

Maintaining postharvest quality of bell pepper (*Capsicum annuum* L. cv. California Wonder) using cactus (*Opuntia stricta* L.) mucilage coating

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Bell pepper (*Capsicum annuum* L.) experiences significant qualitative and quantitative loss during postharvest. This study aimed at providing an alternative postharvest handling technology for bell pepper. The factor studied was cactus (*Opuntia stricta* L.) mucilage coating at four levels: 0% (distilled water), 1, 2, and 3%. The fruits were stored under ambient conditions ($25 \pm 2^\circ\text{C}$ temperature and $65 \pm 2\%$ relative humidity) until senescence. Weight loss and total soluble solids content were determined at an interval of 3 days whereas iron and ascorbic acid content were determined at an interval of 4 days. Shelf life elapsed when fruit lost 25% of their initial weight on average. Cactus mucilage coating reduced weight loss by up to 21.64%, maintained total soluble solids by up to 14.93%, iron by up to 6.46%, ascorbic acid by up to 19.46% and extended shelf life by up to 6 days. Cactus mucilage coating at 1% was the best treatment and therefore can be used by bell pepper growers, retailers, and consumers to maintain postharvest quality and extend shelf life of bell pepper.

1. Introduction

Postharvest losses in horticultural produce in developing countries is as high as 45% due to poor postharvest handling (Kitinoja and Kader, 2015); and is even higher in Sub-Saharan Africa (SSA) (Kitinoja and Kader, 2015). In bell pepper (*Capsicum annuum* L.), losses of 28.6% and 38.7% have been reported during dry and wet seasons, respectively in Nigeria (Tsegay *et al.*, 2013). A short shelf life, even under the most favourable conditions is a major postharvest limiting factor in bell pepper handling (Ilić *et al.*, 2017). Since bell pepper is a non-climacteric fruit, its senescence is mainly accelerated by excessive water loss through respiration.

There is increasing interest in edible fruit and vegetable coatings to extend postharvest life. Cactus [*Opuntia ficus-indica* (L.) Mill.] mucilage has potential in postharvest preservation of horticultural commodities such as minimally processed cactus pear fruits (Liguori *et al.*, 2021),

mango (*Mangifera indica* L.) (Abera *et al.*, 2019) and papaya (Oluwaseun *et al.*, 2014). According to Oluwaseun *et al.* (2014), papaya fruits that were dipped for 30 seconds in cactus mucilage + glycerol and in pure cactus mucilage recorded a significantly lower weight loss, lower increase in fruits' TSS content and higher ascorbic acid content as compared to uncoated fruits at the end of storage period at a temperature $27 \pm 2^\circ\text{C}$ and RH of 55-60%.

Low temperature storage and Modified Atmosphere Packaging (MAP) have been successfully used in maintaining quality and extending shelf life of bell pepper (Manolopoulou *et al.*, 2012; Bayogan *et al.*, 2017). The most effective method has been rapid cooling after harvest followed by storage at low temperature and high relative humidity (Bayogan *et al.*, 2017). Bell pepper being a tropical fruit, suffers chilling injury at temperatures below 7°C , which favors development of fungal diseases (Ilić *et al.*, 2017). The cost of purchasing, installing and running a cold storage facility is also high and unaffordable for most small-scale bell pepper growers, retailers and consumers in developing countries hence rendering the technology untenable.

Modified Atmosphere Packaging (MAP) using plastic bags has also been used for a long time for maintenance of quality of bell pepper. However, their use may trigger development of anaerobic microorganisms (Manolopoulou *et al.*, 2012). These together with the restricted use of plastics in several countries due to environmental pollution has made the technology unreliable. Therefore, the objective of this study was to determine the effects of cactus mucilage coating, an alternative postharvest treatment, on postharvest quality and shelf life of bell pepper.

