STORAGE TEMPERATURE INFLUENCES POSTHARVEST QUALITY OF WILD PLUM (*XIMMENIA AMERICANA L.*) FRUIT

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Abstract
Wild plum has many medicinal, therapeutic and nutritive uses, but its fruits are collected in the wild and less researched. The aim of this study was to evaluate the effects of storage temperature on postharvest fruit quality of wild plum. The fruits of wild plum were subjected to different storage treatments at 0, 5, 10 and 15 ± 1°C in a completely randomized design. The results showed that as storage temperature decreased below 15°C, the incidence and severity of chilling injury significantly ($p<0.05$) increased. As storage temperature increased from 0 to 15°C fruit titratable acidity (TTA) and vitamin C content significantly ($p< 0.05$) decreased, but juice pH, soluble solids content (SSC) and weight loss significantly ($p< 0.05$) increased. The decrease in TTA and vitamin C content, and increase in SSC and juice pH was attributed to fruit ripening. The increase in fruit weight loss was attributed to higher transpiration and respiration. It was concluded that to extend the storage-life and marketing period of wild plum fruits, the fruits should be stored at 15°C and 90-95% RH.

Keywords: Ximenia americana, Chilling injury and incidence, Vitamin C, Soluble solids content, Acidity

Introduction

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 *Ximenia*, is part of the Olacaceae family of plants that comprises of eight species (Orwa *et al*., 2009). Wild plum is widespread throughout the tropics in Africa, India and South East Asia, and also Australia, New Zealand, Pacific Islands, West Indies, Central and South America (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).

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). Bahru *et al*. (2010) reported detailed uses and

management of wild plum in the semi-arid regions of Afar and Oroma of Ethiopia. Wild plum is broadly used in non-traditional medicine in regions where it is distributed (Le *et al*., 2012).

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). Wild plum has been reported to have medicinal properties, displayed by its antimicrobial, antifungal, anticancer, antioxidant, pesticidal and haematological effects (Feyssa *et al*. 2010). 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 fruit is reported to be rich in antioxidants such as vitamin C, polyphenols, anthocyanins and carotenoids (Lamien-Meda *et al*., 2008; Silva *et al*., 2008; Sarmento *et al*., 2015).

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 & Carrillo-Salazar, 2012). Fruit commercialization of wild plum is however, affected by drop in quality due to inappropriate postharvest handling, which largely determines the economic outcome of fruit marketing (Toivonen & Hodges, 2011). 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). However, most fruits that have originated in the tropical and subtropical regions are sensitive to cold temperatures, especially when exposed to low temperatures that are above the freezing point

(Gross *et al*., 2002). Such produce is injured when stored below their critical temperature, which generally ranges between 10 and 13°C for most varieties. Due to sensitivity of tropical fruits to chilling injury, transport of these fruits for most distant markets is generally only successful by air. No appropriate technology has been developed for storage of wild plum fruit (Feyssa *et al*., 2010). In Botswana and other parts of the world, wild plum is among the least exploited edible wild plant species, but highly consumed by local inhabitants in most settlements. Due to high perishability and unknown storage temperature requirement, marketing of wild plum is uncommon or virtually impossible. This research was undertaken to elucidate the optimum storage temperature for wild plum fruit.

Experimental

Fruit collection site

Wild plum fruits were collected from the outskirts of Molepolole village, in a settlement known as Seherelela, 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 above sea level 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 the average. Due to seasonality of wild plum fruits, the experiment was carried out from December 2016 to March 2017 when fruits were available in the forest.

Experimental site and design

The experiment was conducted in the Crop and Soil Sciences Department in the Crop Physiology Laboratory at The Botswana University of

Agriculture and Natural Resources (BUAN) between December 2016 and March 2017. BUAN is located at Content Farm Sebele (Latitude 24º33'S and Longitude 25º54'E; - altitude 994 m above sea level). The experimental design was a completely randomized design (CRD) replicated three times. The treatment was storage temperature at 0, 5, 10 and 15 ± 1°C. Fresh wild plum fruits were picked randomly at physiological maturity from different wild plum trees clustered in one area exposed to similar environmental conditions. At harvest, the fruit was 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.

