EFFECTS OF STORAGE TEMPERATURE ON POSTHARVEST QUALITY OF WILD PLUM (*SIMENIA AMERICANA* L.)

MASTER OF SCIENCE IN CROP SCIENCE
(HORTICULTURE)

BY

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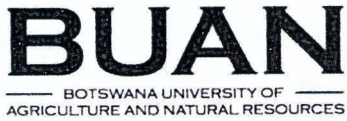

EFFECTS OF STORAGE TEMPERATURE ON POSTHARVEST QUALITY OF WILD PLUM (*Ximenia americana* L.)

A research dissertation submitted to Botswana University of Agriculture and Natural Resources in partial fulfilment of the requirements for the Degree of Master of Science in Crop Science (Horticulture)

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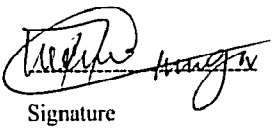
PROF. V. E. EMONGOR		26/06/2018
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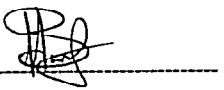
Prof M. BATLANG		26/06/18
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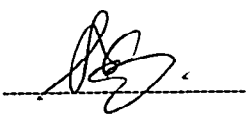
PROF. S. NGWAKO		26/06/18
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STATEMENT OF ORIGINALITY

The work contained in this dissertation was compiled by the author at Botswana University of Agriculture and Natural Resources between August 2017 and March 2018. The work is original except where references have been cited and will not be submitted for the award of any other Degree or Diploma of any University.

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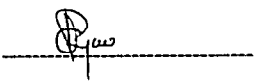
GODIRAONE RAMAGONONO		26/06/2018

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ABSTRACT

Wild plum (*Ximenia americana* L.) is a tropical crop widespread throughout the tropics of Africa, India and South East Asia to Australia, New Zealand, Pacific Islands, West Indies, Central and South America. Despite the widespread distribution of wild plum it remains unexploited and it has potential for commercial exploitation. Wild plum has many medicinal, therapeutic, nutritional and energy uses, but its products are collected in the wild and less researched. A laboratory experiment was undertaken to evaluate the effects of storage temperature on postharvest quality and shelf-life of wild plum fruits. The treatments were storage temperatures at 0, 5, 10 and 15 ± 1°C laid down in a completely randomized experimental design. The results of the study showed that storage temperature significantly ($P < 0.05$) influenced chilling injury incidence and severity of wild plum fruits. As storage temperature decreased below 15°C, the incidence and severity of chilling significantly ($P < 0.05$) increased. Storage temperature also significantly ($P < 0.05$) influenced wild plum fruit quality attributes such as titratable acidity, juice pH, soluble solids content, vitamin C content, weight loss and shelf-life. As storage temperature increased from 0 to 15°C fruit titratable acidity and vitamin C content significantly ($P < 0.05$) decreased, while juice pH, soluble solids content and weight loss significantly ($P < 0.05$) increased. The decrease in fruit titratable acidity and vitamin C content, plus the increase in soluble solids content and juice pH was attributed to fruit ripening. The increase in fruit weight loss with increase in storage temperature was attributed to higher transpiration and respiration rates at the higher temperatures. Storage of wild plum fruit at 15°C maintained fruit quality and fruit had a shelf-life of twenty eight days, instead of two days or less at other temperatures in this study due to chilling injury. Therefore, it was concluded that in order to extend the shelf-life and marketing period of wild plum fruits, the fruits should be stored at 15°C and 90-95% RH.

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LIST OF ABBREVIATIONS

ANOVA – Analysis of variance

AOAC – Association of Official Analytical Chemists

asl – above sea level

BUAN – Botswana University of Agriculture and Natural Resources

CA – Controlled atmosphere

CI – Chilling injury

CII – Chilling injury index

CRD – Completely randomised design

$C_6H_6O_6$ – Ascorbic acid

$C_6H_8O_6$ – Dehydroascorbic acid

FAO – Food and Agriculture Organisation

HSPs – Heat shock proteins

LSD – Least significant difference

MA – Modified atmosphere

0.1 N NaOH – 0.1 Normal Sodium hydroxide

pH – potential Hydrogen

PR – Pathogenesis-related

RH – Relative humidity

SAS – Statistical analysis system

SSC – Soluble solids content

TA – Titratable acidity

TCA – Tricarboxylic acid

CHAPTER I

1.0 INTRODUCTION

1.1 Background

Moretologa (wild plum) scientifically known as *Ximenia americana* (L.) is a bush forming shrub, named after a Spanish Priest Francisco Ximónez (Orwa *et al.*, 2009). The genus name *Ximenia*, is part of the Olacaceae family of plants which comprises of eight species namely *Ximenia roiigi, aegyptiaca, parviflora, coriaceae, aculeata, caffra, americana* (L.) and *aegyptica* (Brasileiro *et al.*, 2008). *Ximenia americana* L. has several common names depending on the language for example in English (sour plum, wild plum, sea lemon, blue sour plum and tallow nut), Afrikaans (kleinsuurpruim), Amharic (inkoy, kol), Arabic (kelto, abu khamira, medica), Bemba (mulebe), French (cerise de mer, macaby, citron de mer, croc), Luganda (museka), Swahili (mtundakula, mtumbui tumbui, tumbui tumbui, mpingi), Setswana (moretologa) (Orwa *et al.*, 2009). Wild plum is a semi-scandent shrub or small tree 2-7 m high, commonly less than 4 m (Kew, 2006; Orwa *et al.*, 2009; Feyssa *et al.*, 2012; Urso *et al.*, 2013). It is characterised by dark brown to pale grey bark, smooth to scaly and produces a rounded to conical crown. Branches normally arching down often armed with straight spines. Leaves are simple, alternate, lanceolate to elliptic, 3-8 to 1.5-4 cm, variable thickness (semi-succulent to thin; obtuse or emarginated, 3-7 pairs of veins, inconspicuous (Maundu *et al.*, 1999; Orwa *et al.*, 2009). The petioles are short, slender, up to 6 mm long, canaliculated. Grey-green, hairless and leathery or thin flesh (Kew, 2006; Orwa *et al.*, 2009). The fragrant white, yellow-green or pink flowers occur in branched inflorescences borne on shortly pedunculate axillary racemes or umbels; pedicels are 3-7 mm long, both pedicels and peduncles are glabrous (Orwa *et al.*, 2009). The fruits are oval shaped about 3 cm long, 1.5-2.5 cm in diameter and contains one large endospermic seed (Maundu *et al.*, 1999; Sacande and Vautier, 2006; Matos, 2007; Orwa *et al.*, 2009). The fruit turns yellow, orange or red when ripe. The fruit is a drupe.

The fruit is thin skinned plum like fruit, picked and consumed immediately from the tree as the fruit quickly perishes, with almond like flavour (Matos, 2007; Urso *et al.*, 2013). It has been observed that during its developmental and growth stages the plant varies in morphology, that is when the plant is young its leaves are very much hairy as compared to when the plant is older, the leaves become smooth and shiny (Maundu *et al.*, 1999; Sacande and Vautier, 2006).

1.2 Distribution

Wild plum is widespread throughout the tropics in Africa, India and South East Asia to Australia, New Zealand, Pacific Islands, West Indies, Central and South America (Sacande and Vautier, 2006; Souza, 2008; Saeed and Bashier, 2010; Sarmento *et al.*, 2015). The fruit tree is mostly solitary, distributed in open lands, savannahs, galley forests, along coastal areas, dry forests, semi-arid bushland and even in sandy woodlands (Feyssa *et al.*, 2010, Maundu *et al.*, 1999) and is characterised as a plant of diverse habitats. It grows in altitudes of 0-2000 m above sea level (asl) (Maundu *et al.*, 1999; Orwa *et al.*, 2009). Wild plum is frequently found on coastal dunes, along water courses and on stony slopes and is adapted to growing in a variety of soils, often poor and dry soils (Sacande and Vautier, 2006; ICRAF, 2010) which include clays, clay loams, loamy sands, sandy clay loams and sands (FAO, 1986), as well as in dry hilly areas (Maundu *et al.*, 1999). It grows in areas with mean annual temperature and rainfall of 14-30°C and 300-1,250 mm, respectively (Maundu *et al.*, 1999; Orwa *et al.*, 2009).

1.3 Propagation

Wild plum is cultivated by seed and grows well in full sun to light shade and is drought tolerant (Orwa *et al.*, 2009; Feyssa *et al*, 2012). It prefers soil pH range of 4.5-7.5. (Orwa *et al.*, 2009; FAO, 1993). Germination is hypogeal. Seeds readily germinate between 26 and 36°C, with the germination rate being fastest at 31°C (Sacande and Vautier, 2006). Seed germination takes eight to thirty days depending on the climatic conditions and whether the seed coats are intact

or removed (Scanade and Vautier, 2006). When seed coats are intact the seeds germinate in approximately sixteen days, removing the seed coat reduces the germination time to about ten days. However, it is recommended not to remove the seed coat before planting as it may damage the seed and reduce germination (Sacande and Vautier, 2006). The plants can be semi-parasitic, absorbing water and nutrients from other plants through their roots, but not solely dependent on this for their survival (Sacande and Vautier, 2006). Under cultivation, a dioecious species is preferred for fruit and seed production (Sacande and Vautier, 2006). The wild plum takes three to four years to start fruiting after cultivation yielding 15-17 kg of fresh fruit per tree. Seeds are sown fresh for good germination (Sacande and Vautier, 2006; Orwa *et al.*, 2009).

