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Effects of Storage Temperature on Postharvest Quality of Marula Fruits (*Sclerocarya birrea spp. Cafra*)

ABSTRACT

A laboratory experiment was undertaken to investigate the effects of storage temperature on storage life and quality of morula fruits. The experimental design was a completely randomized design with three replications. Marula tree landraces growing around Sebele area of Gaborone were used for the study. The storage temperatures were 0, 4, 8 and 12 ± 1 °C. Some fruits were also left at room temperature (25 ± 3 °C). The results of the study showed that storage temperature and duration of storage significantly ($P \leq 0.05$) affected the incidence and severity of chilling injury in marula fruit. Chilling injury developed in all the fruits stored at temperatures of ≤ 12 °C, though the severity of chilling inhury significantly ($P \leq 0.01$) varied with storage temperature. The marula fruit stored well for 25 days at 12 °C without the development of physiological disorders. Upon removal from cold storage (12 °C) and held for seven days at room temperature to simulate marketing conditions, the fruit underwent normal ripening process with colour (carotenoids and anthocyanins) and aroma development, high total soluble solids and low titratable acidity. It was concluded that in order to extend the shelf-life and marketing period of marula fruit, the fruit should be stored at 12 °C and 90-95% RH, because the fruit will not suffer from chilling injury and will undergo normal ripening process.

Keywords: Morula fruit, storage temperature, cultivars, chilling injury, postharvest shelf life

1.0 INTRODUCTION

Fruits are living tissues and are diverse in morphology, structure, composition and general physiology. Due to high moisture content, active metabolisms, tender nature and rich in nutrients, they are vulnerable to dehydration, physiological disorders, environmental stress, mechanical injury and pathological breakdown therefore usually considered to be highly perishable [1]. Therefore, these characteristics limit the storage life of the fruits and vegetables and cause significant deterioration following harvest [2]. Postharvest loss can occur at any point in production and market chain. It is estimated that the magnitude of these

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losses due to inadequate postharvest handling and storage in fresh fruits and vegetables is relatively high but varies in accordance with the country and commodity [1]. In a hungry and increasingly competitive world, reducing postharvest food losses is a major agricultural goal. 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 and modified atmosphere storages.

Temperature being the most important environmental factor that influences the deterioration of harvested horticultural commodities, most perishable commodities last longest at temperature near 0 °C. Temperature influences how other internal and external factors influence the commodity [3]. Temperature management is the most effective tool for maintaining quality and safety, and for extending the postharvest life of fresh horticultural commodities. 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 down to extend shelf life by reducing the fruit temperature. Low temperature storage is considered the most effective method of extending postharvest life and maintaining the quality of the fruits [4]. However, most fruits that originated from the tropical and subtropical regions are chilling sensitive when exposed to low temperatures that are above the freezing point [5]. Such crops including marula 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. Due to sensitivity of tropical and some sub-tropical produce to chilling injury, their transport to 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 of 13 ^{O}C is adequate to avoid the onset of chilling injury in tropical and some sub-tropical horticultural horticultural produce for at least three weeks [6]. There is hardly any literature available on the storage temperature of marula fruit, except it was reported that marula juice stores well at 4 °C [7]. Therefore, the objective of this study was to evaluate the effects of storage temperature on postharvest quality of marula fruit.

2.0 MATERIALS AND METHODS

2.1 Experimental site

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The experiment was conducted in the Crop Science and Production Department Laboratories of the Botswana College of Agriculture between January and April 2015. Botswana College of Agriculture is located at Sebele (latitude 24 34^{0}S and longitude 25 57°E, altitude of 994 m above sea level) and 10 km from Gaborone.

2.2 Experimental design

The experimental design was a completely randomized design (CRD). Fresh marula fruits were picked randomly at the green mature stage (physiological maturity) from 10 different marula trees around Sebele. The fruits were stored at 0, 4, 8 and 12°C. Four refrigerators were set at different temperatures of 0, 4, 8 and 12 ^{o}C ± 1°C. Fresh fruits were collected when still firm, green, uniform and free from bruises and defects as judged subjectively based on epidermal colour. Fruits were washed to remo soil and other external material. Three hundred fruits were stored in each storage temperature. All analyses were done in triplicate.