Dependent variables determined
The dependent variables determined were fruit development, fruit juice soluble solids content (SSC), total fruit titratable acidity (TTA), pH, vitamin C content, chilling injury (CI) (incidence and severity) and storage life of the fruit in cold storage and after storage at room temperature. Fruit development was determined by measuring fruit diameter and length (mm) of ten tagged fruits every week for 10 weeks using a veneer calliper, starting one week after full bloom.

Titratable acidity
The fruit titratable acidity was determined according to AOAC (1996). Ten fruit were cut and mixed. A composite sample of 100 g was weighed, then 100 ml of distilled water was added. The sample was homogenized and filtered through 5-layers of cheese cloth. Then 20 ml of filtrate (juice) was put in a 50-ml conical flask and two drops of 1% phenolphtha-

lein indicator added. The samples were titrated with 0.1 N NaOH to end point (pH 8.2). The results were expressed as total titratable acidity equivalents using the formula given below. Total titratable acidity (g/100 ml juice) = (ml base x normality base x 1x 100 x 2)/ml sample

Soluble solids content
The soluble solids content (SSC) was determined according to AOAC (1996) using a hand refractometer (Atago Model N1, American Optical, New York). Ten fruits per treatment were pinched using a scapel. Three drops of juice were directly placed on the prism surface of the refractometer and the average sugar content was determined in Brix but expressed as % SSC.

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

Vitamin C (ascorbic acid) content
Vitamin C 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.

Chilling injury (incidence and severity)
Chilling injury was evaluated daily for incidence and severity in storage according to the method of Zhao *et al*. (2006). Chilling injury (CI) incidence was assessed from all stored fruits per treatment. Fruit showing symptoms of CI were counted and expressed as a percentage. Chilling injury 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 was calculated by the following equation;

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

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

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.

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 $p \leq 0.05$.

Results and discussion

Fruit development
The results of the study showed that wild plum fruit significantly ($p \leq 0.05$) increased both in length (2.64 cm) and diameter (2.14 cm) with increase in time reaching a maximum size at nine weeks after full bloom (Fig. 1). Fruit growth and development followed a simple sigmoid growth curve (Fig. 1). Fruits can display simple, double or triple sigmoid growth curves depending on the fruit species (Jackson *et al*., 2011; Emongor & 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 (Jackson *et al*., 2011). The size of fruit growth greatly depends on the cultivar and early maturing cultivars with a short period of fruit growth are generally smaller than fruit of later maturing cultivars of the same species that have a longer period for growth (Jackson *et al*., 2011). The fruit size of wild plum is relatively small (2.7-3 cm long, 1.5-2.5 cm in diameter) due to the short fruit growth period of 8-9 weeks after full bloom, which is genetically pre-determined (Maundu *et al*., 1999; Orwa *et al*., 2009).
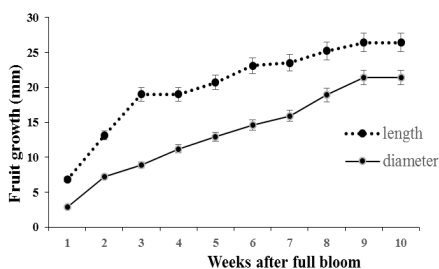
Fig. 1: Fruit growth pattern of wild plum; Error bar: Mean ± standard error.