1.4 Economic importance of wild plum

Many wild plants have economic, medicinal and forage values in addition to preserving cultural heritages and maintaining ecological balance by providing various ecosystem services (Feyssa *et al.*, 2012). Indigenous people have knowledge of the use and management of wild plum. Over-harvesting and lack of proper management can lead to the extinction of a species (Feyssa *et al.*, 2012). Even though Bahiru (2010) and Yohanis (2009) have given inadequate account of uses and management of wild plum, other researches have revealed that it is broadly used in non-traditional medicine in regions where it is distributed (Mwangi and Malii, 1994; Gronhaug *et al.*, 2008; James *et al.*, 2008; Le *et al.*, 2012).

1.4.1 Fruit

When ripe, the fruit is eaten as a dessert and can be used to make juice, jams, jellies, or an intoxicating alcoholic drink (Orwa *et al.*, 2009; Feyssa *et al.*, 2012; Badimo *et al.*, 2015). Sandune people rely on the fruit as their staple food. The fruit juice contains considerable

amount of natural sugars. However, the pulp of seed and fruit contains hydrocyanic acid (Benoit and Santillana, 2000) and it is advisable not to chew the seed.

1.4.2 Medicinal uses

According to research that has been done in the past ten years, wild plum has medicinal properties (Feyssa *et al.*, 2010), displayed by its antimicrobial, antifungal, anticancer, antioxidant and pesticidal and haematological effects. These properties are complimented by chemical compounds of wild plum fruit, seed, leaves and roots which are phenolics, dietary fibres, minerals, vitamin C (antioxidants), saponins, glycosydes, flavonoids, carotenoids, tannins, alkaloids, quinones and terpenoids (Feyssa *et al.*, 2010; 2012; Orwa *et al.*, 2009). Wild plum has been reported to be rich in antioxidants such as vitamin C, polyphenols, anthocyanins and carotenoids, thus representing a good source of such compounds for humans (Rezanka and Sigler, 2007; Silva *et al.*, 2008; Lamien-Meda *et al.*, 2008; Mora *et al.*, 2009; Sarmento *et al.*, 2015).

The seed is very rich in fatty acids and glycerides as well as cyanide (Orwa *et al.*, 2009). At least ten fatty acids have been identified in the seed oil of wild plum constituting 92.42% of unsaturated fats (Eromosele and Eromosele, 2001). The essential fatty acids present included linoleic at 1.34%, linolenic at 10.31% and arachidonic at 0.60%, and conferred on the considerable nutritional value (Eromosele and Eromosele, 2001). Linoleic is known for its importance in the metabolic synthesis of prostaglandins (Al-Jassir *et al.*, 1995). Wild plum oil is a suitable base material for alkyd resin synthesis and paint formulation (Eromosele and Eromosele, 2001).

Constituents used in folk medicines are found in fruits, fruits pulp, leaves, twigs and roots (Mwangi and Malii, 1994; Omer and Ali, 1998; Benoit and Santillana, 2000; Saeed and Bashier, 2010). Fruits serve a crucial role in treatment of habitual constipation, used in bath

water for sick children and kidney and heart problems are manageable together with skin ulcers (Teo, 1997). The fruits eaten in large quantities act as a vermifuge (Niemi *et al.*, 2005). Leaves and twigs are being used for fever and colds treatment as well as laxative and eye lotion (Omer and Ali, 1998). Leaves are reported to be beneficial for the treatment of headaches especially in children as well as poison antidote (Feiberger and Vanderjagt, 1998; Feyssa *et al.*, 2012).

In Ethiopia, wild plum is used in the treatment of cobra bite; the fresh or dried stem is boiled with water then the decoction is drunk by the person bitten by the cobra and the residue from the decoction is applied to the wound to hasten healing (Feyssa *et al.*, 2012). The bark which contains approximately 17% oils (Fatope and Adam, 2005) can also be chewed for the treatment of swollen pancreas (Feyssa *et al.*, 2012). The crushed bark is also used in the treatment of malaria and hepatitis (Feyssa *et al.*, 2012).

Wild plum roots are used to eliminate skin problems and aches, haemorrhoids, guinea worm, sleeping sickness, oedema, treat headaches, and venereal diseases (Teo, 1997). In animals, dysentery can be treated in calves with a decoction of roots or fruits (Hines and Eckman, 1993; Kew, 2006).

1.4.3 Firewood

In many African countries, firewood is the main source of energy for rural households, and is also an important source of cooking fuel in some towns. In rural areas many people rely solely on firewood for cooking, lighting and charcoal production. Wild plum wood is generally the preferred fuel for cooking main meals and heating water mainly because the trunk is rather small (FAO, 1986; 1993; Kew, 2006).

1.4.4 Aesthetic value

Wild plum produces attractive foliage and flowers hence adds value to the landscape and suitable for use as a hedge plant and to create boundaries (Feyssa *et al.*, 2012).

1.5 Postharvest losses

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 fruits and vegetables 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 increases the gastronomic diversity as they provide new flavours, aromas, colours, and attractive appearance for consumers (Ortiz-Hernández and Carrillo-Salazar, 2012). Fruit commercialization is however, affected by quality drop due to inappropriate postharvest handling, which largely determines the economic outcome of fruit and vegetable marketing (Toivonen and Hodges, 2011).

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 usually considered to be highly perishable (Kader, 2005; Emongor, 2010). These characteristics limit the storage life of fruits and vegetables, and cause significant deterioration following harvest.

Postharvest loss can occur at any point in the production and market chain. It is estimated that the magnitude of these losses due to inadequate postharvest handling and storage of fresh fruits and vegetables is relatively higher, but varies in accordance with the country and commodity (Kader, 2005). Postharvest losses vary greatly across commodity types, with production areas and the season of production. Losses of fresh fruits and vegetables in developed 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 being never consumed by

humans (Kader and Rolle, 2004). In a hungry and increasingly competitive world, reducing postharvest food losses is a major agricultural goal. So postharvest handling can play a major role in reducing losses. Postharvest losses in wild plum are not known. In order to reduce these losses, postharvest technology which delays senescence and maintain quality must be applied. These technology systems include temperature management, controlled atmosphere (CA) and modified atmosphere (MA) storages (Emongor, 2010).

1.5.1 Temperature

Temperature is the most important environmental factor that influences the deterioration of harvested horticultural commodities. Most perishable commodities last longest at a temperature near 0°C (Emongor, 2010; Kader, 2013). Temperature influences how other internal and external factors influence product quality (Kader, 2013). Temperature management is the most effective tool for maintaining quality and safety, and for extending the postharvest life of fresh horticultural commodities (Emongor, 2010; Kader, 2013). Harvesting cuts a vegetable and fruit off its source of water, but because life processes continue after harvest, they will lose water and turgor through transpiration. Field heat accelerates the rate of respiration and with it the rate of quality loss. Proper cooling protects quality and extends both 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, 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, reducing water loss and delay ripening. However, most fruits that originate from the tropical and subtropical regions are chilling sensitive when exposed to low temperatures that are above the freezing point (Gross *et al.*, 2002). Such produce are injured when stored below their critical temperature which generally ranges between 10 and 13°C for most varieties. Damage is often induced by a very brief exposure, but may not become apparent for several

days until produce is transferred to warmer temperature depending on the duration and severity of chilling. Due to sensitivity of tropical fruits to chilling injury (CI), transport of these fruits for most distant markets is generally only successful by air. Therefore, advances in the improvement of fruits to resist low temperature storage could be enhanced to prolong their shelf-life even their trade over long distance. However, it has been suggested that the temperature range close to 13°C was adequate to avoid the onset of CI in most tropical fruits for at least three weeks (Hatton, 1990).

1.6 Justification

The botanic description, biology and ecology of the wild plum have been observed and documented (Orwa *et al.*2009, Feyssa *et al.* 2012, Sarmento *et al.* 2015). Research has been done on the uses and management of the wild plum fruit (Feyssa *et al.* 2012 and Orwa *et al.* 2009). The bioactive compounds and antioxidant activity of the wild plum have been investigated (Sarmento *et al.* 2015). No findings are available on the storage of wild plum fruits despite its essentiality in the industry. No appropriate technology has been developed for storage of wild plum (Feyssa *et al.*, 2009). In Botswana and worldwide, wild plum is among the least exploited edible wild plant species, but highly consumed by local inhabitants in most settlements. In all traditional settings in Botswana, wild plum is picked and freshly consumed. Picked fruits usually develop a bitter taste if not eaten immediately. Due to high perishability and unknown storage temperature requirement currently, marketing of wild plum is uncommon or virtually impossible. Temperature is an important postharvest treatment factor in fruit storage, which determines durability and availability of fruit in the market after harvest, however, the storage temperature of wild plum is currently not known. This research seeks to investigate the optimum storage temperature for wild plum, a step to proper utilisation, cultivation and preservation of this natural plant species.

1.7 Objective

To determine the effects of storage temperature on postharvest quality of wild plum fruit.

1.8 Hypothesis

H_a: Storage temperature has an influence on postharvest quality of wild plum fruit.

H_0: Storage temperature has no influence on postharvest quality of wild plum fruit.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Postharvest losses

Due to the fact that there is hardly any literature available on the storage temperature of wild plum fruit or its relatives in the Olacaceae family, this review is general on effects of temperature on storage of fruits in general.

The quality of 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 (Molnar *et al*, 1995). Past studies have shown that the most important criteria for selecting a fruit are the freshness, taste and appearance (Wandel and Bugget, 1997). Commodities should be in excellent condition and quality if maximum storage life is desired. The commodities should be free from skin breaks, bruises, decay and other deterioration. Bruises and other mechanical damage not only change the appearance of the produce, but are good avenues of entrance for decay micro-organisms. 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). Horticultural produce for storage should be harvested at optimum physiological maturity, because storage life may be reduced if they are immature or over mature (Emongor, 2010).

