

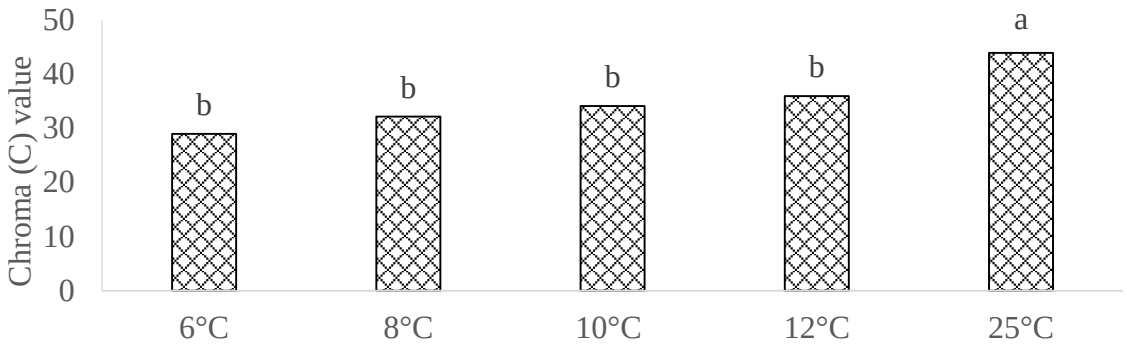

Figure 19: Effect of storage temperature on marula fruit chroma. LSD = 5.0157

Figure 20a: Green marula fruit at harvest

Figure 20b: Onset of marula fruit ripening

Figure 20c: Ripe marula fruit 4 days after harvest

4.4 Titratable Acidity

The interaction of storage temperature and atmosphere was not significant ($P > 0.05$) on fruit juice titratable acidity (Figure 21). Also MAP alone had no influence on fruit juice titratable acidity. However, storage temperature significantly ($P < 0.05$) influenced fruit juice titratable acidity (TTA) (Figure 22). Decreasing storage temperature from 25 to 6ºC significantly ($P < 0.05$) increased fruit juice TTA (Figure 22). Fruit stored at 25ºC had significantly ($P < 0.05$) lower TTA of 6.69 mg/100 ml as compared to TTA of 41.46 mg/100 ml of fruit stored at 6ºC (Figure 22). Fruit stored at 6 and 8ºC did not significantly ($P > 0.05$) differ in their TTA, but was significantly ($P < 0.05$) higher than TTA of fruit stored at 10, 12 and 25ºC (Figure 22).

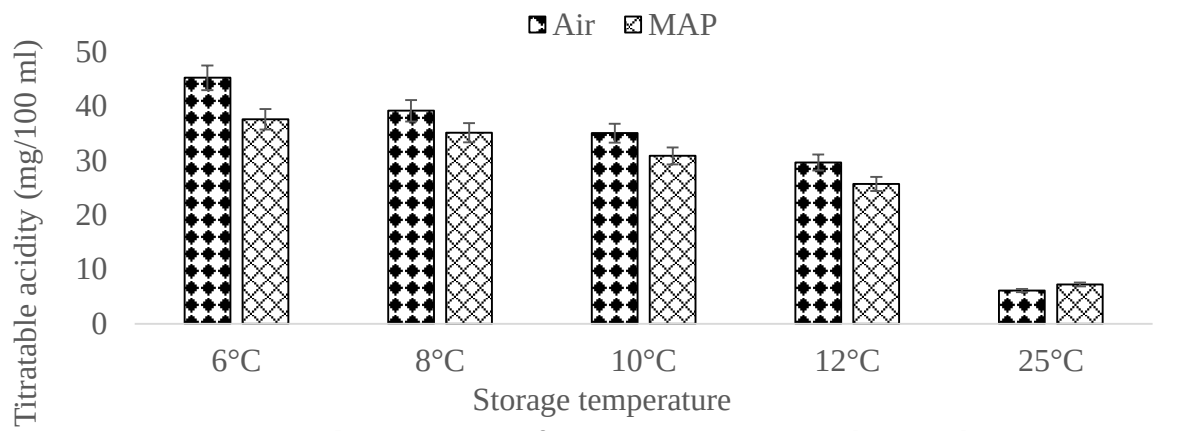

Figure 21: The interaction of storage temperature and atmosphere on titratable acidity of marula fruit, bars are standard error

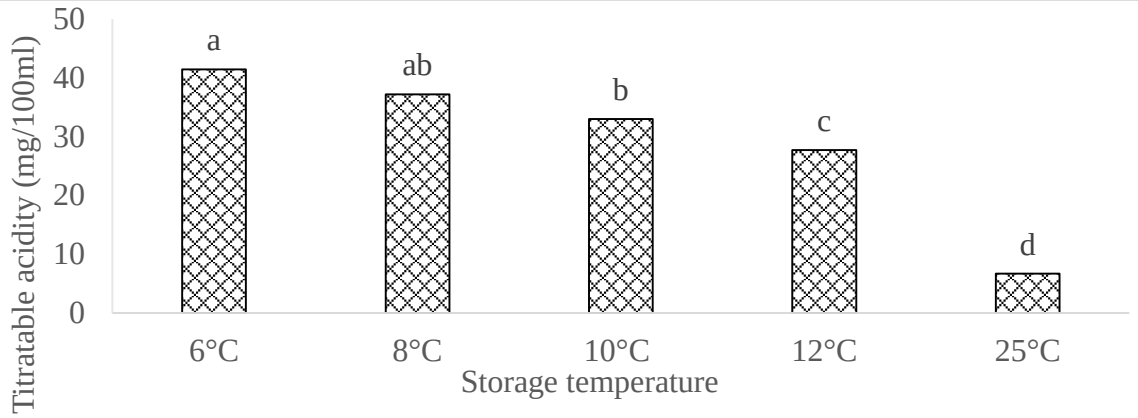

Figure 22: Effect of storage temperature on titratable acidity of marula fruit. LSD = 2.7306

4.5 Fruit Juice pH

Storage temperature and atmosphere had no significant ($P > 0.05$) interaction on marula juice pH (Figure 23). Also MAP alone had no significant ($P > 0.05$) influence on marula juice pH. However, storage temperature significantly ($P < 0.05$) influenced fruit juice pH (Figure 24). Marula fruit stored at 6ºC had significantly ($P < 0.05$) lower juice pH of 3.16 than fruit stored at 25ºC which had a juice pH of 5.6 (Figure 24). Fruit stored at 6, 8, 10 and 12ºC did not signicantly ($P > 0.05$) differ in their juice pH (Figure 24).