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 (Fig. 2, 3). As storage temperature decreased below 15°C, the incidence and severity of chilling significantly (p ≤ 0.05) increased (Fig. 2, 3). Chilling injury symptoms observed were shrivelling, dark scald discoloration, pitting, sunken lesions and poor colour development on wild plum fruits stored at storage temperatures of 0, 5, and 10°C, though the incidence and severity varied with the storage temperature (Fig. 2, 3). Fruit stored at ≤ 10°C developed CI after 24 hours of storage (Fig. 2). After 2 days of storage at 0 and 5°C, the CI severity had an index greater than 2, implying the fruit was unmarketable. Fruit stored at 10°C attained CI injury severity index higher than 2 after 7 days of storage (Fig. 3). Wild plum fruit stored at 15°C did not develop chilling injury and had a storage-life of 28 days, respectively, (Figure 2, 4). Similar results have been reported concerning other tropical and subtropical fruits (Wang, 1993; Gross *et al*., 2002; Emongor, 2015; Emongor & Tautsagae, 2016; Senewa, 2016). Several events have been suggested to be the primary cause of chilling injury, including a phase transition in membrane lipids, an alteration in the substrate specificity of a regulatory enzyme, a change in the cyto-

skeletal structure, or an increase in cytosolic calcium which are genetically controlled (Han *et al*., 2006; Aghdam *et al*., 2012).
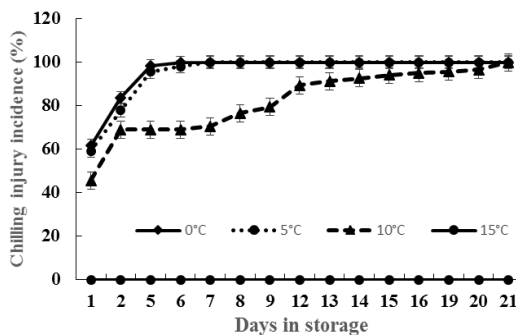

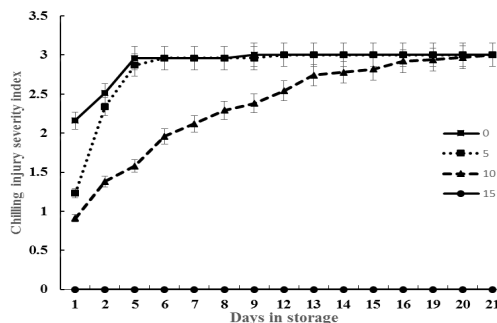Fig. 2: Effect of storage temperature on chilling injury incidence; Error bar: Mean ± standard error

Fig. 3: Effect of storage temperature on chilling injury severity of wild plum; Error bar: Mean ± standard error

Storage-life
Storage temperature significantly (p ≤ 0.05) influenced wild plum fruit shelf-life (Fig. 4). Wild plum fruit stored at 15°C had significantly (p ≤ 0.05) higher storage-life of 28 days than fruit stored at 0, 5 or 10°C which had a storage-life of 1.5, 2 and 7 days, respectively (Fig. 4). Fruit stored at 0 and 5°C suffered severe CI after 2 days of storage making them

unmarketable (Fig. 2, 3, 4). Temperature is the most important environmental factor that influences the deterioration of harvested horticultural commodities, most perishable commodities last longest at temperature near 0°C, if not chilling sensitive (Emongor, 2010; Kader, 2013). However, most tropical and subtropical fruits are chilling sensitive when exposed to low temperatures that are above the freezing point (Gross *et al*., 2002; Emongor, 2015; Emongor & Tautsagae, 2016; Senewa, 2016). Such crops, including wild plum, are injured when stored below the critical temperature between 10-13°C for most varieties. The results of the current study showed that in order to extend the postharvest life of wild plum fruits for about 28 days, the fruits should be stored at 15°C to avoid CI.
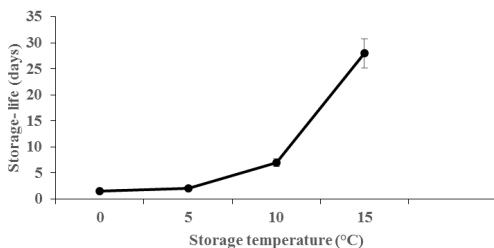

Fig. 4: Effect of storage temperature on shelf life of wild plum; Error bar: Mean ± standard error.