2.2 Storage temperature of horticultural produce

The function of a fruit or vegetable storage is to provide an environment that will permit vegetable produce to be stored as long as possible without deterioration of quality. Fruit or vegetable quality is measured by flavor, texture, moisture content and other factors associated with edibility (Emongor, 2010). A desirable environment can be obtained by controlling temperature and composition of the atmosphere.

Temperature management plays a critical role in ensuring that high quality harvested horticultural produce are delivered. Avoiding high temperature and reducing temperature to the optimum reduces the rate of physiological and biochemical changes that occur in horticultural produce after harvest, minimises water loss from fruit and slows the growth of decay causing microorganism like anthracnose (Brencht, 2009). However, there is a limit to the low temperature that tropical and sub-tropical fruits can tolerate due to their susceptibility to chilling injury, a disorder that results in flavor loss, surface blemishes and inhibition of ripening (Emongor and Tautsagae, 2016; Emongor, 2015; Wang, 1982; Raison and Orr, 1990; Saltveit and Morris, 1990).

Harvesting stimulates metabolic changes associated with ripening and senescence. Depending on temperature there may be increased respiration rate, accelerated softening, water loss, and changes in chemical constituents such as pectins, starch, sugars and acids (Hardenburg *et al.*, 1986; Emongor, 2010). The quality and storage life of horticultural produce may be seriously affected within a few hours after harvest if the crop has not been precooled promptly to control deterioration (Emongor, 2010).

2.3 Effects of temperature on storage of fruits

2.3.1 Chilling injury

Low temperature storage is a major postharvest technology used widely to extend the postharvest life of fresh horticultural produce (Hardenburg *et al.*, 1986; Emongor, 2010; Mworia *et al.*, 2012). Low temperature slows down most cell metabolic activities and delays fruit ripening and plant senescence (McGlasson *et al.*, 1979; Hardenburg *et al.*, 1986; Emongor, 2010). Specific fruit species and cultivars require specific storage conditions and temperature for optimal quality. Their temperature requirement is influenced mostly by their origin and growing conditions (Jobling, 2000; Emongor, 2010). Fruits from the tropics would not require temperature as low as 0°C, but higher (above 12°C), while fruits of temperate origin can tolerate temperature as low as 0°C because they are adapted to growing in cool environments. Most fruits that originated from the tropical or subtropical regions are chilling sensitive (Gross *et al.*, 2002; Emongor, 2015; Emongor and Tautsagae, 2016). These crops are injured after a period of exposure to chilling temperature below 10-15°C, but above their freezing points. Some horticultural crops of temperate origin are also susceptible to low temperature injury, but have lower critical threshold temperature generally less than 5-10°C. Keeping tropical or subtropical fruits under low temperature (critical minimum temperature) causes CI. Wild plum fruit being tropical in origin may suffer from CI (Emongor, 2015, Emongor and Tautsagae, 2016; Pholoma, 2016). The severity of CI on chilling depends on the cultivar, duration and temperature of exposure, degree of fruit maturity, environmental conditions during and after low temperature storage (Lim *et al.*, 2009; Emongor, 2015; Emongor and Tautsagae, 2016; Pholoma, 2016).

The CI is known to significantly change the microstructure of the tissue which in severe cases may lead to tissue breakdown due to failure to carry normal metabolic processes (Han *et al.*,

2006). 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 cascade to development of CI symptoms such as skin surface pitting, sunken or surface lesions, uneven ripening, pulp discoloration, grayish-scald discoloration of the skin, water-soaking of the tissue, off-flavor, susceptibility to fungal decay, reduced aroma, carotenoids (Saltveit and Morris, 1990; Ding *et al.*, 2007; Emongor, 2015; Pholoma, 2016). These CI symptoms become more serious when fruits are transferred to room temperature (Saltveit and Morris, 1990; Ding *et al.*, 2007; Emongor. 2015). 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). 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. It is generally accepted that the storage of tropical fruits below 10-13˚C render the fruit susceptible to develop CI although the time required to show visible symptoms varies between cultivars (Mukherjee, 1958; Akamine, 1963; Lutz and Hardenburg, 1968; Couey, 1986; Emongor, 2015). Fruits held for a longer period at low temperature will not ripen satisfactorily. Chilling susceptibility varies with fruit cultivar, origin of the fruit crop, genetic makeup of the cultivar and environmental factors such as temperature, humidity, light and atmospheric composition and tolerance increase during ripening (Medlicott *et al.*, 1990).

2.3.2 Control of chilling injury symptoms

The CI in tropical and subtropical fruits can be alleviated successfully by temperature preconditioning, intermittent warming, heat treatment, CA storage and plant regulators treatments (Wang, 1993). The reduction of CI can be achieved by either retarding the symptoms developing in sensitive crops or improving chilling stress tolerance of the crop produce (Emongor, 2015; Pholoma; 2016). Polyamines being positively charged aliphatic amines are reported to act as free radical scavengers and membrane stabilisers (Nair and Singh, 2004). Heat treatment induces heat shock proteins (HSPs) which protect the produce from both heat and CI, suppresses oxidative activity and maintain membrane stability (Timperio *et al.*, 2008). Low temperature and oxidative stress cause HSPs biosynthesis and accumulation (Timperio *et al.*, 2008).

Methyl salicylate and jasmonate among the growth regulators methods of alleviation of CI, stimulates the synthesis of some stress proteins such as HSPs, pathogenesis-related (PR) proteins which activate lipoxygenase gene expression and reduce synthesis of polyamines (Wang, 2010). Packaging has also been reported to delay CI in bananas, lemons, tomatoes and apricot (Miller *et al.*, 1990). Film packaging modify oxygen and carbon dioxide concentration surrounding the produce thereby inhibiting the epidermal and underlying cells collapse hence preventing pitting formation. The length of shelf-life of mango fruits varies markedly with cultivar, calcium sprays and exposure to ethylene dip in 4-6% calcium chloride can significantly increase shelf-life of some cultivars, with the response varying with season, field management practices and soil type (Nakasone and Paull, 1998).

2.3.3 Fruit quality

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 (Emongor, 2010; Khorshidi *et al.*, 2010). Many studies on the effect of storage temperature on quality and storage life of fruits have been done which showed that temperature plays an important role on quality of fruits after harvest (Khorshidi *et al.*, 2010). Temperature and storage duration have been reported to affect the vitamin C content of fruits and vegetables (Lee and Kader, 2000; Kadzere *et al.*, 2006; Emongor; 2015; Emongor and Tautsagae, 2016). Emongor (2015) and Emongor and Tautsagae (2016) reported that mango and marula fruits stored at 0°C (twenty eight days) had significantly ($P < 0.05$) higher vitamin C content than fruits stored at 25°C (five days). Storage of fruits and vegetables at temperatures below 5°C has been reported elsewhere in literature to decrease loss of vitamin C (Hardenburg *et al.*, 1986; Kays and Paull, 2004). Higher temperatures can completely destroy vitamin C in fruits and vegetables (Igwemmar *et al.*, 2013; Emongor, 2015; Emongor and Tautsagae, 2016). Loss of vitamin content especially vitamin C, pro-vitamin A, thiamine (vitamin B1) and nicotinic acid is common in horticultural produce poorly handled. Pholoma (2016) reported that the interaction of storage temperature and water temperature, and storage temperature and hot water treatment duration significantly influenced the vitamin C content of mango fruits. As the storage and hot water temperature increased the mango fruit vitamin C content significantly decreased immediately after storage and seven days after storage on fruit held at room temperature (25 ± 2°C) (Pholoma, 2016). Mango fruits dipped in water at 25°C and stored at 4°C had vitamin C content of 35.86 mg/100 g, while fruit dipped in hot water at 55 °C and stored at 25°C had vitamin C content of 21.56 mg/100 g, immediately after storage, accounting for 39.9% reduction of vitamin C content (Pholoma, 2016). Similarly, seven days after storage

on fruit held at room temperature, but dipped in hot water at 55°C and stored at 25°C had a lower vitamin C content of 40.58% than fruit dipped in water at 25°C and stored at 4°C (Pholoma, 2016). 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) reported that loss of vitamin C in fresh commodities is 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 ten minutes. Mgaya-Kilima (2014) reported that the vitamin C content of 'Roselle' mango, papaya, guava juices at processing was 54.4, 53, 74.4 mg/100 g fresh weight, respectively, but reduced to 24.5, 23.2 31.3 mg/100 g fresh weight at 28°C. But after six months of storage at 4°C, the vitamin C content of mango, papaya and guava juices was 31.5, 31.5 and 42.6 mg/100 g fresh weight. The rate of loss of vitamin C is higher with higher storage temperature, an effect associated with loss of acidity (Kays and Paull, 2004; Pholoma, 2016). Pholoma (2016) reported that decrease in vitamin C content with increase in storage and water temperature was due to decrease in juice pH and titratable acidity. Loss in vitamin C content of fruits and vegetables is pH and temperature dependent, having rapid loss of vitamin C at higher fruit pH and temperature (Hardenburg *et al.* 1986; Kays and Paull., 2004; Pholoma, 2016).

Temperature has a direct effect on the physiological and biochemical processes of the fruit such as respiration, deterioration, water loss (transpiration) and ripening (McGlasson *et al.*, 1979; Hardenburg *et al.*, 1986; Emongor, 2010). All these metabolic processes influence fruit quality. If the cold storage is maintained along the market chain, ripening, transpiration, respiration and deterioration rates can be slowed down and the shelf-life extended and fruit quality maintained

(Hardenburg *et al.*, 1986; Emongor, 2010; Punitha *et al.*, 2010; Mworia *et al.*, 2012; Emongor; 2015). It has been established that for every 10°C increase in temperature, the rate of metabolic processes and biochemical reactions roughly double or even treble and vice versa in biological systems (Hardenburg *et al.*, 1986). For example an apple held at 10°C ripens and respires about three times as fast as one held at 0°C (Hardenburg *et al.*, 1986). 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 (Hardenburg *et al.*, 1986). It is important to note that there is a significant improvement in shelf-life by storing horticultural produce at 0°C compared to 3 or 5°C, provided they are not chilling sensitive (Emongor, 2010), for example, asparagus is chilling sensitive and so the shelf-life is actually reduced by storing it at 0°C as the optimum storage temperature is 2°C The storage life of products can be adversely affected by storing them at the wrong temperature. It is important that chilling sensitive products are stored at the correct temperature as CI will make them unsalable.