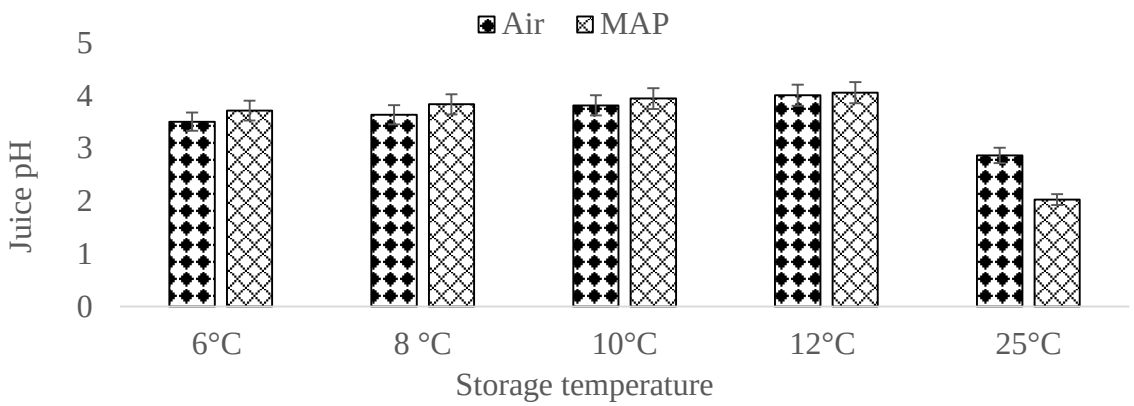

Figure 23: The interaction of storage temperature and atmosphere on the pH of marula fruit juice, bars are standard error

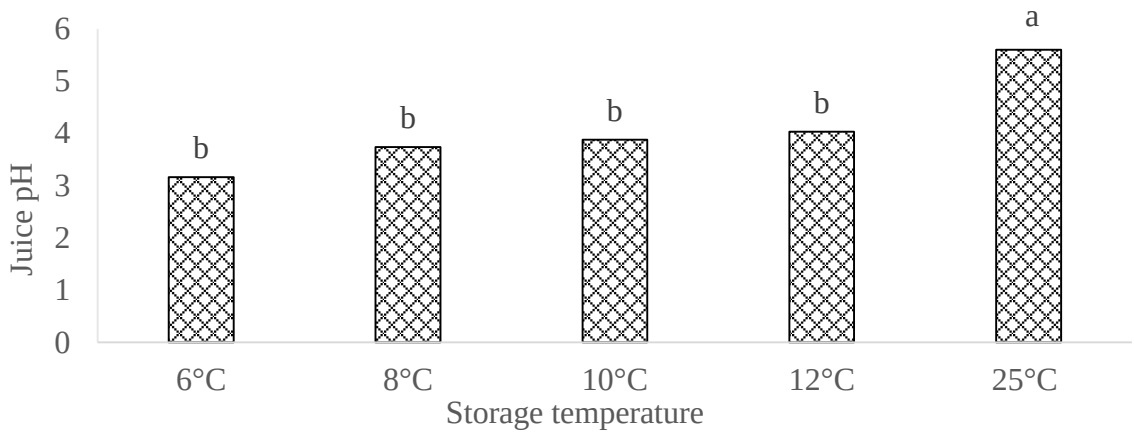

Figure 24: Effect of storage temperature on the pH of marula fruit juice. LSD = 0.53

4.6 Soluble Solids Content

There was no significant interaction ($P > 0.05$ effect between storage temperature and atmosphere on fruit soluble solids content (SSC) (Figure 25). Also MAP alone had no significant ($P > 0.05$) influence on fruit SSC. Storage temperature had a significant ($P < 0.05$) influence on fruit SSC (Figure 26). Fruit stored at 12ºC had SSC of 12% which was significantly ($P < 0.05$) higher than SSC of fruit stored at 6, 8, 10 and 25ºC (Figure 26). Fruit stored at 25ºC had significantly ($P < 0.05$) the lowest SSC of 6% at the end of storage (Figure 26). Fruit stored at 8 and 10ºC did not significantly ($P > 0.05$) differ in their SSC, but significantly ($P < 0.05$) higher than SSC of fruit stored at 6ºC (Figure 26).

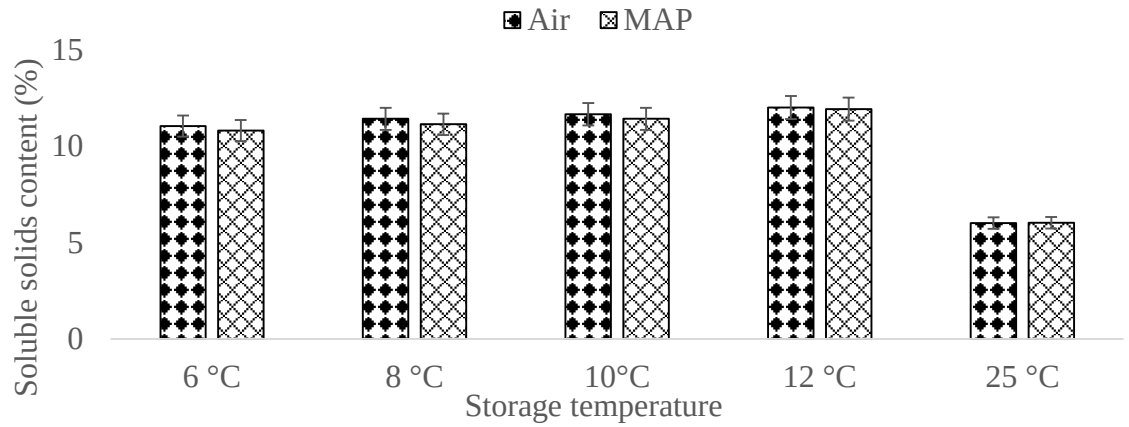

Figure 25: The interaction of storage temperature and atmosphere on soluble solids content of marula fruit, bars are standard error

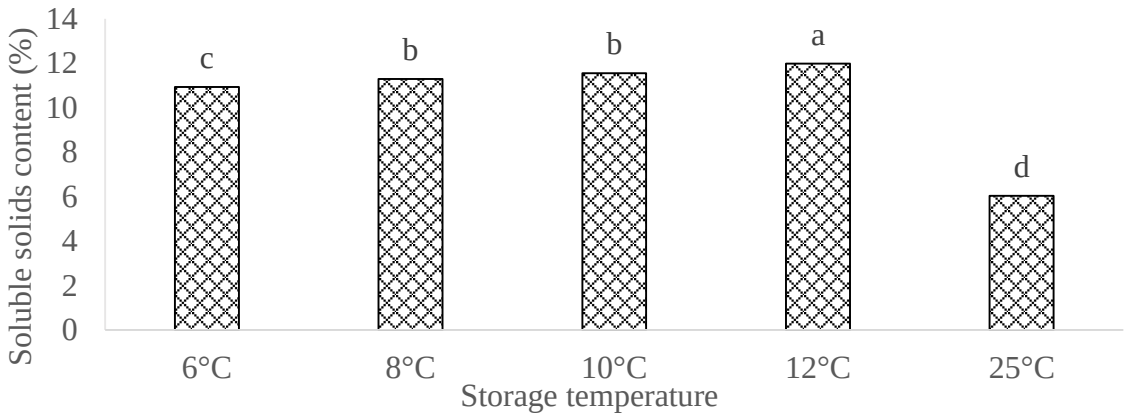

Figure 26: Effect of storage temperature on soluble solids content of marula fruit. LSD = 0.2227

4.7 Changes in Vitamin C Content

Storage temperature and atmosphere had no significant ($P > 0.05$ influence on fruit vitamin C content (Figure 27). Modified atmosphere packaging had no significant ($P > 0.05$) influence on fruit vitamin C content. However, storage temperature significantly ($P < 0.05$) influenced fruit vitamin C content (Figure 28). Fruit stored at 6ºC had significantly ($P < 0.05$) the highest vitamin C content of 92 mg/100 ml juice than fruit stored at 8, 10, 12 and 25ºC (Figure 28). Marula fruit stored at 25ºC had significantly ($P < 0.05$) the lowest vitamin C content of 29.1 mg/100 ml juice at the end of storage (nine days) (Figure 28). Fruit stored at lower temperatures significantly ($P < 0.05$) retained more vitamin C content than fruit at 25ºC (Figure 28). At harvest the average vitamin C content of marula fruit was 171 mg/100 ml juice.