2.3 Dependent variables determined

The dependent variables analysed were fruit development, fruit chlorophyll content, fruit anthocyanin and carotenoid content, fruit juice soluble solids content (SSC), total titratable acidity (TTA), vitamin C content, chilling injury (incidence and severity) and storage life of the fruit in cold storage and after storage at room temperature (25 ± 3 °C).

2.3.1 Titratable acidity

The fruit and skin was cut and 100 g of sample weighed, then 200 ml of distilled water was added to the sample. The mixture was then blended for five minutes. The sample was homogenized and filtered through 5-layers of cheese cloth. 20 ml of filtrate (juice) was then 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, and this was done in triplicates. The results were expressed as total titratable acidity equivalents using the formula given below.

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

2.3.2 Soluble solids content

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Soluble solids content (SSC) was determined using a refractometer. Ten fruits per treatment per replication were pinched using a razor blade and the juice squeezed directly into the hand refractometer (0-32%) and then the average sugar content was determined in ^{o}Brix.

2.3.3 Vitamin C (ascorbic acid) determination

Vitamin C content was determined using 2, 6-dichloroindophenol titrimetric method [8]. Ascorbic acid reduces oxidation-reduction indicator dye (2, 6-dichloroindophenol) to colourless solution. At end point, excess unreduced dye is rose pink in acid solution. Vitamin C was extracted and titration performed in the presence of metaphosphoric acid-acetic acid solution to maintain proper acidity for reaction and to avoid antioxidation of ascorbic acid at high pH [8].

2.3.4 Fruit chlorophyll, anthocyanin and carotenoid contents

Anthocyanin plus carotenoids and chlorophyll content of the fruit skin were measured from two discs (11 mm diameter) per fruit (10 marula fruits per replicate/treatment), cut from the yellowish and greenest portions using a cork borer and the scalpel. The 10 discs were then extracted in 4 ml of 0.1 N HCL in methanol at 25 ± 2 ^{o}C in the dark for 24 hours. Absorbance of extracts was measured using a UV Visible spectrophotometer (UV-1602 I PC, Shimadzu, RSA Pty Ltd.Co.Reg.NO: 90/07641/07) at various wavelengths of 530, 620,645, 650 and 663 nm.

Anthocyanins plus carotenoid content was determined according to an established method [9], using a molar extinction coefficient of 4.62 x 10^4 [10].

$$\text{Anthocyanins plus carotenoids (nmole/cm}^2\text{ marula skin)} = \frac{[(A_{530}-A_{620}) - 0.1(A_{650}-A_{620})]}{4.62 \times 10^4}$$

Skin chlorophyll content was measured as absorbance at 663 nm [11]. The following equation was used to calculate the relative total chlorophyll content [11].

$$\text{Chlorophyll (mg/cm}^2\text{ of morula skin)} = 24.88 \times A_{653}$$

2.3.5 Chilling injury (incidence and severity)

Chilling injury was evaluated daily for incidence and severity of chilling in storage. Chilling injury (CI) incidence was determined from a sample of 20 fruit/replicate/treatment. Fruit

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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 2/3 of the fruit showing injury depending on the peel damage).

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.

2.3.6 Fruit development

Fruit development was measured from 10 tagged fruits every week starting from one week after full bloom to 21 weeks after full bloom using a digital electronic vernier calliper.

2.3.6 Data analysis

Analysis of variance was performed on the data collected using the general linear Models, procedure of the Statistical Analysis System (SAS) Programme. Multiple comparisons among means was performed using the Protected Least Significant Difference at P=0.05.

3.0. RESULTS

3.1. Fruit development

The results of this study showed that marula fruit growth and development showed a typical simple sigmoid growth curve (Figure 1). Fruit diameter increased with time and reached maximum diameter at 18 weeks after full bloom (Figure 1).