Temperature management is a key tool for preventing the development of postharvest rots. The growth rate of micro-organism (bacteria and fungi) that cause postharvest rots is controlled by temperature. These disease causing organisms grow faster at warmer temperatures, therefore, if storage temperatures are low, the rate of disease development can be considerably reduced and the storage life and quality of fresh products can be assured (Emongor, 2010; Punitha *et al.*, 2010). Storage temperatures can also influence which type of disease develops, for example in oranges, the fungi that cause the blue mould grows at low temperatures and the bacteria that causes the soft rot grows best in warmer conditions. Coates *et al.* (1994, 1995) and Jonhson and Sangchote (1994), reported that among tropical fruits, litchi is highly susceptible to postharvest decay. A wide range of fungal pathogens affect the litchi fruits, with many of them

also being found to infect other tropical fruits. The decay causing fungi isolated from litchi fruit after harvest included: *Alternaria sp., Aspergillus sp., Cladosporium sp., Colletotrichum gloeosporoides, Fusarium sp., Geotrichum candidum, Geotrichum ludwigii, Lasiodiplodia theobromae, Penicillium sp., Peronophythora litchii, Phomopsis sp.* and *Rhizopus sp.* (Coates *et al.* 2005; Prasad and Bilgrami 1973; Scott *et al.* 1982; Tandon and Tandon, 1975; Tsai and Hsieh 1998). *Botryodiplodia sp.* infects fruit in the field and through the cut stem end during harvest and handling (Jiang *et al.* 2003). *Peronophythora litchii* is a major pathogen of harvested litchi fruit in China (Qu *et al.* 2001). Bacteria have also been isolated from decaying litchi fruit (Roth, 1963). Postharvest approaches to pathogen control that have been investigated for litchi fruit include temperature management, application of fungicides and biological control agents, heat treatments, various packaging options and modified MA or CA storage (Jiang *et al.* 2003; Underhill *et al.* 1997). These approaches apply in storage of a wide range of fruits.

2.3.4 Fruit ripening

Fruit ripening is a highly coordinated, genetically programmed, and an irreversible process involving a series of physiological, biochemical, and organoleptic changes that lead to the development of a soft and edible ripe fruit with desirable quality attributes (Seymour *et al.*, 1993). A wide spectrum of biochemical changes such as increased respiration, chlorophyll degradation, biosynthesis of carotenoids, anthocyanins, essential oils, and flavor and aroma volatiles, increased activity of cell wall hydrolases, and a transient increase in ethylene production are some of the major changes involved during fruit ripening (Seymour *et al.*, 1993; Brady, 1987; Prasanna *et al.*, 2007). Based on ripening regulation, fruits are largely divided into two groups: climacteric fruit that ripen with ethylene and non-climacteric fruit that ripen independently of ethylene (Mworia *et al.*, 2012). In climacteric fruit, ethylene is critical for the induction of fruit ripening since most ripening-related events are regulated or accelerated by

ethylene (Saltveit, 1999). In tomatoes and melons, suppression of ethylene biosynthesis or the transcription factor for ethylene signaling by transgenic engineering resulted in the inhibition or significant delay of most ripening related events (Hamilton *et al.*, 1990; Murray *et al.*, 1993; Ayub *et al.*, 1996; Gius *et al.*, 1997; Yokotani *et al.*, 2009). Fruit ripening is accepted as a protracted form of senescence due to the breakdown of the cellular integrity (Brady, 1987; Barry and Giovannoni, 2007; Kays and Paull, 2004).

Temperature influences several biochemical pathways and enzyme activity related to ripening (Medlicott and Jeger, 1987; O'Hare, 1995; Emongor, 2015), and each of these have different temperature optima. Optimum ripening temperatures for many fruits have been reported in the range of 18-25°C (Singh and Mathur, 1952; Hatton *et al.*, 1965; Medlicott *et al.*, 1986; Hardenburg *et al.*, 1986; Emongor, 2010; 2015). Storage temperature influences fruit ripening (Esguerra *et al.*, 1992; Ahmad *et al.*, 2001; Seymor *et al.*, 2013). An increase in storage temperatures between 14-30°C enhanced the rate of fruit ripening (Smith, 1989; Ahmad *et al.*, 2001; Wu, 2010). Mworia *et al.* (2012) reported that low temperature (4°C) storage of kiwifruit modulated fruit ripening. The respiration rate and ethylene production have been reported to increase with increase in storage temperature (Emongor, 2010; Emongor, 2015). Healthy kiwifruit stored at 4°C for twenty eight days did not produce any detectable ethylene (Mworia *et al.*, 2012). High temperature also results in damage to ripening fruit (Smith and Thompson, 1987; Semple and Thompson, 1988). Temperatures less than 14°C can cause uneven ripening in chilling sensitive fruits (Stover and Simmonds, 1987; Emongor, 2010; Emongor, 2015; Emongor and Tautsagae, 2016; Pholoma, 2016).

Temperature influences the catalytic activity of enzymes and due to the integrity of the three dimensional structure of the enzyme it can be denatured at high temperatures (Trejo *et al.*, 2010). Fruits stored at low temperatures present rapture of cell structure, increase the solubility

of polyphenol oxidase and makes easier for its contact with phenolic substrates (Trejo *et al.*, 2010). In banana ripening, the golden yellow colour of ripe fruit is due to chlorophyll breakdown, which unmasks carotenoid pigments in the plastids (Yang *et al.*, 2009; Emongor, 2010). When ripening of bananas was done at temperatures above 24°C, banana fruit failed to develop a fully yellow peel as they retained high levels of chlorophylls in their peel (Blackbourn *et al.*, 1990; Li *et al.*, 2006).

Respiration is the central process that results in fruit ripening and deterioration of produce leading to loss of nutritional value, changes in texture and flavor and loss of weight due to continued breakdown of carbohydrates, associated with oxygen consumption and carbon dioxide, water and energy production (Emongor, 2010; 2015). Paull and Chen (1987) reported that the respiration rate of 'Chenzi' litchi fruit declined from 103 to 39 mg/kg/hour after eight days of storage at 5°C, whereas Nagar (1994) showed that the respiration rate of 'Calcutta' litchi fruit declined from 36.3 to 18.1 mg/kg/hour after six days of storage at 5°C compared to room temperature of 22-25°C. Kader (2000) reported that respiration rate ranges for litchi fruit are 9.5–15.1 mg/kg/hour at 5°C, 18.6–28.5 mg/kg/hour at 10°C and 47.2–74.4 mg/kg/hour at 20°C. Thus, storage at low temperatures markedly reduces fruit and other horticultural produce respiration rates (Kader, 2000). Kassim (2013) described respiration as the process predominantly responsible for the ripening of avocados. Starrett and Laties (1991), Jeong *et al.* (2002), Yahia (2002), Jeong *et al.* (2003) and Wu *et al.* (2011) described avocados as being climacteric, characterised by a surge in ethylene production and respiration at the start of the climacteric ripening. The shelf-life of fresh commodities is inversely related to respiration and ethylene rates (Perez *et al.*, 2004). An increase in the respiration rate hastens senescence contributing to poor fruit quality (Maftoonazad and Ramaswamy, 2008). Improvement in handling of avocados once harvested is essential in lowering the respiration rate by reducing

the temperature, increasing carbon dioxide and reducing oxygen concentrations within limits in the storage atmosphere (Kader, 2000; Emongor, 2010).

Tomato fruit quality is determined by a number of biochemical and developmental processes that result in changes in the quality attributes (Žnidarčič *et al.*, 2010). Mordy and Atta (1992) and Giovannoni (2007) revealed that an increase in temperature from 5 to 20°C increased respiration rate, sugar accumulation and colour (lycopene) development of tomato during ripening. Increasing storage temperature from 15 to 30°C significantly increased carbon dioxide production of tomato fruits resulting in internal quality reduction (Mordy and Atta, 1992). Tomato fruits lost more weight when their metabolic rate increased due to loss of water when storage temperature was increased from 12 to 20°C (Ashby, 2000; Mutari, 2011; Luengwilai and Beckles, 2009). A decrease in carbohydrates which are the source of energy for the respiratory process leads to decrease in water soluble solids. For example the water soluble solids and total sugars of the gabiroba fruits were affected by temperature and time of storage (Pablo da Silva, 2013). Emongor and Tautsagae (2016) reported that increasing storage temperature from 0 to 25°C, increased marula fruit soluble solids content (SSC), carotenoid and anthocyanin (colour) biosynthesis and chlorophyll degradation, but titratable acidity (TA) decreased. Decreased acidity (organic acids) and accumulation of sugars (SSC) in fruits during ripening results in an excellent sugar/acid blend for edibility as fresh fruit or for processing into various products (Kudachikar *et al.*, 2001; Srinivasa *et al.*, 2002; Emongor, 2010; Emongor, 2015; Emongor and Tautsagae, 2016; Pholoma, 2016). Fruit flavor is reported to be influenced by the ratio of sugar to acid which are influenced by temperature (Paull, 1999; Workneh and Osthoff, 2010; Paull and Duarte, 2011). Grapefruit stored at 8°C displayed sugar and acid decline as compared to those stored at 12°C. It is also imperative that fruits be harvested at the right time because premature harvesting can lead to an undesirable taste

(Brown, 1972; Perez *et al.*, 2004 and Wu *et al.*, 2011) or lack of flavor (Gamble *et al.*, 2010; Osuna-Garcia *et al.*, 2010). Ahmad (2001) reported that bananas stored at higher temperatures showed greater SSC than those stored at lower temperatures. Those that were ripened at 14 and 20°C showed significant differences in their total soluble solids, with no significant differences between bananas ripened at 16, 18 and 20°C (Ahmad, 2001).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Fruit collection site

Wild plum fruits were collected in the outskirts of Molepolole village, in a settlement known as Scherelela in Botswana, where the fruit trees were found in clusters or scattered on the hills and rocky soils of the area. The area has an altitude of 1,112 m asl with an average rainfall of 484 mm per annum. The rainfall pattern is comprised of the driest period in the month of July which records 3 mm of rainfall and the wettest month of January with a precipitation of 87 mm on average. The average annual temperature is 19.5°C. The lowest temperature is recorded in July at an average of 12.2°C, while January records the warmest temperature of 24.7°C on average. Due to seasonality of wild plum fruits, the experiment was done from December 2016 to March 2017 when fruits were available in the forest.