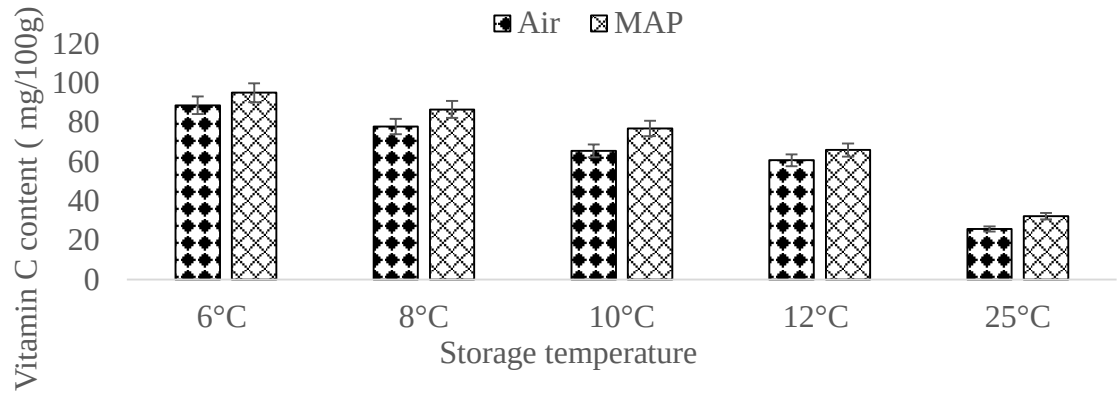

Figure 27: The interaction of storage temperature and atmosphere on vitamin C content of marula fruit, bars are standard error

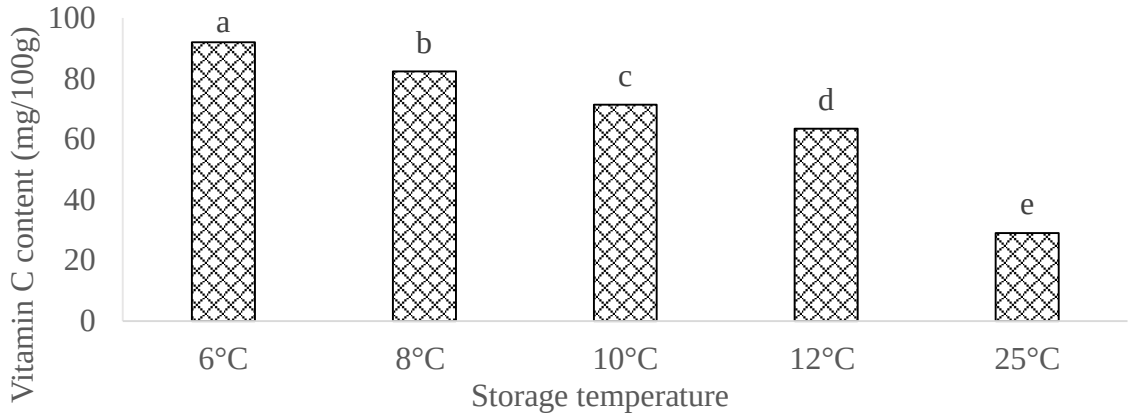

Figure 28: Effect of storage temperature on vitamin C content of marula fruit during storage. LSD = 3.5867

4.8 Weight Loss

Storage temperature and atmosphere significantly ($P < 0.05$) interacted to influence fruit weight loss (Figure 29). Marula fruit in MAP and stored at lower temperatures had significantly ($P < 0.05$) lower weight loss than fruit stored in Air but stored at the same temperatures. As storage temperature increased from 6 to 25ºC, fruit weight loss increased irrespective of the storage atmosphere (Figure 29). Fruit in MAP and stored at 6ºC had the lowest weight loss of 1400 g and fruit stored in Air and stored at 25ºC had the highest weight loss of 2362 g at the end storage life (Figure 29).

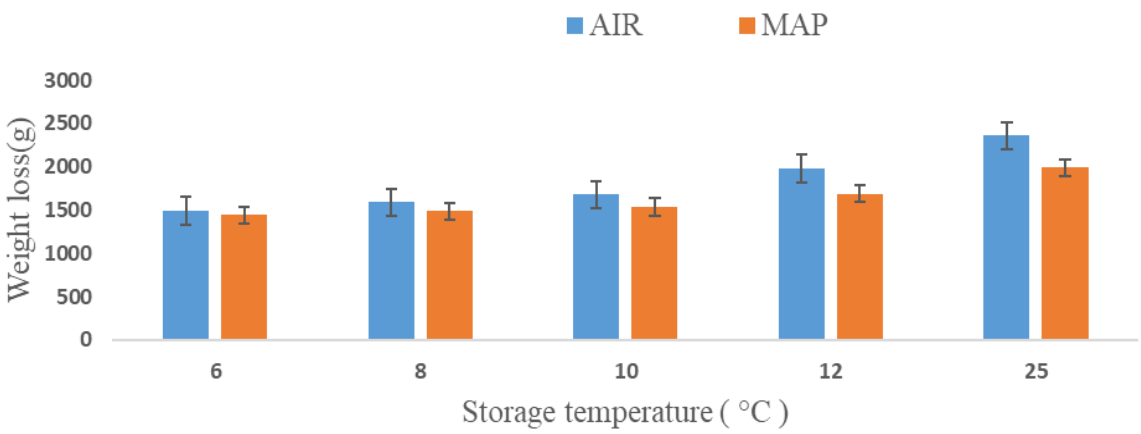

Figure 29: Effect of tempereature and MAP on weight loss of marula fruit, bars are standard error

Figure 30: Marula fruit weight loss

4.9 Shelf-life

Storage temperature and atmosphere had significant ($P < 0.05$) interaction on the marula fruit shelf-life (Figure 31). Marula fruit stored at 12˚C on MAP had significantly ($P < 0.05$) higher shelf-life of 21 days than fruits stored in Air stored at 6, 8, 10 and 12˚C which had a shelf-life of 12, 13, 15 and 19 days, respectively (Figure 31). Marula fruit held in MAP had significantly ($P < 0.05$) higher shelf-life than fruit stored in Air and stored at the same temperatures under study (Figure 31). Marula fruits stored at 25˚C as control had the shortest shelf-life of 9 days when stored in Air (Figure 31).