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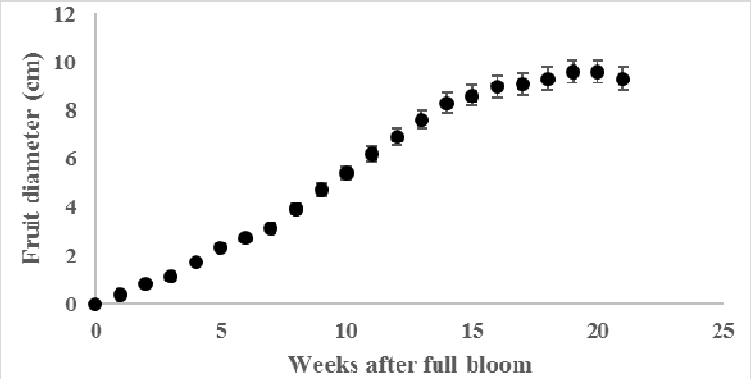

Figure 1. Marula fruit growth and development

3.2 Chilling injury

The results of this study showed that storage temperature significantly ($P \leq 0.05$) influenced the incidence (Figure 2) and severity (Figure 3) of chilling injury of marula fruit. All marula fruit stored at temperatures ≤ 8 °C developed chilling injury, but the severity was significantly ($P \leq 0.01$) greater on fruit stored at 0 and 4 °C (Figure 3). Marula fruit storage at 8 °C had severity index of ≥ 2 (67 % or above of the fruit is chilled) after 15 days of storage, while fruit stored at 0 and 4 °C attained a severity index ≥ 2 after 7 and 8 days, respectively (Figure 3). Marula fruit stored at 12 °C did not suffer from chilling injury (Figure 2, 3).

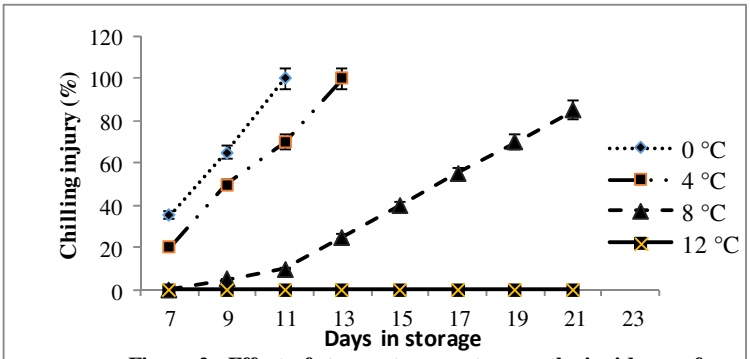

Figure 2: Effect of storage temperature on the incidence of chilling injury

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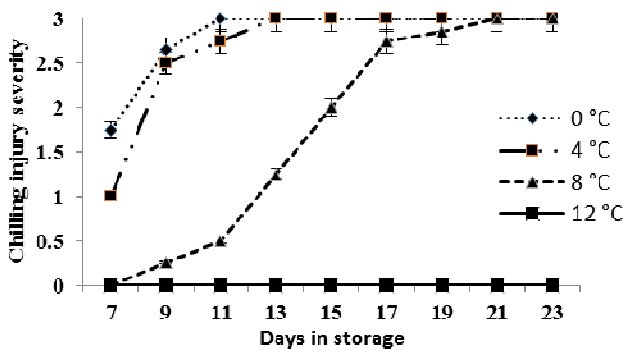

Figure 3: Effect of storage temperature on severity of chilling injury

3.3 Titratable acidity

During fruit growth and development**,** tritratable acidity increased with time and a reached a maximum after 21 weeks after full bloom and decreased thereafter (Figure 4). During storage for 8 and 16 days total titratable acidity significantly ($P \leq 0.05$) decreased with increase in storage temperature (Figure 5). Storage temperature and duration of storage significantly ($P \leq 0.05$) influenced the decrease in fruit titrable acidity (Figure 5). Fruit stored for 8 days had significantly ($P \leq 0.05$) higher titratable acidity than fruit stored for 16 days at 0, 4, 8 and 12 °C, respectively (Figure 5).