3.2 Experimental site

The experiment was conducted in the Crop Science and Production Department in the Crop Physiology laboratory at The Botswana University of Agriculture and Natural Resources (BUAN). BUAN is located at Content Farm Sebele (Latitude 24°33'S and Longitude 25°54'E, altitude 994 m asl.

3.3 Experimental design

The experimental design was a completely randomized design (CRD). The treatment was storage temperature at 0, 5, 10 and 15 ± 1°C. Fresh wild plum fruits were picked randomly at mature stage from different wild plum trees clustered in one area exposed to similar environmental conditions. Fresh fruits were collected when still firm, deep orange/yellow, uniform and free from bruises and defects judged subjectively based on epidermal colour. Fruits were washed to remove soils and other external material. Three hundred and forty fruits

were harvested for the study and stored in various temperatures stated above. Four refrigerators were set at temperatures of 0, 5, 10, and 15 ± 1°C.

3.4 Dependent variables determined

The dependent variables determined were fruit development, fruit juice SSC, TA, pH, vitamin C content, CI (incidence and severity) and storage life of the fruit in cold storage and after storage at room temperature.

3.4.1 Fruit Development

Fruit development was determined by measuring fruit diameter and length (mm) of ten tagged fruits every week for ten weeks using a veneer calliper, starting one week after full bloom.

3.4.2 Titratable acidity

The fruit TA was determined according to AOAC (1996). The fruit and skin were cut and 100 g of sample weighed, then 100 ml of distilled water was added to the sample. The sample was homogenized and filtered through five layers of cheese cloth. Then 20 ml of filtrate (juice) was put in a 50-ml conical flask and two drops of 1% phenolphthalein indicator was added. The samples were titrated with 0.1 N NaOH to end point (pH 8.2) and this was done in triplicates. The results were expressed as total titratable acidity equivalents using the formula given below.

$$\text{Titratable acidity (g/100 ml juice)} = \frac{\text{(ml base x normality base x 1x 100 x 2)}}{\text{ml sample}}$$

3.4.3 Soluble solids content

The SSC was determined using a hand refractometer (Atago Model N1, American Optical, Buffalo, New York). Ten fruits per treatment were pinched using a scalpel. Three drops of juice was directly placed on the prism surface of the refractometer and then the average sugar content was determined in Brix but expressed as percentage SSC.

3.4.4. 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.

3.4.5 Vitamin C content

Vitamin C (ascorbic acid) content was determined using 2, 6-dichloroindophenol titrimetric method according to AOAC (1996). Ascorbic acid reduces oxidation-reduction indicator dye (2, 6-dichloroindophenol) to colourless solution. The fruit pulp was cut and mixed to form a composite sample. Then 100 g of the composite sample was weighed. Then 100 g of the composite sample was homogenized with 100 ml of metaphosphoric acid-acetic acid solution. The sample was filtered with five layers of cheese cloth. Then 20 ml of the filtrate was pipetted into a 100 ml conical flask and two drops of thymol blue (0.04%) indicator added. The sample was titrated with 2,6-dichloroindophenol solution to end point. Also three sample aliquots containing the standard ascorbic acid solution (20 ml) with metaphosphoric acid-acetic acid solution (for correction or blank) was titrated with 2,6-dichloroindophenol. Calculation of vitamin C content was done according to AOAC (1996) following the formula given below and expressed as mg/g.

$$\text{mg ascorbic acid/g} = (X - B) * \left(\frac{F}{E}\right) * \left(\frac{V}{Y}\right),$$

where X = average ml for sample titration, B = averege ml for blank titration, F = ml of standard ascorbic acid soulution, E = weight of sample ground, V = volume of initial sample solution and Y = volume of aliquot titrated.

3.4.6 Chilling injury

The CI was evaluated daily for incidence and severity on fruits in storage. Its incidence was assessed from all stored fruits per treatment. Fruit showing symptoms of CI were counted and

expressed as a percentage. The CI severity was evaluated on a predetermined scale of 0-3: 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 being severe injury (determined by more than 67% of the fruit showing injury depending on the peel damage).

Chilling injury index (CII) was calculated by the following equation;

CII (score 0-3) = $\sum_{I=0}^{N} (CI \times n) \div N$

Where CI = Chilling injury level; n = Number of fruit at the CI level; and N = Total number of fruit in the treatment.

3.4.7 Shelf-life

Shelf-life was determined by the number of days fruits maintained their fresh physiological status at different temperatures until they become unacceptable due to development of physiological disorders.

3.5 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 (Cochran and Cox, 1992).

CHAPTER 4

4.0 RESULTS

4.1 Fruit growth

The results of the study showed that wild plum fruit growth and development followed a simple sigmoid growth curve (Figure 1). Fruit longitudinal and cross sectional size increased with time (Figure 1). The highest fruit length (26.4mm) and fruit diameter (21.4mm) was observed nine weeks after full bloom (Figure 1). Thereafter both fruit length and diameter decreased.

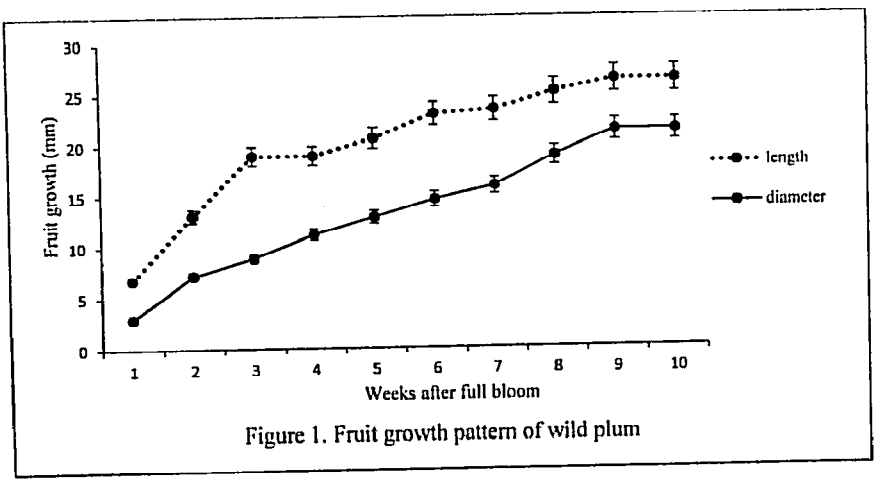

Figure 1. Fruit growth pattern of wild plum

4.2 Chilling injury incidence and severity

The results of the study showed that storage temperature significantly ($P < 0.05$) influenced CI incidence and severity of wild plum fruits (Figures 2, 3). As storage temperature decreased below 15°C, the incidence and severity of chilling significantly ($P < 0.05$) increased. The CI symptoms such as shrivelling, dark scald discoloration, pitting or sunken lesions and poor colour development were observed during the study on wild plum fruits stored at storage temperatures of 0, 5, and 10°C, although the incidence and severity varied with storage temperature (Figures 2, 3). Nevertheless, wild plum fruits stored at 0, 5 and 10°C developed

CI after twenty four hours of storage (Figure 2). After two days of storage at 0 and 5°C, the CI severity had an index greater than two, implying the fruit was unmarketable. Fruits stored at 10°C attained CI severity index higher than two after seven days of storage (Figure 3). Wild plum fruit stored at 15°C did not develop CI (Figures 2,3).