Titratable acidity and juice pH
Storage temperature significantly ($p \leq 0.05$) influenced wild plum fruit TTA (Fig. 5) and juice pH (Fig. 6). As storage temperature increased from 0 to 15°C, fruit TTA and juice pH significantly ($p \leq 0.05$) decreased (Fig. 5) and increased (Fig. 6), respectively. The response of wild plum fruit TTA to increasing storage temperature was a linear decrease with a correlation coefficient of 0.99. At harvest, the

fruit juice pH was 2.31, however, after 28 days of storage, fruit juice pH ranged between 2.54 to 2.8 (Fig. 6). The flavour of fruits depends on the interaction of sugars, organic acids, phenolics, tannins and aroma volatiles (Prasanna *et al*., 2007; Paliyath & 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 & Murr, 2008; Emongor; 2010; Seymour *et al*., 2013; Emongor, 2015). The decrease and increase in wild plum fruit TTA and juice pH, respectively, with increasing storage temperature in the current study was attributed to increased level of fruit ripening. Immature fruits contain more acids that may decline during maturation and ripening due to gluconeogenesis (Prasanna *et al*., 2007; Paliyath & Murr, 2008; Emongor, 2010; Seymour *et al*., 2013). Sarmento *et al*. (2015) reported that the TTA 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). 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 TTA and pH was 67 mg/ 100 ml juice and 3.0, respectively, but after 28 days of storage, the fruit juice TTA and pH ranged between 35-55 mg/100 ml and 2.54-2.8, respectively. 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 flavour and promotes a high dilution factor in the formulation of juices leading to a greater yield while low acidity dismisses any acidification during processing (Chakraverty *et al*., 2003).
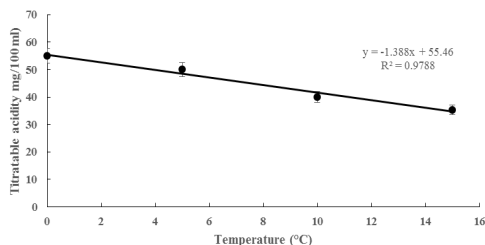
Fig. 5: Effect of storage temperature on titratable acidity 28 days after storage; Error bar: Mean ± standard error
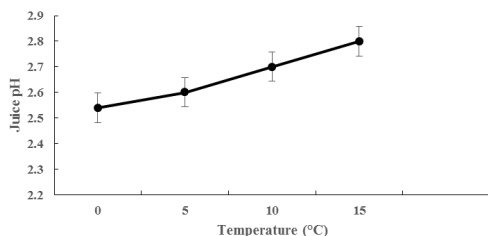

Fig. 6: Effect of storage temperature on juice pH at the end of storage; Error bar: Mean ± standard error.

Soluble solids content

The result of the study showed that storage temperature significantly ($p \leq 0.05$) influenced fruit soluble solids content (SSC) 28 days after storage (Fig. 7). Fruit stored for 28 days at 0, 5, 10 and 15°C had fruit SSC of 20.2, 23.6, 25.7 and 26.8%, respectively (Fig. 7). The increase in fruit SSC of wild plum with increase in storage temperature was attributed to fruit ripening which promoted glucogenesis and hydrolysis of polysachharides especially starch (Aivalakis & Katinakis, 2008; Paliyath & Murr, 2008). The relatively low SSC on wild plum fruit stored at low temperatures of 0, 5 or 10°C was attributed to CI. Chilling injury negatively affects fruit ripening and caus-

es uneven ripening (Emongor, 2015; Emongor & Tautsagae, 2016). From the results of the current study, the lowest safe temperature for long term exposure of mature wild plum fruits is 15°C. Lowering the temperature further could inhibit ripening due to chilling injury hence abbreviated storage life (Kader, 2013).
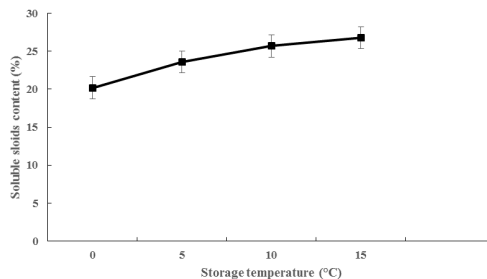

Fig. 7: Effect of storage temperature on fruit soluble solids content 28 days after storage; Error bar ± standard error.