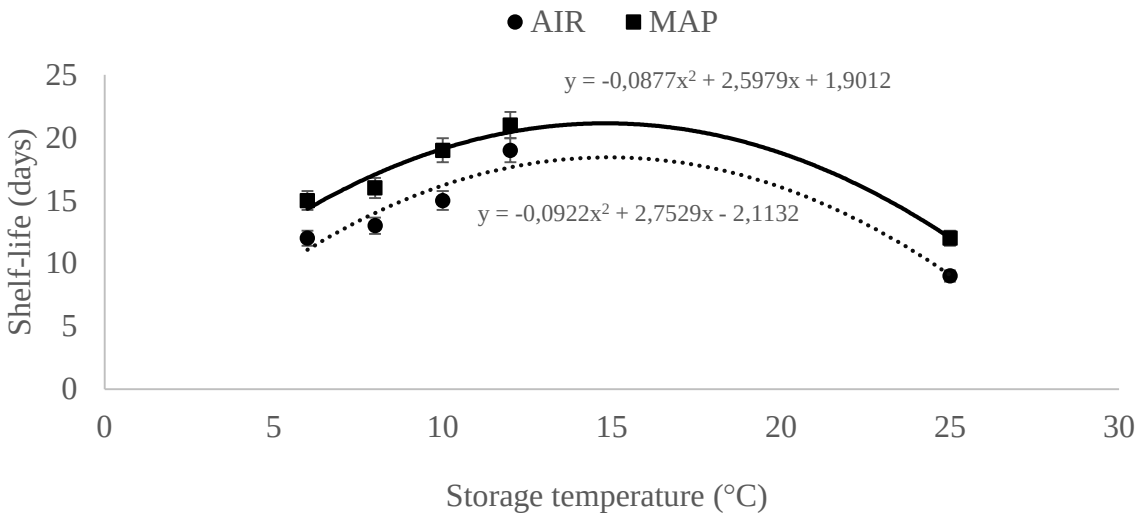

Figure 31: Effects of storage temperature and MAP on marula fruit shelf-life, bars are standard error

Figure 32a: Green marula fruit at harvest

Figure 32b: Marula fruit at the end of the storage period

CHAPTER 5

5.0 DISCUSSION

Temperature affects shelf-life of most horticultural produce since it is estimated that deteriorative processes increases 2-3 fold for every 10°C increase in temperature (KMUTT, 2007). Poor temperature management is a critical component to maintaining perishable horticultural produce in good quality for a longer period of time (Bachmann and Earles, 2000). Temperature is a major contributor to the high postharvest losses (ranging between 45-50%) experienced along horticultural commodities value chain (Atanda *et al*., 2011) hence the need for cold chain maintenance. However, expensive conventional cold rooms are out of reach for small holder farmers (Huyskens-Keil *et al*., 2015).

5.1 Modified Atmosphere Packaging and Respiration

5.1.1 Modified Atmosphere Packaging

Modified atmosphere packaging relies in modification of the atmosphere inside the package, which is achieved by respiration of the produce and the exchange of gases through the packaging which leads to an atmosphere high in CO_2 and low in O_2. The gas concentrations in the polymeric package depends on package permeability characteristics, package dimensions, surface area and produce mass. Oxygen inside the package is consumed by the produce as it respires and approximately equal amount of CO_2 is produced (Kader *et al*., 1989; Fonseca *et al*., 2002; Mangaraj and Goswami, 2009). In the current study, the steady state equilibrium in the MAP was significantly influenced by storage temperature. The steady state equilibrium was reached in 4, 6, 8 and 9 days in MAP fruit stored at 12, 10, 8 and 6ºC. The variation in days to reach steady state due to temperature was attributed to the role of temperature in the metabolic activity of the marula fruit. High temperature (25ºC) had high respiration rate, thus increased CO_2 production and O_2 consumption hence high CO_2 and low O_2 in the package. The steady state equilibrium in MAP fruit stored at 25ºC occurred in less than 24 hours because of high respiration rate in the current study. Temperature has been reported to affect the metabolic activity of the commodity and consequently affected the rate of attaining the steady state equilibrium or the desired modified atmosphere (Kader *et al*., 1989; Parry, 1993; Das, 1993; Jacxsens *et al*., 2000; Mahajan *et al*., 2007).

5.1.2 Respiration

Respiration is a pivotal metabolic process that provides the energy (adenosine triphosphate-ATP) and carbon skeletons for biosynthetic and maintenance processes (Emongor, 2010). It's the main process of deterioration of fresh produce that leads to the oxidative breakdown of organic substrates into simple molecules such as CO_2 and H_2O with the production of energy including ATP and carbon skeletons (Fonseca, 2002; Emongor, 2010; Fagundees, 2012). In the current study, storage temperature interactered significantly with storage atmosphere to lower the respiration rate of marula fruit compared to fruit stored in Air. In each storage temperature, fruit stored in MAP had significantly lower respiration rate than fruit stored in Air at the same temperature. The reduction in respiration rate induced by MAP and storage temperature was attributed to the combined effects of lowering of O_2 and increase of CO_2 and low temperature in the metabolism of marula fruit. In the current study, the atmosphere composition in MAP ranged between 0-16.8% O_2 and 0.03-52.3% CO_2 depending on the storage temperature and days in storage. Reduced O_2 (below 10-12%) and elevated CO_2 (above 0.9%) has been reported to reduce respiration rate due to the overall reduction in the metabolic activity of horticultural produce such as apples, pears, tomatoes, lemons, strawberries and broccoli (Kader, 1987; Solomos and Kanellis, 1989; Kader *et al*., 1989; Mahajan and Goswami, 2001; Fonseca *et al*., 2002; Saltveit, 2003; Rocculi and Romani, 2006; Mangaraj and Goswami, 2009). The reduction of respiration rate in response to low O_2 concentrations has been reported not due to the result of cytochrome-c-oxidase activity, which has got high affinity to O_2, but due to a decrease in the activity of other oxidases such as polyphenoloxidase, ascorbic acid oxidase and glycolic acid oxidase, whose affinity for O_2 is much lower (Kader, 1986; Solomos and Kanellis, 1989; Zagory, 1998).

Fruits stored in lower temperatures either in MAP or Air had significantly lower respiration rates than fruit stored at 25^{o}C in the current study. Temperature has been shown to be the most important external factor influencing respiration (Hardenburg *et al*., 1986; Zagory and Kader, 1988; Kays, 1997; Paliyath and Murr, 2008; Emongor; 2010; Seymour *et al*., 2013). According to Hardenburg *et al.* (1986), for every 10^{o}C increase in temperature, the rate of respiration roughly doubles or even trebles. For example, an apple held at 10^{o}C ripens and respires about three times as fast as one held at 0^{o}C. This increase in respiration has a direct impact on the shelf-life of fresh produce. The storage life of commodities varies inversely with the rate of respiration. Products with a high rate of respiration generally have a shorter shelf-life than those

with a lower rate of respiration. The lower the storage temperature the longer the shelf-life (Hardenburg *et al*., 1986; Emongor, 2010; Kader, 2013; Emongor and Ramagonono, 2019). It is often critical that fresh produce rapidly reach the optimal pulp temperature for short-term storage if it is to maintain its highest visual quality, flavor, texture and nutritional content (Kader, 2013). For most produce maintaining cool temperature will increase storage life by lowering respiration rate, decreasing sensitivity to ethylene and reducing water loss.

The respiration rate (CO_2 production) increased with increase in days of storage reaching the climacterick peak on different days depending on storage atmosphere and temperature, with exception of fruit in MAP at 6 and 8ºC in the current study. The fruit in MAP stored at 25, 12 and 10ºC reached the climacteric peak after 7, 9 and 10 days of storage respectively. Marula fruit stored in Air at 25, 12, 10, 8 and 6ºC reached the climacteric peak after 5, 7, 11, 11 and 15 days of storage. The results of the current study confirm those of Nerd and Mizrahi (1993), Weinert *et al*. (1990) and Redelinghuys (1976) that marula fruit are climacteric. The results of the current study showed that at the climacteric peak the CO_2 production of marula fruit was 169.97 ml/kg/hr are stored in Air which are in agreement with those of Redelinghuys *et al*. (1976) and Weinert *et al*. (1990), but in disagreement with those of Nerd and Mizrahi (1993) who reported CO_2 production of 30 ml/kg/hr which is low. The difference in the current results and those of Nerd and Mizrahi (1993) with respect to the amount of CO_2 produced during marula fruit ripening could be attributed to climatic differences between Botswana (natural habitat) and Israel (trying to domesticate marula) where marula fruit were grown. The similarity of the current results with respect to CO_2 production during marula fruit ripening with those of Redelinghuys *et al*. (1976) and Weinert *et al*. (1990) could be attributed to the similarity of the climate between Botswana and the Republic of South Africa where the two studies were done. The increase in respiration at the onset of fruit ripening and reaching peak (climacteric peak) thereafter it declines is a distinct feature of the climacteric fruit (Brady, 1987; Gamage and Rehman, 1999; Prasanna *et al*., 2007; Paliyath and Murr, 2008; Emongor; 2010; Seymour *et al*., 2013).