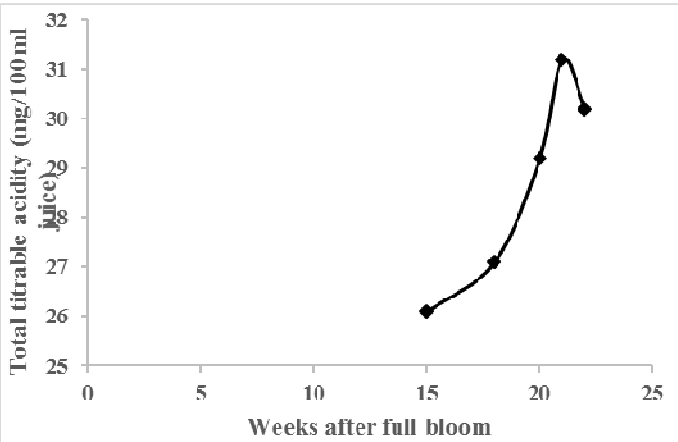

Figure 4. Titratable acidity of marula fruit during growth and development

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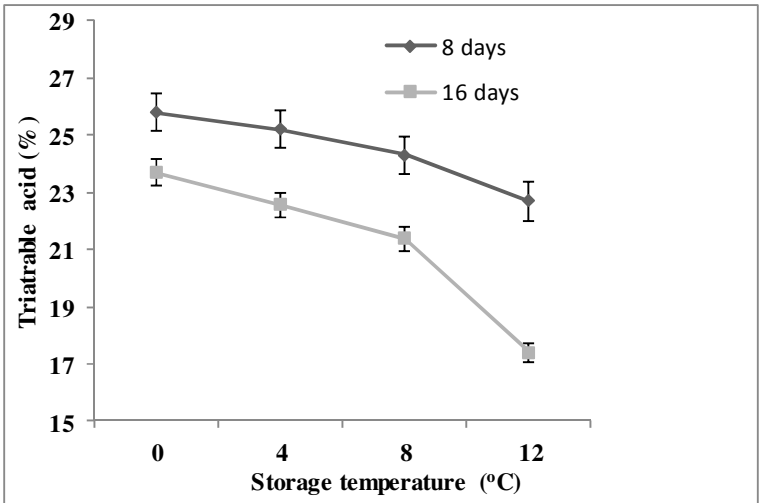

Figure 5. Effect of storage temperature on marula fruit titratable acidity

3.4 Total soluble solids

During fruit growth and development, the fruit total soluble solids content increased with time (Figure 6). During storage at room temperature (25 ± 3 °C) fruit soluble solids content (SSC) increased and reached a maximum after six days in storage, thereafter the fruit SSC decreased (Figure 7). Storage temperature and duration of storage significantly ($P \leq 0.05$) influenced the marula fruit SSC (Figure 8). As storage temperature increased from 0 to 12 °C, fruit SSC significantly ($P \leq 0.05$) increased, irrespective of storage duration (Figure 8). Fruit stored for 16 days had significantly ($P \leq 0.05$) higher SSC than fruit stored for 8 days (Figure 8).

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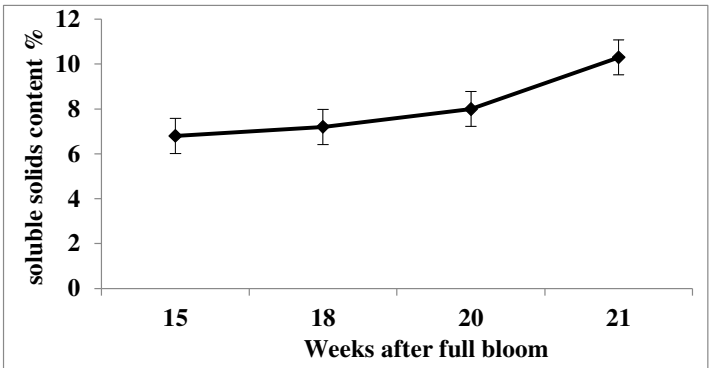