Vitamin C

Storage temperature had a significant ($p \leq 0.05$) influence on the vitamin C content of wild plum 28 days after storage (Fig. 8). As storage temperature increased from 0 to 15°C, fruit vitamin C content significantly ($p \leq 0.05$) decreased (Fig. 8). In the current study, the fruit vitamin C content at harvest was 76 mg/100 g. However, after 28 days of storage at 0, 5, 10 and 15°C, fruit vitamin C content decreased from 76 to 8 mg/100 g (Fig. 8). 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). The difference in vitamin C content of the current study and that 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 (Kays & Paull, 2004).

Loss of vitamin C content in fruits and vegetables during storage at various temperatures is reported in literature (Kays & Paull, 2004; Emongor, 2010; Emongor & Tautsagae, 2016; Senewa, 2016). In the current study TTA and juice pH decreased and increased (loss of acidity), respectively, with increase in storage temperature which correlated with loss of vitamin C, explaining the loss of vitamin C. The loss of vitamin C is pH dependent, being slow in acid pH, rapid at neutral pH and extremely rapid at alkaline pH (Kays & Paull, 2004; Paliyath & Murr, 2008; Emongor, 2010).
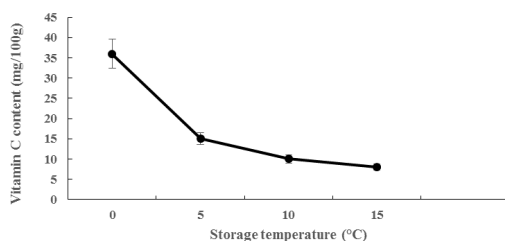

Fig. 8: Effect of storage temperature on fruit vitamin C content after 28 days of storage; Error bar: Mean ± standard error.

Weight loss
Storage temperature significantly (p ≤ 0.05) influenced wild plum fruit weight loss in storage (Fig. 9). As storage temperature increased, fruit weight loss significantly (p ≤ 0.05) increased (Fig. 9). Fruit stored at 0°C had significantly (p ≤ 0.05) lower fruit weight loss than fruit stored at 5, 10 or 15°C (Fig. 9). In general, the higher the temperature the shorter the storage life of horticultural products and the greater the amount of 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 (Ball, 1997; Emongor, 2010). This applies to the rate of growth of spoilage microorganisms, the rate of indigenous physio-logical change, and physical processes such as water loss and wilting (Emongor, 2010). In the current study, as storage temperature increased from 0 to 15 °C, wild plum fruit weight loss increased from 1.02% to 26.75%, respectively, after 28 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 (Ball, 1997; Kays & Paull, 2004). Senewa (2016) reported that as mango fruit storage temperature increased from 4 to 25 °C fruit weight loss increased from 10.6 to 15.9%. 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).
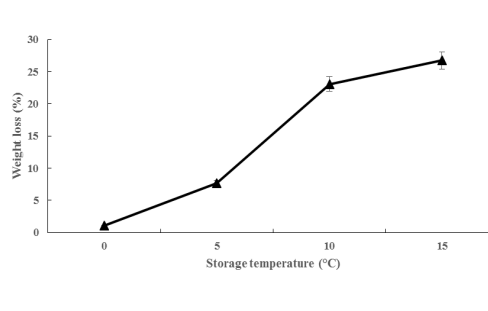

Fig. 9: Effect of storage temperature on weight loss of wild plum fruits 28 days after storage; bars are standard error.

Conclusion
Postharvest temperature management of wild plum fruits is important for their successful storage, extension of storage-life and marketing. This study showed that storage of wild plum fruits in temperatures below 15°C led to the development of CI. It is concluded that in order to extend the storage-life and marketing period (28 days) of wild plum fruits, the fruits should be stored at 15°C and 90-95% RH. It is

recommended that this study on storage temperature of wild plum be repeated using other varieties and temperatures in the range of 10 to 15°C.

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