Also in the current study, the respiration rate of fruit in MAP and held at 6 and 8ºC increased continuously and never a maxium during the time of study. This increase in respiration was accompanied by CI in fruits stored at 6 and 8ºC. Stress has been shown to induce ethylene production which induces increase in respiration (Adams and Yang, 1979; Yang and Hoffman, 1984; Alexander and Grierson, 2002; Guo and Ecker, 2004). Both electrolyte leakage and

respiration rate depend on fruit tissue integrity and increase in these should be expected at the end of ripening or when the fruit is exposed to severe chilling stress conditions (Pholoma, 2016).

5.2 Chilling Injury and Incidence

The results of the current study showed that storage temperature below 12ºC significantly increased CI incidence and severity of marula fruit. As storage temperature decreased below ≤ 12°C, the incidence and severity of CI increased. The increase in CI incidence and severity in marula fruit stored at temperatures below 12ºC was attributed to stress induced by the low temperatures leading to the development of CI symptoms such as water soaked areas, skin pitting, poor colour development and aroma, uneven ripening and sunken depressions. Results of the current study are in agreement with those of Emongor and Tautsagae (2016) and Nerd and Mizrahi (1993) in marula fruit, Tasneem (2004), Emongor (2015) and (Pholoma, 2016) in mango fruit, Saltveit (2005) and Shang *et al*. (2011) in tomato and peach fruit, respectively. Emongor and Ramagonono (2019) reported that storage temperature significantly influenced CI incidence and severity of wild plum (*Ximmenia americana* L.) fruits. As storage temperature decreased below 15°C, the incidence and severity of chilling significantly increased in wild plum fruits (Emongor and Ramogonono, 2019). Emongor and Tautsagae (2016) reported that the optimum temperature for marula fruit storage was 12ºC, below which CI developed. Marula fruits like any other tropical and subtropical fruits, are susceptible to CI when stored at temperature below their critical minimum temperatures (Nerd *et al*., 1993; Nerd *et al*., 2000; Dube *et al*., 2012; Emongor and Tautsagae, 2016). Although low temperature storage is considered the most effective method of extending postharvest life and maintaining the quality of most fruits (Willes *et al*., 1998; Pholoma, 2016; Ramagonono, 2018), low temperature storage may be detrimental to the storage of tropical and subtropical fruits. Mango fruits are injured after a period of exposure to chilling temperature below 10-15°C, but above their freezing temperatures (Pholoma, 2016). Chill-injured fruits suffer from physical and physiological changes induced by low temperatures. These problems limit the use of low storage temperature to manage postharvest ripening of chilling sensitive horticultural produce because temperatures that are low enough to delay ripening, decay and senescence may also be damaging to the fruit (Emongor, 2015; Emongor and Tautsagae, 2016).

The CI is also associated to enzymatic browning of activities of polyphenoloxidase (PPO) and peroxide (PO) and the increase of phenolic compounds (Trejo-Marquez, 2010). The CI is also known to significantly change the micro-structure of the tissue which in severe cases may lead to tissue breakdown due to failure to carry normal metabolic processes (Wang, 1982; Kader, 2005). Various physiological, biochemical alteration and cellular dysfunction occur in chilling sensitive species in response to chilling stress (Wang, 1982). These alterations include increased membrane permeability and alteration of activities of membrane proteins. If chilling stress is prolonged, these alterations will result in development of chilling injury symptoms such as skin surface pitting, sunken or surface lesions, uneven ripening, pulp discoloration, greyish-scald discoloration of the skin, water-soaking of tissues, off-flavour, susceptibility to fungal decay, reduced aroma and carotenoids (Saltveit *et al*, 1990; Ding *et al*, 2001; Emongor and Tautsagae, 2016). Marula fruits stored at 12°C, in Air or MAP did not develop chilling injury.

In the current study, MAP alleviated CI incidence and severity. This was attributed to the role of plastic film in helping to maintain high relative humidity and modify the concentrations of O_2 and CO_2 in the atmospheres surrounding the marula fruit. Saltveit (1997), Wang (2013) and Peng *et al*. (2013) reported that the major effects of MAP are the maintenance of high humidity and modification of atmospheric composition surrounding the horticultural produce. The reduction of water loss from the tissues in MAP was reported to inhibit the collapse of epidermal and underlying cells and prevents pitting formation (Wang, 2010). Packaging with low density polyethylene film has been reported to alleviate CI in cucumber fruit (Wang and Qi, 1997). Wrapping fruit individually in heat-shrinkable film has also been reported to reduce pitting and scalding in chilled grapefruit (Miller *et al*., 1990). The MAP has also been shown to delay CI in bananas, pineapples, Japanese apricots, lemons, and tomatoes (Wang, 2010), loquat (Zheng and Xi, 2000; Ding *et al*., 2002a) and litchi fruits (Somoboonkaew and Terry, 2010).

The reduction in CI incidence and severity by MAP observed in the current study could also be attributed to the role of MAP in acting as a barrier to marula fruits hence protecting it from CI. Induction of chilling tolerance by physical treatments or exposure to other stresses such as high and low temperature, becomes a great potential approach for protecting harvested fruit from CI and enhancement of membrane integrity by regulating plasma membrane proteins and lipids (Zhang and Tian, 2010; Li *et al*., 2012), improvement of antioxidant system and

suppression of reactive oxygen species (Aghdam *et al*., 2012; Chen and Yang, 2012), proline accumulation by modulating its synthesis and degradation (Shang *et al*., 2011; Aghdam *et al*., 2012; Cao *et al*., 2012), and maintenance of higher ATP content and energy charge (Chen and Yang, 2012; Jin *et al*., 2012; Zhu *et al*., 2012) are considered as the mechanisms being involved in the acquisition of chilling tolerance. Dysfunction of cell membrane and excess production of the reactive oxygen species are two primary events involved in CI development (Ben-Amor *et al*., 1999; Chongchatuporn *et al*., 2013; Luo *et al*., 2015).