Figure 6. Marula Soluble solids content during growth and development

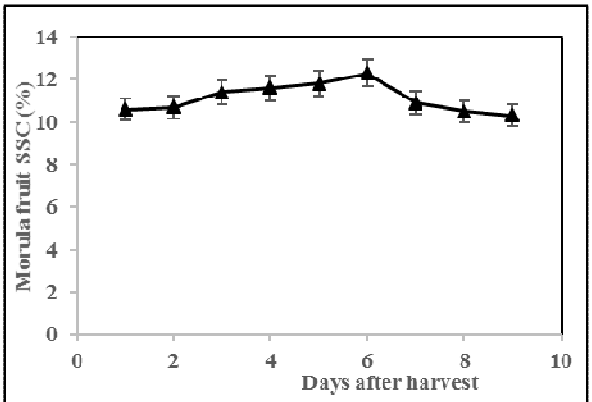

Figure 7. Morula fruit sluble solids content after harvest at room temperature

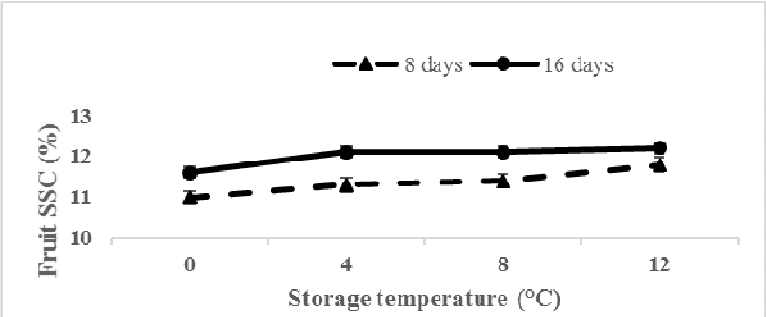

Figure 8. Effect of storage temperature on morula fruit soluble solids content

3.5 Fruit anthocyanin and carotenoid content (colour)

The fruit colour (anthocyanin and carotenoid) content increased significantly ($P \leq 0.05$) with increase in storage temperature (Figure 9). After 22 days of storage at lower temperatures (≤ 12 °C), maximum fruit colour developed in fruit stored at 12 °C (Figure 9). Though fruit stored at 25 ± 3 °C, developed the best colour, but it was not significantly different from fruit stored at 8 and 12 °C (Figure 9). Maximum fruit colour was attained after 8 and 25 days of storage at 25 ± 3 and 12 °C, respectively (Figure 9).

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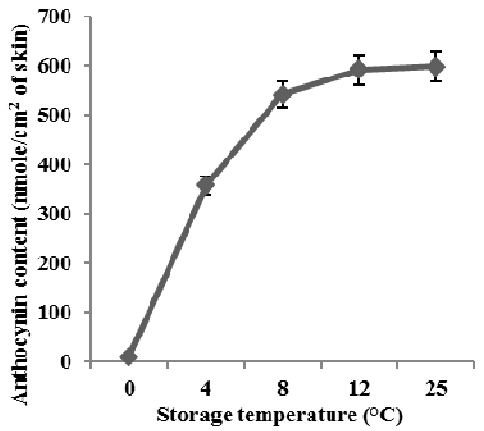

Figure 9: Effect of storage temperature on fruit anthocyanin content

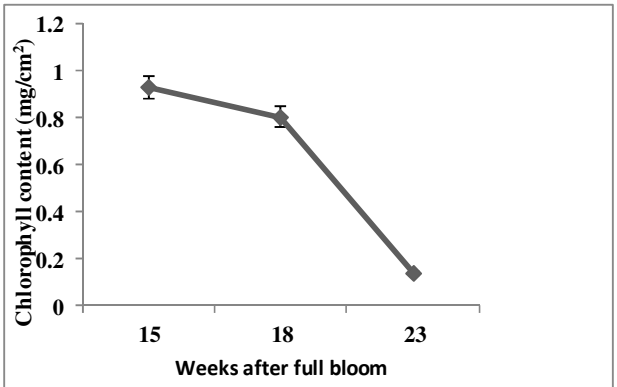

Figure 10: Chlorophyll content during fruit growth and development

3.6 Fruit chlorophyll content

The results of the current study showed that during fruit growth and development, chlorophyll content was high but significantly ($P \leq 0.05$) decreased as fruit maturation increased (Figure 10). In storage, storage temperature significantly ($P \leq 0.05$) affected fruit chlorophyll content (Figure 11). Increase in storage temperature from 0 to 25 °C significantly ($P \leq 0.05$) increased the degradation of fruit chlorophyll content of marula fruit (Figure 11).