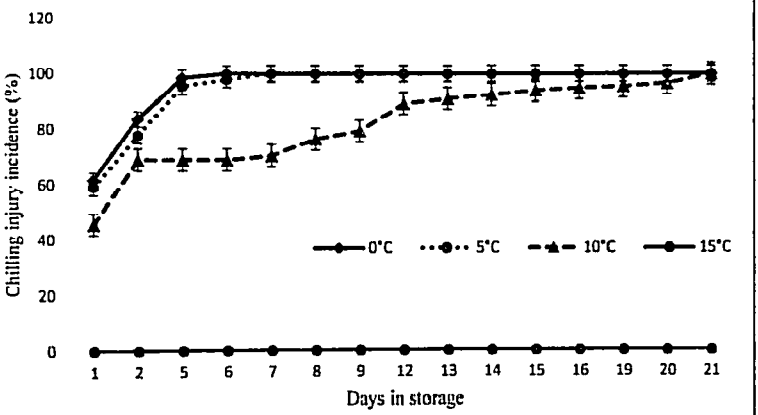

Figure 2. Effect of storage temperature on chilling injury incidence; bars are standard error

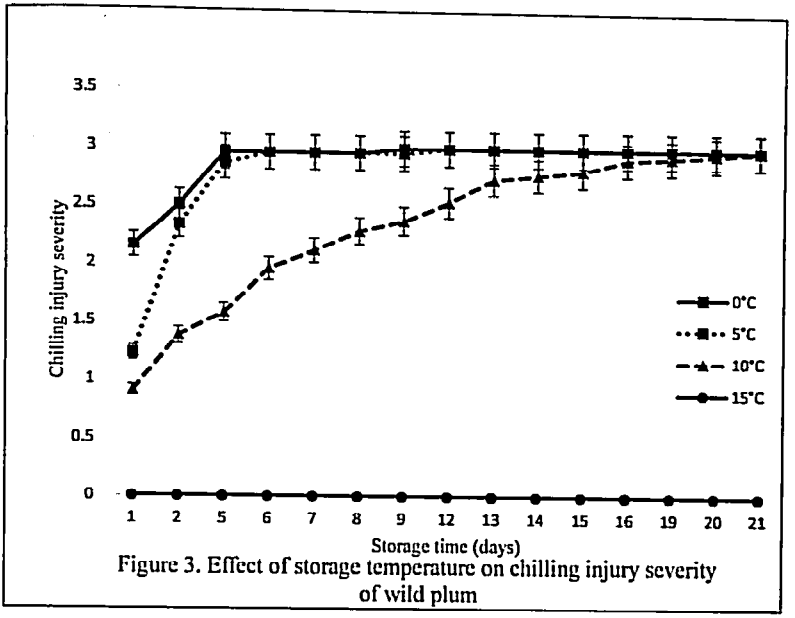

Figure 3. Effect of storage temperature on chilling injury severity of wild plum

4.3 Shelf-life

In this study, the results showed that storage temperature significantly ($P < 0.01$) influenced wild plum fruit shelf-life (Figure 4). Wild plum fruit stored at 15°C had significantly ($P < 0.01$) higher shelf-life of twenty eight days than fruit stored at 0, 5 or 10°C with a shelf-life of one and half, two and seven days, respectively (Figure 4). Wild plum fruits stored at 0 and 5°C suffered severe CI after two days of storage making them unmarketable (Figure 2, 3, 4).

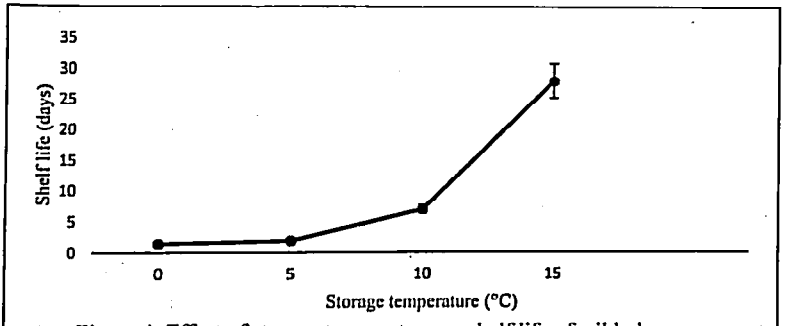

Figure 4. Effect of storage temperature on shelf life of wild plum; bars are standard error

4.4 Titratable acidity

Storage temperature significantly ($P < 0.05$) influenced wild plum fruit TA (Figure 5). As storage temperature increased from 0 to 15°C fruit TA significantly ($P < 0.05$) decreased (Figure 5). The response of wild plum fruit TA to increasing storage temperature was linear with a correlation coefficient of 0.99 ($r = 0.99$).

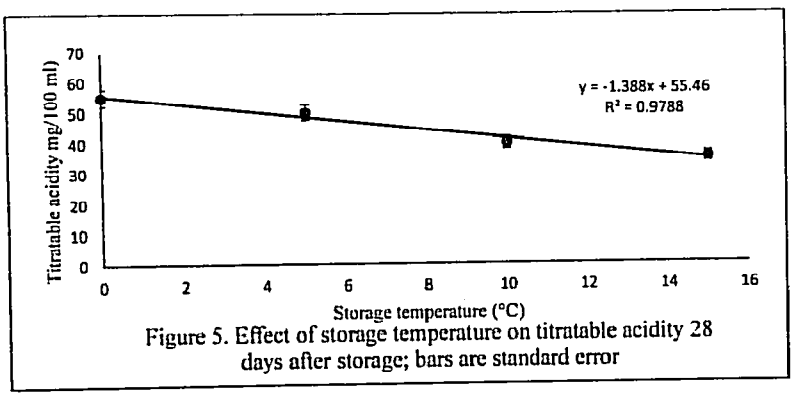

Figure 5. Effect of storage temperature on titratable acidity 28 days after storage; bars are standard error

4.5 Fruit juice pH

Storage temperature significantly ($P < 0.05$) influenced fruit juice pH (Figure 6). As storage temperature increased, fruit juice pH significantly ($P < 0.05$) increased (Figure 6). At harvest the fruit juice pH was 2.31, however, after twenty eight days of storage, fruit juice pH increased with increase in temperature (Figure 6).

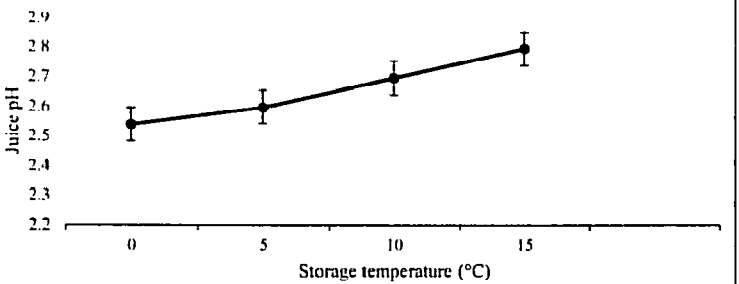

Figure 6. Effect of storage temperature on juice pH at the end of storage; bars are standard error

4.6 Soluble solids content

The result of the study showed that storage temperature significantly ($P < 0.05$) influenced fruit SSC after twenty eight days of storage (Figure 7). The wild plum fruit SSC significantly ($P < 0.05$) increased with increase in storage temperature (Figure 7). Fruit stored at 0, 5, 10 and 15°C had fruit SSC of 20.2, 23.6, 25.7 and 26.8%, respectively, after twenty eight days of storage (Figure 7).

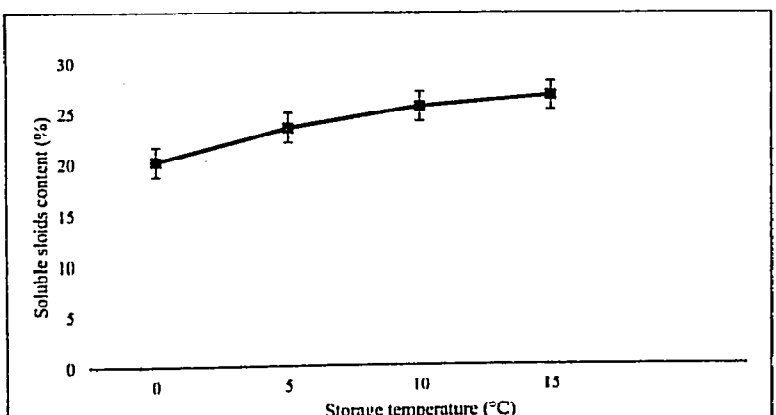

Figure 7. Effect of storage temperature on fruit soluble solids content 28 days after storage; bars are standard error

4.7 Vitamin C

Storage temperature had a significant ($P < 0.01$) influence on the vitamin C content of wild plum after twenty eight days of storage (Figure 8). As storage temperature increased from 0 to 15°C, fruit vitamin C content significantly ($P < 0.05$) decreased (Figure 8). Vitamin C content at harvest was 76 mg/100 g, during storage at any temperature after twenty eight days, fruit vitamin C content decreased (Figure 8). Fruit stored at 0°C significantly retained more Vitamin C (36 mg/100 g) content than fruits stored at 5, 10 or 15°C (Figure 8).

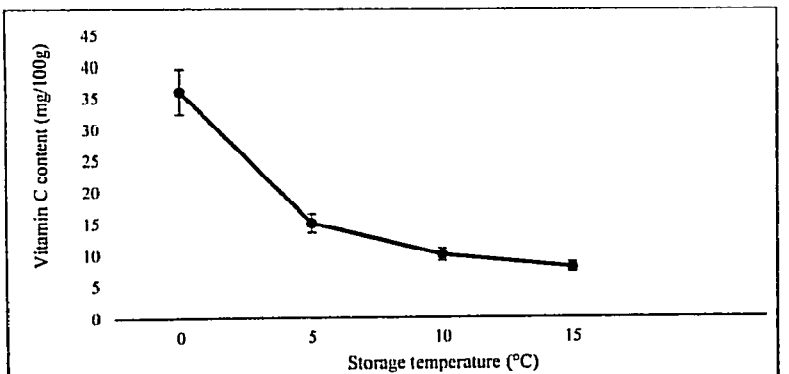

Figure 8. Effect of storage temperature on fruit vitamin C content after 28 days of storage; bars are standard error

4.8 Weight loss

Storage temperature significantly ($P < 0.05$) influenced wild plum fruit weight loss in storage (Figure 9). As storage temperature increased, fruit weight loss significantly ($P < 0.05$) increased. Fruits stored at 0°C had significantly ($P < 0.05$) lower fruit weight loss than fruits stored at 5, 10 or 15°C (Figure 9). The highest fruit weight loss was 26.8% in fruits stored at 15°C, while the lowest fruit weight loss was 1% in fruits stored at 0°C (Figure 9).