5.2.1 Proline Content

As the storage temperature decreased, the proline content in marula fruits increased significantly irrespective of duration of storage. Marula fruits stored at 6˚C, had the highest proline content while those stored at 12 and 25˚C had the lowest proline content may be due to lack of chilling stress on the fruit. The high proline content in fruit stored at 6ºC in the current study was attributed to low temperature stress. Proline and γ-aminobutyric acid (GABA) production are commonly reported to be induced by environmental stresses (water stress, nutrient deficiency, temperature stress and salinity) in plant parts and/or organs (Vurayai *et al*., 2011; Cao *et al*., 2012; Pholoma, 2016). Proline has been thought to enhance plant and/or plant organ resistance to environmental stresses (Hare *et al*., 1999; Shelp *et al*., 1999; Kinnersley and Turano, 2000). Proline is thought to have antioxidant activity as a hydroxyl radical scavenger, in regulation of the NAD^+/NADH ratio and as a protein-compatible hydrotrope thereby enhancing the adaptability of plants and/or plant parts or organs to environmental stress such as low temperature in mango fruits (Hare and Cress, 1997; Ashraf and Harris, 2004). Some studies have attributed high proline content in chilled mango fruit to either enhanced protein degradation at chilling temperatures and/or proline synthesis (Kumar *et al*., 2003; Shang *et al*., 2011; Cao *et al*., 2012; Pholoma, 2016).

5.2.2 Electrolyte Leakage

In the current study, there was a significant interaction between storage temperature and atmosphere on fruit electrolyte leakage. Marula fruit in MAP and stored in various temperatures had significantly lower electrolyte leakage than fruit stored in Air. Fruit stored in Air and stored at 6ºC had the highest electrolyte leakage of 67.7%. It is reported in literature that MAP could be useful method in controlling CI development in various kinds of fruits (Kader *et al*., 1989; Saltveit, 1997; Fonseca *et al*., 2002; Kader, 2013; Peng *et al*., 2013;

Bodbodak and Moshfeghifar, 2016). Cucumber fruit packaged in perforated or sealed low density polyethylene (LDPE) bags had lower CI than non-wrapped fruit in storage at 5°C (Wang and Qi, 1997). Loquat fruit kept in LDPE bags and stored at 1 and 5°C for 30 days had higher fruit quality and lower CI than unwrapped fruit (Zheng and Xi, 2000; Ding *et al*., 2002a). Litchi fruit packaged in LDPE and stored at 13°C for 9 days had higher sugar and organic acids contents and lower browning of pericarps induced by CI than control fruit (Somoboonkaew and Terry, 2010). Pulp browning, softness, and flavour of MAP-banana fruits were better than control fruits, which was associated with lower activities of phenylalanine ammonia lyase (PAL) and polyphenoloxidase (PPO) induced by chilling temperatures (Nguyen *et al*., 2004). The major effects of MAP are the maintenance of high humidity and modification of atmospheric composition (Saltveit, 1997; Wang, 1993; 2013; Peng *et al*., 2013).

Phase transitions and lateral phase separation of membrane lipids are the primary molecular events leading to development of CI symptoms (Lurie *et al*., 1987; Raison and Orr, 1990; Stanley, 1991; Mirdehghan *et al*., 2007). Lateral phase separation of bilayer lipids results in membrane leakiness (Murata and Yamaya, 1984; Sharom *et al*., 1994; Aghdam *et al*., 2012). Evidence for membrane damage and loss of cell membrane integrity as the cause of CI includes increased electrolyte leakage (King and Ludford, 1983; Autio and Bramlage, 1986; Woods *et al*., 1991: Zhao *et al*., 2009; Junmatong *et al*., 2012). The stage of fruit ripeness, duration to exposure to low temperatures, storage temperature and their interaction may influence the fruit electrolyte leakage. Both leakage and respiration rate depend on fruit tissue integrity and increase in these should be expected at the end of ripening or when the fruit is exposed to severe stress conditions (Pholoma, 2016). Membrane damage can be measured by the ion leakage, which in the present study, the electrolyte leakage was significant in marula fruit stored in temperatures of ≤ 10°C than fruit stored at 25°C. In the current study, electrolyte leakage increased with a decrease in storage temperature from 12 to 6°C. According to Forney and Lipton (1990), electrolyte leakage is a useful index of CI as it is affected by lower storage temperatures. McCollum and McDonald (1991) reported that electrolyte leakage was an effective index of CI in grapefruit.

Waxing and coating are a form of MAP widely-used in various kinds of fruits during postharvest treatment (Kader *et al*., 1989; Wang, 1993; Peng *et al*., 2013). Waxing not only improves the appearance of fruits and vegetables but also restricts gas exchange and retards transpiration of fresh produce (Kader, 2013). Edible coating combined with MAP is beneficial

in reducing respiration, delay oxidative reaction and therefore, improved the CI tolerance of apple and banana (Pranoto *et al*., 2005; Vilas-Boas and Kader, 2006). Waxing of grapefruit and limes has been reported to reduce CI (Wang, 2013). Rojas-Graü *et al*. (2007) also reported that alginate and gellan edible coatings containing N-acetylcysteine prevented browning and extended the shelf-life of fresh-cut 'Fuji' apples.

5.3 Fruit Quality

Fruit ripening involves changes in tissue metabolism making the fruit attractive for consumption by organisms that help in seed dispersal (Brady, 1987; Seymour *et al*., 1993; Prasanna *et al*., 2007; Aivalakis and Katinakis, 2008; Emongor, 2010). Fruit ripening is a genetically controlled process that involves physiological, biochemical, and flavor changes that lead to the development of a soft edible fruit with acceptable quality components (Brady, 1987; Seymour *et al*., 1993; Prasanna *et al*., 2007; Aivalakis and Katinakis, 2008; Paliyath and Murr, 2008; Emongor, 2010). Some of the biochemical changes that take place during fruit ripening includes increased respiration, chlorophyll breakdown, biosynthesis of carotenoids, anthocyanins, flavor and aroma volatiles, essential oils, synthesis of cell wall-degrading enzymes, and increase in ethylene production (Brady, 1987; Seymour *et al*., 1993; Prasanna *et al*., 2007; Aivalakis and Katinakis, 2008; Paliyath and Murr, 2008; Emongor, 2010). Fruit ripening is affected by internal and external factors, including developmental genetic regulation, phytohormones, light and temperature (Brady, 1987; Seymour *et al*., 1993; Prasanna *et al*., 2007; Aivalakis and Katinakis, 2008; Paliyath and Murr, 2008; Emongor, 2010).

5.3.1 Fruit colour

In the current study, storage temperature significantly increased fruit colour development. Fruit stored at 25ºC had significantly better colour development during ripening than fruit stored at 6, 8, 10 and 12ºC. Low temperatures (< 12ºC) delayed or retarded colour change and led to uneven ripening during fruit ripening in cold storage in the current study. Storage temperature has been reported to influence the ripening changes in fruits (Esguerra *et al*., 1992; Ahmad *et al*., 2001; Prasanna *et al*., 2007; Aivalakis and Katinakis, 2008, Paliyath and Murr, 2008; Emongor, 2010). An increase in storage temperatures above 12ºC enhanced the rate of ripening and softening of marula fruit in the current study. The results of the current study are in agreement to those reported in papaya (Rohani *et al.*, 1997; Pesis *et al*., 2002; Githiga *et al*.,

2014), Loquat (Amoros *et al*., 2008), mango (Emongor, 2015; Pholoma, 2016), banana (Smith and Thompson, 1987; Semple and Thompson, 1988; Ahmad *et al*., 2001), tomato (Aivalakis and Katinakis, 2008, Paliyath and Murr, 2008), wild plum (Emongor and Ramogonono, 2019) and pepper (Aivalakis and Katinakis, 2008). The increase in marula fruit colour development with increase in storage temperature was attributed to degradation of chlorophyll and biosynthesis of anthocyanins and carotenoids plus unmasking of carotenoids due to chlorophyll breakdown (Brady, 1987; Tucker and Grierson, 1987; Lizada, 1993; Blackenship and Dolle, 2003; Aivalakis and Katinakis, 2008, Paliyath and Murr, 2008; Emongor, 2010; Emongor, 2015). Colour change is a dramatic event that occurs in fleshy fruits at the start of ripening. A rise in storage temperature increased fruit skin colour in marula fruits. Chroma of mangoes tended to increase during storage particularly in fruits stored at temperatures higher than 5˚C (Nunes *et al*., 2007).