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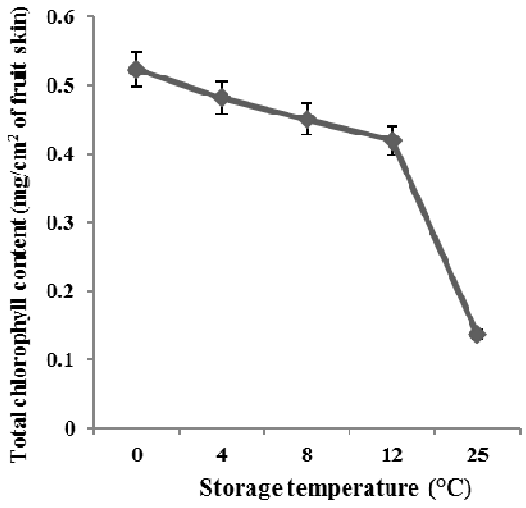

Figure 11: Effect of storage temperature on fruit chlorophyll content

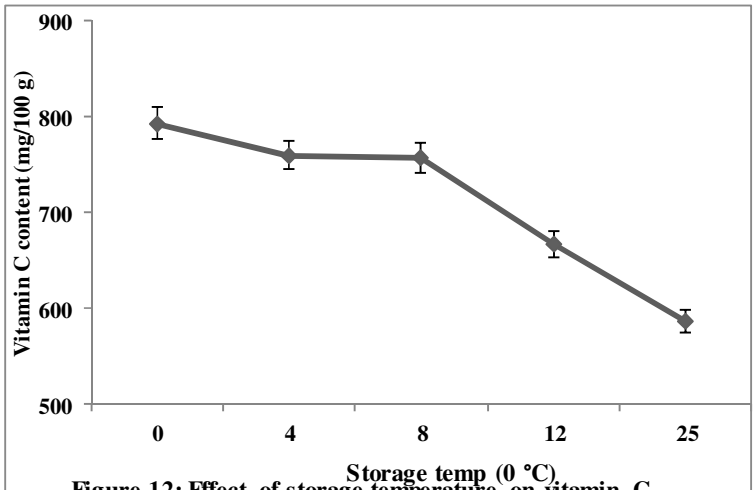

Figure 12: Effect of storage temperature on vitamin C content

3.7 Vitamin C content

The vitamin C content of marula fruit was significantly ($P \leq 0.05$) influenced by storage temperature (Figure 12). Retention of marula fruit vitamin C content was significantly ($P \leq 0.05$) increased as storage temperature was lowered (Figure 12). The vitamin C content of marula fruit stored at 25 ± 3°C for 8 days was 1.35 times lower than the vitamin C content of fruit stored at 0 °C for 25 days (Figure 12).

4.0 DISCUSSION

4.1 Fruit Development

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The results of this study showed that marula fruit displayed a simple sigmoid growth curve. 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 [12, 13, 14, 15]. Fruit can increase in mass or volume by 100-fold or more from fertilization to maturity and such changes follow simple or double-sigmoid growth curve depending on the fruit species [13, 16].