2. Materials and Methods

Experimental materials

Bell pepper, cv. California Wonder, fruit was produced at the Horticulture Teaching and Research Field of Egerton University, Njoro, Kenya under white agro net covers. The University lies at a latitude $0^\circ 23'$ South, longitude $35^\circ 56'$ East; and is 2,227 m above sea level (Jaetzold *et al.*, 2006). Fruit was harvested at mature green stage (Díaz-Pérez *et al.*, 2007), packed in plastic buckets, and taken to the laboratory. Average minimum temperature, maximum temperature and relative humidity of the laboratory site

was 11°C , 24.5°C and 64.7%, respectively (Egerton Meteorological Weather Station, 2020), where fruit free from bruises and blemishes were selected and used for the study. Cactus (*Opuntia stricta* L.) stems were also harvested from the field at Egerton University, packed in plastic buckets and transported to the laboratory.

Extraction of cactus mucilage and preparation of cactus mucilage treatments

Cactus mucilage was extracted at room temperature ($25 \pm 2^\circ\text{C}$) using the method described by Sepulveda *et al.* (2007). Cactus stems were washed using 2% volume per volume (v/v) sodium hypochlorite (NaClO) to remove dirt and for disinfection. Stems were peeled and chopped into small pieces using a sharp knife. Distilled water was added to the chopped pieces in a ratio of 1:1 (w/v) (200 g of the chopped pieces in 200 mL distilled water) and blended for 3 min using a blender (PPS SB-4171, Sayona, China) to obtain slurry which was gravity filtered through muslin cloth. The filtrate was precipitated using 20% isopropyl alcohol in a ratio of 1:1 (v/v) (1 L of the filtrate in 1 L of 20% isopropyl alcohol). The precipitated filtrate was centrifuged for 10 min at $2,683 \times g$ using a centrifuge (DL-5-D). The supernatant was drained off and precipitates at the bottom of the Eppendorf tubes dried in a forced air oven at 70°C for 4 h to obtain dried cactus mucilage. To obtain 1, 2 or 3% mucilage solution, 1, 2 or 3 g, respectively, of the dried cactus mucilage was weighed using an electronic weighing balance (Denver Instrument XL-1810) and dissolved in 80 mL of distilled water. To each solution, 2 mL of glycerol plasticizer was added, volume made to 100 mL mark using distilled water and blended for 3 min to obtain complete dispersion. The solutions were centrifuged for 10 min at $2,683 \times g$ using a centrifuge (DL-5-D) to obtain a supernatant; which was used to coat fruit. The different concentrations of cactus mucilage coating (1%, 2% and 3%) were chosen for the current study based on past research that were done on effects of cactus mucilage on other fruits and fruit vegetables (Alikhani, 2014; Zegbe *et al.*, 2013)

Treatments application

Before treatment application, all fruits were disinfected by washing for 5 min using 0.5% (v/v) NaClO (Lerdthanangkul and Krochta, 1996). This was followed by air drying of fruit at room temperature ($25 \pm 2^\circ\text{C}$) until the disinfecting solution on fruit skin was completely dry. The fruit were dipped in 1 litre of

cactus mucilage solutions for 5 min based on the treatments (Alikhani, 2014) after which the excess coating was allowed to drain off. The fruits were air dried until the cactus mucilage on skin was completely dry allowing formation of a layer of coating on the fruit surface. Control fruit were dipped in distilled water for 5 min, removed and allowed to air-dry at room temperature ($25 \pm 2^\circ\text{C}$) until distilled water on fruit skin was completely dry. After treatments application, all fruit were stored on plastic trays under ambient conditions ($25 \pm 2^\circ\text{C}$ temperature and $65 \pm 2\%$ relative humidity) until they senesced.

Experimental design

The experiment was a single factor experiment arranged in a randomized complete block design, with 3 replications. Blocking was done against different harvesting times; harvesting of the 3 blocks was done at 1 month interval. In total, there were 12 experimental units with each experimental unit represented by a plastic tray containing 30 fruits.

Data collection

Data collection commenced immediately after treatments application and continued until fruit lost 25% of their initial weight (Sibomana *et al.*, 2015). Data collection was done on fresh weight loss and total soluble solids (TSS) at 3 days intervals; and iron and ascorbic acid content at 4 days intervals. Three fruits per experimental unit, were selected at random at the onset of the study, marked and used for data collection throughout the study for non-destructive variables which were fresh weight loss and shelf life. On the other hand, three fruits per experimental unit were also randomly selected from the remaining fruits and used to collect data for the destructive variables (TSS, iron and ascorbic acid content). Data for each destructive variable was collected from the three fruits with a new set of fruits used on each sampling date. The variables were determined as described below.