5.3.2 Titratable Acidity and Juice pH

Acidity of fruit, is determined by titratable acidity and it's associated with both sweetness and sourness of fruit (Lobit *et al*., 2002). Acidity level of fruit has a major impact on internal fruit quality and consequently affects the time when the fruit reaches the minimum market standard (Marsh *et al*., 2003). The flavour of fruits and vegetables depends on the interaction of sugars, organic acids, phenolics, tannins and aroma volatiles (Prasanna, 2007; Paliyath and Murr, 2008; Emongor, 2010; Seymour *et al*., 2013).

The results of the current study showed that storage temperature significantly influenced fruit juice titratable acidity (TTA). Decreasing storage temperature from 25 to 6ºC significantly increased marula fruit juice TTA. Fruit stored at 25ºC had significantly the lowest TTA of 6.69 mg/100 ml compared to TTA of 41.46 mg/100 ml of fruit stored at 6ºC indicating that fruit at 25ºC was more ripe than fruit at 6ºC. The results of the current study showed that storage temperature significantly influenced fruit juice pH. Marula fruit stored at 6ºC had significantly lower juice pH of 3.16 than fruit stored at 25ºC which had a juice pH of 5.6. The decrease and increase in marula fruit TTA and juice pH, respectively, with increasing storage temperature was attributed to increased level of fruit ripening. Fruit ripening is a complex, genetically programmed process that culminates in dramatic physiological, biochemical and organoleptic changes that lead to the development of a soft and edible ripe fruit with desirable quality attributes (Brady, 1987; Prasanna *et al*., 2007; Paliyath and Murr, 2008; Emongor, 2010;

Seymour *et al*., 2013). Immature fruits contain more acids that may decline during maturation and ripening due to their conversion to sugars (gluconeogenesis) (Brady, 1987; Kays and Paull, 2004; Prasanna *et al*., 2007; Paliyath and Murr, 2008; Emongor, 2010; Seymour *et al*., 2013). Total acidity in fruits decreases with ripening due to their utilization as respiratory substrates especially in the Krebs (TCA) cycle (Kays and Paull, 2004; Prasanna *et al*., 2007; Emongor, 2010). Emongor and Tautsagae (2016) reported a decrease and increase in marula fruit TTA and juice pH, respectively, with increase in storage temperature. Similar results have been reported in mangoes (Srinivasa *et al*., 2002; Yousef *et al*., 2012; Emongor, 2015; Pholoma, 2016), wild plum (Ramagonono, 2018), banana (Ahmad *et al*., 2001; Wachiraya *et al*., 2006; Mohapatra *et al*., 2016), tomatoes (Carrari and Fernie, 2006; Aivalakis and Katinakis, 2008), and strawberries (Holcroft and Kader, 1999). Emongor and Ramogonono (2019) reported that storage temperature significantly influenced wild plum fruit TTA and juice pH. As storage temperature increased from 0 to 15°C, fruit TTA and juice pH significantly decreased and increased, respectively (Emongor and Ramagonono, 2019). The response of wild plum fruit TTA to increasing storage temperature was a linear decrease with a correlation coefficient of 0.99 (Emongor and Ramagonono, 2019). Pholoma (2016) reported that TTA acidity of mango significantly decreased from 12.8 mg/100 ml juice in fruit stored at 4°C to 6 mg/100 ml juice fruit stored at 25°C. The reduction in acidity during ripening plays a great role in the acid-to-sugar balance and consequently in influencing the taste and flavour of the fruits (Prasanna, 2007; Paliyath and Murr, 2008; Emongor, 2010; Seymour *et al*., 2013). High temperature in storage is reported to enhance starch and polysaccharides hydrolysis into sugars and decreased total acidity in fruits hence enhanced fruit quality (Kudachikar *et al*., 2001; Srinivasa *et al*., 2002; Paliyath and Murr, 2008; Seymour *et al*., 2013; Emongor and Tautsagae, 2016).

5.3.3 Soluble Solids Content

Emongor (2010) described quality as the degree of excellence or superiority which is the combination of attributes, properties, or characteristics that give each commodity value in terms of its intended use. The relative importance given to a specific quality attribute varies in accordance with the commodity concerned and with the individual (producer, consumer, and handler) or market concerned with quality assessment. To producers, high yields, good appearance, ease of harvest, and the ability to withstand long-distance shipping to markets are important quality attributes (Emongor, 2010). Appearance, firmness, and shelf-life are

important from the point of view of wholesale and retail marketers. According to consumers, quality of fresh fruits is judged on the basis of appearance at the time of initial purchase.

Fruit ripening renders fruit attractive and palatable to a variety of seed dispersing organisms and typifies non-dehiscent (fleshy) fruits (Giovannoni, 2001; Prasanna *et al*., 2007; Emongor, 2010). Starch degradation is another biochemical process linked to fruit ripening and contributes to fruit soluble solids content (Luengwilai and Beckles, 2009a). Soluble solids content indicates the level of acids and sugars in the fruit; the biochemical pathways that produce these compounds are stimulated by climacteric ethylene in climacteric fruit, but their initiation precedes this event in fruit development (Jeffery *et al*., 1984).

Results of current study showed that storage temperature had a significant influence on fruit SSC. Fruit stored at 12ºC had SSC of 12% which was significantly higher than SSC of fruit stored at 6, 8, 10 and 25ºC. The increase in marula fruit SSC with increase in storage temperature from 6 to 12ºC was attributed to fruit ripening which enhanced solubilization of cell wall polysaccharides such as pectins and cellulose (Tucker and Grierson, 1987), and hydrolysis of starch and other storage polysaccharides (Selvaraj *et al*., 1989; Giovannoni, 2001; Prasanna *et al*., 2007; Aivalakis and Katinakis, 2008; Emongor, 2015). The taste development in ripe fruit is due to a general increase in sweetness, which is a result of increased glycogenesis, hydrolysis of polysaccharides, especially starch, decreased acidity as evidenced in the current study, and accumulation of sugars and organic acids resulting in an excellent sugar/acid blend (Selvaraj *et al*., 1989; Lizada, 1993; Giovannoni, 2001; Prasanna *et al*., 2007; Aivalakis and Katinakis, 2008; Siddique *et al*, 2010; Seymour *et al*., 2013; Emongor, 2015; Emongor and Tautsagae, 2016). Fruit stored at 25ºC had significantly the lowest SSC of 6% at the end of storage (six days) this was attributed to high respiration compared to the low respiration observed at the lower storage temperatures observed in the current study.

5.4 Changes in Vitamin C Content

Vitamin C occurs naturally in many fruits and vegetables, but is easily destroyed by cooking, canning or by exposure to air and light. Vitamin C acts as an antioxidant, binds and neutralizes free radicals which damages the tissues (Rickman *et al*., 2007). Vitamin C being one of the important vitamins that keeps human beings healthy, is vital for growth and maintenance of healthy bones, teeth, gum, ligaments and blood vessels which increases the body's resistance

to infection (Rickman *et al.*, 2007). Because of its role in the formation of collagen, the body's major building proteins, vitamin C is the central component of all body organs (Njoku *et al*., 2011). The factors that affect the vitamin C content of fruits include production factors, climatic conditions, maturity stage of fruits, handling and storage (Naggy, 1980; Padayatty *et al*., 2003; Ajibola *et al*., 2009).