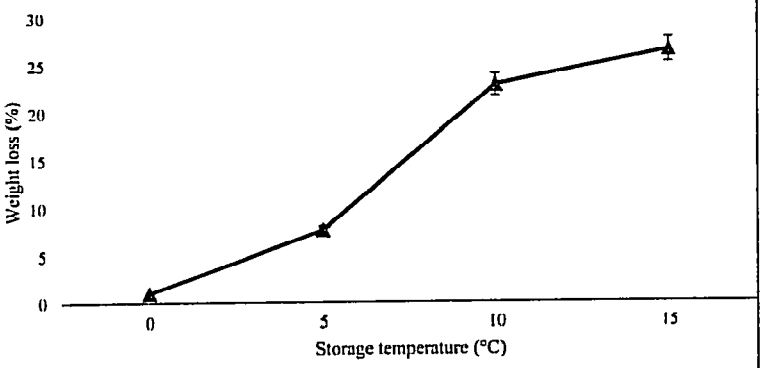

Figure 9. Effect of storage temperature on weight loss of wild plum fruits 28 days after storage; bars are standard error

CHAPTER 5

5.0 DISCUSSION

5.1 Fruit growth and development

The results of the current study showed that wild plum fruit displayed a simple sigmoid growth curve. Fruits can display simple, double or triple sigmoid growth curves depending on the fruit species (Faust, 1989; Emongor, 1995; Jackson *et al.*, 2011; Zadravec *et al.*, 2014; Emongor and Tautsagae, 2016). The growth curve of pome fruits, stone fruits and Chinese goose berry are simple, double and triple sigmoid, respectively (Faust, 1989; Emongor, 1995; Jackson *et al.*, 2011; Zadravec *et al.*, 2014; Emongor and Tautsagae, 2016). The increase in fruit size was attributed to cell division in the first four weeks after full bloom and then followed by cell elongation and expansion (McGlasson and Adato, 1977; Harker *et al.*, 1987; Emongor, 1995; Jackson *et al.*, 2011; Seymour *et al.*, 2013). Fruit can increase in mass or volume by hundred-fold or more from fertilization to maturity and such changes follow simple or double-sigmoid growth curve depending on the fruit species (Harker *et al.*, 1987; Emongor, 2010; Jackson *et al.*, 2011; Zadravec *et al.*, 2014). Air space formation occurs to a different degree and at different times in the various fruits (Faust, 1989; Jackson *et al.*, 2011). The size of fruit growth greatly depends on the cultivar (Faust, 1989; Jackson *et al.*, 2011; Seymour *et al.*, 2013). Early maturing cultivars with a short period of fruit growth are generally smaller than fruit of later maturing cultivars of the same species that has a much long period of growth (Faust, 1989; Jackson *et al.*, 2011). The fruit size of wild plum are relatively small implying they have a short fruit growth period which is genetically pre-determined. Wild plum fruit trees flowered the first week of November, but reached physiological maturity and ripening stage eight and nine weeks after full bloom, respectively.

5.2 Chilling injury incidence and severity

The results of this study showed that storage temperature significantly influenced the wild plum CI incidence development and severity. As storage temperature decreased below 15°C, the wild plum CI incidence and severity significantly increased. The following CI symptoms were observed: shrivelling, water soaked areas, dark scald discoloration, browning, uneven ripening, pitting or sunken lesions and poor colour development in wild plum fruits stored at 0, 5 and 10°C though the incidence and severity varied with the storage temperature. The CI incidence occurred twenty four hours after storage at 0, 5 and 10°C, though the severity of CI was high in fruits stored at 0 and 5°C than fruits stored at 10°C. Wild plum fruits stored at 0 and 5°C reached 100% CI incidence after five and seven days of storage, respectively. However, wild plum fruits stored at 0 and 5°C attained a chilling severity index higher than two after two days of storage, making the fruit unmarketable. Wild plum fruits stored at 10°C reached 69% CI incidence after two days of storage, but did not attain CI severity index higher than two until seven days after storage making the fruit unmarketable. Fruits stored at 15°C did not develop CI. Similar results have been reported in literature concerning tropical and subtropical fruits (Wardlaw and Leonard, 1936; Abou-Aziz *et al.*, 1976; Chaplin *et al.*, 1991; Wang, 1993; Gross *et al.*, 2002; Tasneem, 2004; Emongor, 2015; Emongor and Tautsagae, 2016; Pholoma, 2016). The CI is a storage disorder that occurs in fruits that are tropical and subtropical in origin (Wang, 1993; Gross *et al.*, 2002; Lim *et al.*, 2009; Emongor, 2015) when stored at temperatures below the critical threshold but non-freezing temperature (Hardenburg *et al.*, 1986; Mercer and Smittle, 1992; Sharom *et al.*, 1994). The problem limits the use of low storage temperature to manage postharvest ripening and senescence of fruits of tropical and subtropical origin, because the temperatures that are low enough to delay ripening, decay and senescence may also be damaging to the fruit (Emongor and Tautsagae, 2016; Pholoma, 2016; Emongor, 2015). The CI causes economic losses during storage and transport of tropical and subtropical fruits

(Emongor, 2015). Several events have been proposed as likely candidates for the primary cause of CI, including a phase transition in membrane lipids, an alteration in the substrate specificity of a regulatory enzyme, a change in the cytoskeletal structure, or an increase in cytosolic calcium (Wang, 1982; Raison and Orr, 1990; Han *et al.*, 2006; Ding *et al.*, 2007) which are genetically controlled (Craig *et al.*, 1994; Lee, 1995; Sun *et al.*, 2002; Aghdam *et al.*, 2012). Factors that have been reported to influence the susceptibility of tropical and subtropical horticultural produce to CI include cultivar, growing conditions, maturity at harvest and postharvest handling techniques, and duration of exposure to the chilling temperature (Wang, 1982; Snowdown, 1990; Saltveit and Morris, 1990; Raison and Orr, 1990; Brecht and Cecilia, 2012; Emongor; 2015).

5.3 Shelf-life

Wild plum fruits like other tropical and subtropical fruits, have a short shelf-life when held at ambient temperatures and are very sensitive to CI when stored at low temperatures. Storage is essential for extending consumption period of fruits (Wang, 1993; Lizada, 1991; Emongor, 2015). The results of the current study showed that wild plum fruits stored at 15°C had a shelf-life of twenty eight days, while fruit stored at 0, 5 or 10°C had a shelf-life of one and half, two and seven days, respectively. Wild plum fruit stored at 0 and 5°C suffered severe CI after two days of storage making them unmarketable. Wild plum fruits held at room temperature (25 ± 3°C) had a shelf-life of two days, indicating that wild plum fruits are very perishable, have a short postharvest life and suffer from CI when stored at temperatures below 15°C. There is no literature reported on the storage of wild plum fruits. Temperature is the most important environmental factor that influences the deterioration of harvested horticultural commodities and most perishable commodities last longest at temperature near 0°C if not chilling sensitive (Emongor, 2010; Kader, 2013). Temperature management is the most effective tool for

maintaining quality and safety, and for extending the postharvest life of fresh horticultural commodities (Wills *et al.*, 2007; Kader, 2013). Field heat can accelerate the rate of respiration and with it the rate of quality loss. 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, 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. However, most fruits that have originated from the tropical and subtropical regions are chilling sensitive when exposed to low temperatures that are above the freezing point (Gross *et al.*, 2002; Emongor, 2015; Emongor and Tautsagae, 2016; Pholoma, 2016). Such crops including wild plum when stored below its critical temperature generally 10-13°C for most varieties are injured. Damage often is induced by a very brief exposure but may not become apparent for several days or until transfer to warmer temperature depending on the duration and severity of chilling (Wang, 1993; Gross *et al.*, 2002; Kader, 2013; Emongor, 2015). The results of the current study indicated that in order to extend the postharvest life of wild plum fruits for about twenty eight days, the fruits be stored at 15°C to avoid CI.

5.4 Titratable acidity and juice pH

Acidity of fruit, measured by titratable acidity (Lobit *et al.*, 2002) is associated with both sweetness and sourness of fruit. 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.*, 2000). The flavor 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). In general, the concentration of acids decline during

ripening, but the total number of acids increase (Paliyath and Murr, 2008; Emongor, 2010; Seymour *et al.*, 2013; Emongor, 2015).

In the current study, as storage temperature increased from 0 to 15°C fruit TA and juice pH significantly decreased (55 to 35.2 mg/100 ml) and increased (2.54 to 2.8), respectively. The response of wild plum fruit TA and juice pH to increasing storage temperature was linear with a correlation coefficient of 0.99 (r = 0.99) with respect to TA. The decrease and increase in wild plum fruit TA and juice pH, respectively, with increasing storage temperature was attributed to increased level of fruit ripening. Fruit ripening involves a series of 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). The TA in fruits decreases with ripening due to their utilization as respiratory substrates especially in the Krebs (TCA) cycle (Wills *et al.*, 2003; Kays and Paull, 2004; Prasanna *et al.*, 2007; Emongor, 2010). Emongor (2015) reported a decrease in mango fruit TA with increase in storage temperature irrespective of cultivar. Similar results have been obtained in mangoes (Jacobi *et al.*, 2000; Srinivasa *et al.*, 2002; Yousef *et al.*, 2012; Pholoma, 2016), marula (Emongor and Tautsagae, 2016), 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). Pholoma (2016) reported that TA 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. Kosiyachinda *et al.* (1984) explained that, the reduction in acidity during ripening plays a great role in the acid-to-sugar balance and consequently in influencing the taste and flavor of the fruits. High temperature in storage is reported to enhance

starch and polysaccharides hydrolysis into sugars and decreases 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). Silva *et al.* (2013) reported that during storage of gabiroba fruit, there was an increase in the juice pH and decrease in TA as storage temperature increased.