Fresh weight loss

The fresh weight (g) of the three selected fruits per experimental unit was measured using an electronic weighing balance (Denver Instrument XL-1810) immediately after treatment application (before storage). The same fruits were thereafter weighed at 3 days intervals until they lost 25% of their initial total weight. Progressive % fresh weight loss was determined using the formula by Moneruzzaman *et al.* (2008). Average % fresh weight loss of the 3 fruits was calculated and recorded as average % weight

loss per fruit for the time period (Moneruzzaman *et al.*, 2008). The shelf life of the fruit on the other hand was determined by counting the number of days the fruit took from harvesting to lose 25% of their initial weight (Sibomana *et al.*, 2015).

Total soluble solids content

Total Soluble Solids (%TSS) content was determined using a portable hand-held refractometer RHB-32/ATC (YHEQUIPMENT CO., LIMITED, Shenzhen City, China) as described by Opiyo and Ying (2005). A small piece of pepper fruit was cut, squeezed and the juice obtained dropped onto a refractometer and readings taken. Average %TSS of the 3 fruits was calculated and recorded as average %TSS per experimental unit for the time period.

Iron content

Iron (Fe) content was determined using an Atomic Absorption Spectrophotometer (model 210 VGP, Buck Scientific, Norwalk, CT) following Jones and Case (1990). Dried ground sample (1 g) was weighed into crucibles and ashes were obtained in a furnace at a temperature of 550°C for 2 h. The ash was cooled to room temperature ($25 \pm 2^\circ\text{C}$), transferred into a 100 mL beaker and 10 mL of the digestion mix added. Distilled water (50 mL) was added. Activated charcoal (1 g) was added to obtain a clear sample and stirred. The contents were gravity filtered through Whatman No.5 filter paper into a 100 mL volumetric flask. The filtrate was filled to the mark with distilled water. Into a cuvette, 10 mL of filtrate was pipetted and absorbance read at 248 nm. Iron standard solutions of 0, 5, 10, 15, 20 and 25 $\mu\text{g/g}$ were prepared from iron sulphate. Into a cuvette 10 mL of each standard was pipetted and absorbance read at 248 nm, and a standard curve developed. The amount of iron was calculated against the standards, converted to $\mu\text{g/g}$ and expressed using the formula of Okalebo *et al.* (2002).

Ascorbic acid content

Ascorbic acid (Vitamin C) was determined by titration with 2, 6-dichloro-phenol-indophenol dye following a standard procedure (AOAC, 1990). Using an electronic weighing balance (Denver Instrument XL-1810, USA), 10 g of fruit sample was weighed. The weighed fruit sample was extracted in 20 mL 5% oxalic acid using a mortar and pestle, and then gravity filtered through cotton wool. Ascorbic acid standard solution was prepared by dissolving 0.05 g of pure ascorbic acid in a small volume of 5% oxalic acid solution and then diluting to 250 mL with the same oxalic

acid solution. Ascorbic acid standard solution (10 mL) was titrated with 0.005% indophenol solution to a persistent slight pink colour end point and 10 mL of oxalic acid as a blank. The amount of ascorbic acid corresponding to 1 mL of indophenol solution was calculated. Into a 50 mL flask, 10 mL of the gravity filtered sample extract was pipetted and made to the mark with the 5% oxalic acid solution. The standard indophenol solution was used to titrate 10 ml of the filtrate to a slight pink end point. Vitamin C content was calculated following Obel *et al.* (2019).

Data analysis

All the data were subjected to analysis of variance (ANOVA) in SAS (ver. 9.0, SAS Institute Inc., Cary, NC). Significant means at F-Test were separated using Tukey's Honestly Significant Difference (SAS, 2010).