The results of the current study showed that the storage temperature significantly influenced fruit vitamin C content. Marula fruit stored at 6ºC had significantly higher vitamin C content of 92 mg/100 ml juice than fruit stored at 8, 10, 12 and 25ºC. Fruits stored at 25ºC had significantly the lowest vitamin C content of 29.1 mg/100 ml juice at the end of storage (nine days). High temperature has positively been associated with significant reduction of vitamin C in fruits and vegetables (Lee and Kader, 2000; Kays and Paull, 2004; Ajibola *et al*., 2009; Yousef *et al*., 2012; Emongor and Tautsagae, 2016). The stability of vitamin C is reported to decrease with the increase in temperature, because at high temperatures ascorbic acid (vitamin C) variably degrades and gets converted to dehydroascorbic acid which is heat labile and leaches or escapes out of the fruit (Owusu-Aninkorah and Sefa-Dedeh., 2006). However, in the case of orange, the rind acts as protective covering which prevents excessive loss of ascorbic acid when openly exposed to high temperatures as compared to ambient room temperatures. Emongor and Tautsagae (2016) reported that marula fruit stored at 0°C for three weeks had vitamin C content of 793.6 mg/100 g, while marula fruit stored at 25°C for one week had vitamin C content of 586.5 mg/100 g, high temperature accounted for 26.1% reduction in marula fruit vitamin C content. Lee and Kader (2000) and Ajibola *et al*. (2009) reported that loss of vitamin C in fresh horticultural commodities was enhanced by extended storage and high temperature. Yousef *et al*. (2012) reported that ascorbic acid content decreased gradually and significantly during storage at 8, 10, 13˚C as well as in mango fruits dipped in hot water at 48 and 52˚C for 10 minutes. The rate of loss of vitamin C is higher with higher storage temperature, an effect associated with loss of acidity (Kays and Paull, 2004; Emongor and Tautsagae, 2016, Pholoma, 2016). In the current study, TTA and juice pH of marula fruit decreased and increased (loss of acidity) respectively, with increase in storage temperature which correlated with loss of vitamin C and explaining that the loss of vitamin C was related to loss in fruit acidity.

5.5 Weight Loss

Maintaining quality and extending the postharvest life of fresh fruits and vegetables is the goal of postharvest technologies (Kader *et al.*, 1985; Hardenburg *et al*., 1986; Shewfelt, 1986; Kader *et al*., 1989; Mangaraj and Goswami, 2009; Emongor, 2010; Kader, 2013). Maintaining the initial weight of the produce is vital as an increase in weight loss indicates deterioration and poor quality of produce which may make it unmarketable**.** One purpose of using MAP is to maintain a high relative humidity in the packaging, so dehydration typically is not a problem, however, water permeability of the packaging film is critical in influencing the weight loss of the horticultural produce.

Results of this study showed that storage temperature and atmosphere significantly interacted to influence fruit weight loss. Marula fruit in MAP and stored at lower temperatures had significantly lower weight loss than fruit in Air but stored at the same temperatures. As storage temperature increased from 6 to 25ºC, fruit weight loss increased irrespective of the storage atmosphere. The interaction of low temperature storage and MAP has shown to reduce water loss in fruits and vegetables (Kader *et al*., 1989; Parry, 1993; Prusky and Kee, 1993; Saltveit, 1997; Gorris and Tauscher, 1999; Mahajan *et al*., 2007; Valero and Serrano, 2010; Kader, 2013). The MAP is reported in literature to affect physicochemical and physiological processes in fruits positively or negatively (Fonseca *et al*., 2002; Mahajan *et al*., 2007; Valero and Serrano, 2010). Some of the beneficial effects of MAP include delayed ripening by inhibiting the production of ethylene and sensitivity, reduced transpiration, reduced softening and compositional changes, reduced water loss, reduced decay and physiological changes such as oxidation and reduced respiration (Kader *et al*., 1989; Parry, 1993; Saltveit, 1997; Gorris and Tauscher, 1999; Kader, 2013).

5.6 Shelf-life

Marula fruit like other tropical and subtropical fruits have a short postharvest shelf-life when stored at ambient temperatures and is sensitive to chilling injury (CI) when stored at low temperatures below 12ºC (Nerd and Mizrahi, 1993; Gross *et al*., 2002; Wang, 2013; Peng *et al*., 2013; Emongor, 2015; Emongor and Tautsagae, 2016; Chaudhary *et al*., 2017). Low temperature storage is essential for extending postharvest life of fruits and allows the preservation of fruit quality after harvest (Hardenburg *et al*., 1986; Sevillano *et al*., 2009; Mworia *et al*., 2012; Aghdam, 2013; Emongor, 2010; 2015). Low temperature slows down

most cell metabolic activities and delays fruit ripening and plant senescence (Hardenburg *et al.*, 1986; Sevillano *et al.*, 2009; Mworia *et al*., 2012; Aghdam, 2013; Emongor, 2010; 2015).

The results of the current study showed that marula fruit stored at 12˚C in MAP had significantly longer shelf-life of 21 days than fruits in Air stored at 6, 8, 10 and 12˚C which had a shelf-life of 12, 13, 15 and 19 days, respectively. Marula fruits stored at 25˚C as control had the shortest shelf-life of nine days when held in Air. The increase in marula shelf-life in MAP irrespective of temperature was attributed to the role of MAP in delayed ripening, reducing transpiration and water loss, reducing softening and compositional changes, reducing decay and physiological changes such as oxidation and reducing respiration (Kader *et al*., 1989; Parry, 1993; Saltveit, 1997; Gorris and Tauscher, 1999; Kader, 2013). Low storage temperature of 12ºC and MAP prolonged marula shelf-life to 19 days compared to fruit stored in Air at 25ºC (nine days) because low temperature lowers respiration rate, decreases sensitivity to ethylene, therefore, reduced fruit ripening, reduces the growth of rot causing micro-organisms and reduces water loss (Willes *et al*., 1998; Sevillano *et al*., 2009; Mworia *et al*., 2012; Aghdam, 2013; Emongor, 2015; Emongor and Tautsagae, 2016, Pholoma, 2016).

CHAPTER 6

6.0 CONCLUSSION AND RECOMMENDATIONS

Postharvest management of marula fruits is important for their successful postharvest storage and maintenance of fruit quality during marketing. This study showed that a combination of low temperature storage of 12°C and MAP were effective in maintaining the quality of marula fruits. The MAP and storage temperature of 12ºC significantly reduced respiration rate, electrolyte leakage and weight loss, delayed fruit ripening and increase fruit shelf-life. Storage temperature significantly influenced CI and CI severity, proline content, fruit quality (colour, SSC, pH, SSC, and vitamin C content), fruit weight loss and shelf-life of marula fruit. Storage of fruits in MAP or Air at 12ºC reduced CI and CI severity. It can be recommended that in order to reduce marula fruit CI and CI severity, maintain fruit quality, and extend shelf-life and the marketing period of the fruit; the fruit should be stored in MAP and held at 12°C. It was also recommended that this study be repeated with other MAP technologies such as waxing and low density polymeric films with different porosities.

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