4.2 Effect of storage temperature on postharvest quality of marula fruits

Marula is subtropical in origin, however, most fruits that are of tropical or subtropical in origin are reported to be chilling sensitive [5, 17, 18]. These fruits are injured after a period of exposure to chilling temperature below 10-15°C, but above their freezing temperatures. The results of this study showed that storage temperature significantly influenced the development of chilling injury (incidence) and severity of chilling injury in marula fruit. As storage temperature decreased below 12°C, the incidence and severity of chilling injury increased. Chilling injury symptoms of skin pitting, poor colour development, poor aroma and surface lesions developed in marula fruit stored at temperatures ≤ 12°C, though severity varied with storage temperature and duration of storage. Marula fruit stored at 0, 4 and 8 °C developed chilling injury symptom after 7 and 9 days of storage, but severity of chilling was high in fruit stored at 0 and 4 °C. Marula fruit stored at 12 °C did not develop chilling injury. Similar results have been reported [2, 17], who all reported of chilling injury symptoms such as scalding, surface pitting, poor aroma, uneven ripening, poor colour development and increased susceptibility to diseases in mango stored at temperatures below 12 ^{o}C. Chilling injury can be related to the enzymatic browning of activities of polyphenoloxidase (PPO) and peroxide (PO), and the increase of phenolic compounds [19]. Chilling injury 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 [20]. Various physiological, biochemical alteration and cellular dysfunction occur in chilling sensitive species in response to chilling stress [20]. These alterations include increased membrane permeability and alteration of activities of membrane proteins. If chilling stress is prolonged, these alterations cascade to 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 the tissue, off-flavour, susceptibility to fungal decay, reduced aroma, carotenoids [21, 22] and become more serious when fruits are transferred to room temperature probably because they satisfactorily ripen at 21-24 °C. Factors that have been reported to influence the susceptibility of horticultural produce to chilling injury include

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environmental conditions, maturity when picked and postharvest handling techniques, and duration of exposure to the chilling temperature [2, 23].

4.3 Marula fruit changes during ripening

Fruit ripening is a highly coordinated, genetically programmed, and an irreversible phenomenon 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. 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 [24, 25]. The results of the current study showed that as storage temperature increased from 0 to 25°C, SSC content, carotenoid and anthocyanin biosynthesis and chlorophyll degradation increased, but titratable acidity decreased, irrespective of storage temperature, indicating fruit ripening occurred in all the storage temperatures. The fruit colour change during ripening is due to unmasking of preformed pigments by degradation of chlorophyll and/or biosynthesis of carotenoids and anthocyanins and their accumulation in vacuoles [26, 27]. The increase in SSC and decrease in titratable acidity after three weeks of marula fruit storage at different temperatures was attributed to increased glucogenesis and hydrolysis of polysaccharides especially starch [26,27]. Decreased acidity and accumulation of sugars (SSC) and organic acids in marula fruits results in an excellent sugar/acid blend for edibility as fresh fruit or for processing of marula into various products. In general, total acidity decreases during ripening, though the content of one or more acids may increase. Total acidity decreases with ripening due to their utilization as respiratory substrates especially in the Krebs (TCA) cycle [16].

4.4 Vitamin C content

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Loss of vitamin content especially vitamin C, pro-vitamin A, thiamine (vitamin B1) and nicotinic acid is common in horticultural produce poorly handled. 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 mono-dehydroascorbic acid being catalyzed by any of the oxidases such as ascorbic acid oxidase, cytochrome oxidase, o-diphenol oxidase, ρ-diphenol oxidase and peroxidase [16, 28]. The balanced oxidation and reduction of ascorbic and dehydroascorbic acids leads to no loss of vitamin C. Loss can occur 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 [28]. The results of the current study showed that the loss of vitamin C was significantly reduced when marula fruit was stored at lower temperatures ≤ 12 °C. Storage of fruits and vegetables at temperatures below 5°C is reported to decrease the loss of vitamin C [28, 29]. The rate of loss of vitamin C is higher with higher storage temperature, an effect associated with loss of acidity [28]. Marula fruit according to the current study have very high levels of vitamin C in the range of 586-793 mg/100 g. Mangoes stored at 24-26°C for 6 days has been reported to result in a drop in vitamin C content from the initial 71 mg/100g to 63.9 mg/100g [28].

5.0 CONCLUSION

Postharvest management of marula fruits is important for their successful marketing. The study showed that the most critical factor affecting the postharvest shelf life of marula fruits is storage temperature. It was concluded that in order to extend the shelf life and marketing period of marula fruits, the fruits should be stored at 12 °C and 90-95% RH, because the fruit will not suffer from chilling injury and will undergo normal ripening process. Marula fruit could initially be stored at 8 °C for 9 days and transferred to 12 °C to extend the marketing period and availability of the fruit beyond the harvest period.

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