Sarmento *et al.* (2015) reported that the TA of wild plum ranged between 75.4 (immature fruit) to 65.3 (mature fruit) mg/100 g, while the juice pH ranged between 3.03 (immature fruit) to 2.96 (mature fruit). While Silva *et al.* (2008) in Brazil reported that the juice pH of ripe wild plum fruit was 2.6. In the current study, at harvest, the wild plum fruit TA and pH was 67 mg/ 100 ml juice and 2.31, respectively, but after twenty eight days of storage the fruit juice pH ranged between 2.54 and 2.8. The results of the current study, confirm the results of Silva *et al.* (2008) and Sarmento *et al.* (2015). The results of the current study and those of Silva *et al.* (2008) and Sarmento *et al.* (2015) showed that wild plum juice is acidic, which is a desirable characteristic for the processing industry as acidity contributes to an enhanced flavor and promotes a high dilution factor in the formulation of juices leading to a greater yield while low acidity reduces any acidification during processing (Chakraverty *et al.*, 2003).

5.5 Soluble solids content

Fruit ripening is a highly coordinated, genetically programmed and an irreversible occurrence involving a series of 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; Emongor, 2010). Starch degradation is another biochemical process linked to fruit ripening and contributes to fruit soluble solids content (Luengwilai and Beckles, 2009). The SSC indicate 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; Tasnim *et al.*,

2010). As storage temperature increased, wild plum fruit SSC significantly increased. After twenty eight days of storage wild plum fruit stored at 0, 5, 10 and 15°C had SSC of 20.2, 23.6, 25.7 and 26.8%, respectively, implying fruit ripening was occurring at the high temperatures of storage. The increase in fruit SSC during storage and with increase in storage temperature was attributed to fruit ripening which promotes the conversion of starch to soluble sugars (Brady, 1987; MacRae *et al.*, 1989; Luengwilai and Beckles, 2009; Emongor, 2010; 2015). The increase in fruit SSC during storage and with increase in storage temperature is reported in literature (Brady, 1987; Tasneem, 2004; Paliyath and Murr, 2008; Yousef *et al.*, 2012; Emongor, 2015; Emongor and Tautsagae, 2016; Pholoma, 2016). Pholoma (2016) reported that as mango fruit storage temperature increased from 4 to 25°C fruit SSC significantly increased. While Emongor (2015) reported that as storage temperature increased from 5 to 12°C mango fruit SSC of four mango varieties Tommy Atkins, Haden, Kent and Keitt) significantly increased. Yousef *et al.* (2012) reported that mango fruit SSC significantly increased from 7.3 to 15.6% as mango storage temperature increased from 8 to 13°C. Similar results have been reported in other fruits such as marula (Emongor and Tautsagae, 2016), apples and pears (Hardenburg *et al.*, 1986), bananas (Marin *et al.*, 1996; Kays and Paull, 2004; Mohapatra *et al.*, 2016), kiwifruit (MacRae *et al.*, 1989) and tomatoes (Aivalakis and Katinakis, 2008; Žnidarčič, *et al.*, 2010). The increase in fruit SSC induced by storage temperature in most fruits is attributed to glucogenesis and hydrolysis of polysachharides especially starch (Brady, 1987; Tucker and Grierson, 1987; Lizada, 1993; Kays and Paull, 2004; Aivalakis and Katinakis, 2008; Paliyath and Murr, 2008). The relatively low SSC on wild plum fruit stored at low temperatures of 0, 5 or 10°C was attributed to CI. The CI negatively affects fruit ripening and causes uneven ripening (Wang, 1982; Tasneem, 2004; Emongor, 2015; Emongor and Tautsagae, 2016). Exposure of chilling sensitive fruits such as banana to low temperatures for several days has been reported to cause low accumulation of SSC during ripening (Agopian *et*

al., 2011). The lowest safe temperature for long term exposure of mature wild plum fruits is 15°C. Lowering the temperature further could completely inhibit ripening, but results in shortened storage life due to CI.

Also in the current study, at harvest wild plum fruit had SSC of 24.2-28.7%. Similar results have been reported by Sarmento *et al.* (2015) who found that wild fruit had SSC of 26.9 ± 1.6% (immature fruit) and 26.2 ± 1.8% (mature fruit).

5.6 Vitamin C content

Vitamin C (ascorbic acid) occurs naturally in many fruits and vegetables, but is easily destroyed by cooking or canning food and by exposure to air and light (Emongor, 2010; Njoku *et al.*, 2011). Vitamin C includes both ascorbic acid ($C_6H_8O_6$) and its oxidation product dehydroascorbic acid ($C_6H_6O_6$) both being antiscorbutic. The two forms of vitamin C are largely interchangeable via the unstable monodehydroascorbic acid being catalyzed by any of the oxidases such as ascorbic acid oxidase, cytochrome oxidase, o-diphenol oxidase, p-diphenol oxidase and peroxidase (Kays and Paull, 2004; Emongor, 2010). Vitamin C acts as an antioxidant, a nutrient that chemically binds and neutralizes the tissue damaging effect of free radicals (Rickman *et al.*, 2007). As a result, vitamin C is vital for the growth and maintenance of healthy bones, teeth, gums, ligaments, blood vessels and increases the body's resistance to infection (Rickman *et al.*, 2007). Due to 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). Factors that affect the vitamin C content of fruits include production factors (fertilizer application, irrigation and leaf-to-fruit ratio), climatic conditions, maturity stage of fruits, species, variety), handling and storage (Batchelder, 1936; Naggy, 1980; Padayatty *et al.*, 2003; Ajibola *et al.*, 2009).

In the current study storage temperature significantly influenced the vitamin C content of wild plum after twenty eight days of storage. As storage temperature increased from 0 to 15°C, fruit vitamin C content significantly ($P < 0.05$) decreased from 36 to 8 mg/100 g. Vitamin C content at harvest was 76 mg/100 g, however after storage at 0, 5, 10 and 15°C for twenty eight days, wild plum fruit vitamin C content decreased. Loss of vitamin C content in fruits and vegetables during storage at various temperatures is reported in literature (Hardenburg *et al.*, 1986; Kays and Paull, 2004; Emongor, 2010; Emongor and Tautsagae, 2016; Pholoma, 2016). However, storage of fruits and vegetables at temperatures below 5°C is reported to decrease the loss of vitamin C (Hardenburg *et al.*, 1986; Kays and Paull, 2004). 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 TA and juice pH 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. Loss of vitamin C occurs by irreversible conversion of dehydroascorbic acid to 2,3-dioxo-L-gulonic acid, which is then further metabolized. The reaction is pH dependent, being slow in acid pH, rapid at neutral pH and extremely rapid at alkaline pH (Lee and Kader, 2000; Kays and Paull, 2004; Paliyath and Murr, 2008; Emongor, 2010). 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 commodities is 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 ten minutes.

Wild plum fruit has been reported in the semi-arid regions of Brazil to contain vitamin C of 170 mg/ 100 g (immature fruit) and 188-251 mg/100 g (mature fruit) (Silva *et al.*, 2008; Sarmento *et al.*, 2015). In the current study, ripe wild plum fruit contained 76 mg/100g of vitamin C. The difference in the current results and those of Silva *et al.* (2008) and Sarmento *et al.* (2015) might be due to climatic differences between Botswana and Brazil, stage of fruit maturity at harvest and the species of wild plum (Naggy, 1980; Kays and Paull, 2004).

5.7 Weight loss

In general, the higher the temperature the shorter the storage life of horticultural products and the greater the amount of weight loss within a given time, as most factors that destroy the produce or lower its quality occur at a faster rate as the temperature increases (Hardenburg *et al.*, 1986; Ball, 1997; Emongor, 2010). This applies to the rate of growth of spoilage micro-organisms, the rate of indigenous physiological change and physical processes such as water loss and wilting (Emongor, 2010). For each 10°C rise in temperature above the optimum, the rate of deterioration for fresh harvested commodities increase two to three-fold (Thompson, 1996). When fresh commodities are stored at temperature above 25°C some commodities lose quality rapidly including weight due to high respiration rate and water loss (Emongor, 2010; Pholoma, 2016). Cooling commodities to below 5°C immediately after harvest can greatly reduce the deterioration and incidence of pathogenic rots although CI is a problem at such temperatures.

In the current study, increase in storage temperature significantly ($P < 0.05$) increased fruit weight loss twenty eight days after storage. As storage temperature increased from 0 to 15°C, wild plum fruit weight loss increased from 1.02% to 26.75%, respectively, after twenty eight days of storage. The higher fresh weight loss of wild plum fruit at 5, 10, and 15°C than at 0°C was attributed to higher fruit transpiration and respiration rates at the higher temperatures

(Hardenburg *et al.*, 1986; Ball, 1997; Kays and Paull, 2004). Pholoma (2016) reported that as mango fruit storage temperature increased from 4 to 25°C fruit weight loss increased from 10.6 to 15.9%. Yousef *et al.* (2012) reported progressive increase in fresh mass loss of mango fruit cultivar 'Copania' throughout the storage. Kumah *et al.* (2011) reported that there was a gradual increase in the cumulative weight loss in 'Keitt' mango fruit after the fourth day of storage and continued with rapid increase in weight until twenty one days after storage. Similar results of fruit weight loss with increase in storage temperature have been reported in different fruits such as tangerine (Roongruangsri *et al.*, 2013), squash (Wang, 1993), tomato (Žnidarčič, 2010), and avocado (Perez *et al.*, 2004).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

Postharvest temperature management of wild plum fruits is important for their successful storage, extension of shelf-life and marketing. This study showed that storage of wild plum fruits at temperatures below 15°C leads to the development of CI, a physiological disorder that renders the fruit unmarketable. It was concluded that in order to extend the shelf-life and marketing period (twenty eight days) of wild plum fruits, the fruits should be stored at 15°C and 90-95% RH, because the fruit will not suffer from CI and will undergo normal ripening process. It is recommended that this research on storage temperature of wild plum be repeated using more varieties and temperatures in the range of 10 to 15°C.

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