3. Results

Effect of cactus mucilage coating on fresh weight loss and shelf life of bell pepper fruit

Cactus mucilage treatments significantly reduced fresh weight loss of bell pepper fruits from 3 DAH (days after harvest) to the end of storage (Fig. 1). At 3 DAH, fruits coated with 1, 2 and 3% cactus mucilage recorded a significantly lower weight loss as compared to a higher weight loss observed for the control treatment (distilled water) (Fig. 1). A similar trend was observed at 6 DAH and 9 DAH. At 12 DAH, weight loss of fruits coated with 1% cactus mucilage was significantly lower as compared to weight loss of

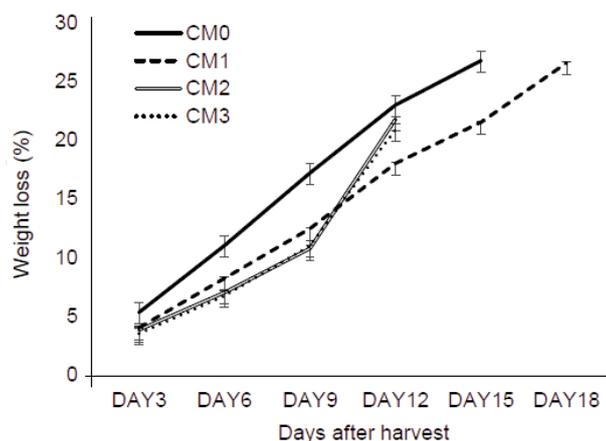


Fig. 1 - Effect of cactus mucilage coating on weight loss of bell pepper. Error bars indicate \pm SE of the mean. CM0 is fruit coated with 0% cactus mucilage, CM1 is fruit coated with 1% cactus mucilage, CM2 is fruit coated with 2% cactus mucilage and CM3 is fruit coated with 3% cactus mucilage.

fruits coated with 0, 2 and 3% cactus mucilage (Fig. 1). Fruits coated with 1% cactus mucilage treatment recorded a significantly lower weight loss as compared to the control fruits at 15 DAH (Fig. 1)

Application of cactus mucilage treatments significantly extended shelf life of bell pepper fruit by up to 6 days during storage (Fig. 2). Fruits coated with 1% cactus mucilage recorded a significantly longer shelf life, followed by a shelf life recorded for the control fruits with the shortest shelf life recorded for the fruits coated with 2 and 3% cactus mucilage (Fig. 2).

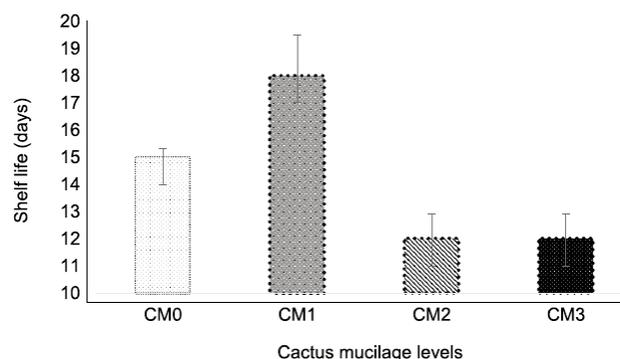


Fig. 2 - Effect of cactus mucilage coating on shelf life of bell pepper. Error bars indicate \pm SE of the mean. CM0 is fruit coated with 0% cactus mucilage, CM1 is fruit coated with 1% cactus mucilage, CM2 is fruit coated with 2% cactus mucilage and CM3 is fruit coated with 3% cactus mucilage.

Effect of cactus mucilage coating on total soluble solids of bell pepper fruit

Cactus mucilage coating had a significant effect on total soluble solids (TSS) content of bell pepper fruit from 3 DAH through the end of storage (Fig. 3). In addition, there was an increase in TSS content of bell pepper fruit as storage duration progressed except for fruits coated with 2 and 3% cactus mucilage where a decrease was observed from 9 DAH (Fig. 3). At 3 DAH, fruits coated with 2 and 3% cactus mucilage treatments recorded a significantly lower TSS content, followed by TSS content recorded for fruits coated with 1% cactus mucilage with the highest TSS content recorded for the control treatment (Fig. 3). The trend was the same at 6, 9 and 12 DAH of storage. At 15 DAH, fruit coated with 1% cactus mucilage recorded a significantly lower TSS content as compared to a higher TSS content recorded for the control treatment (Fig. 3).

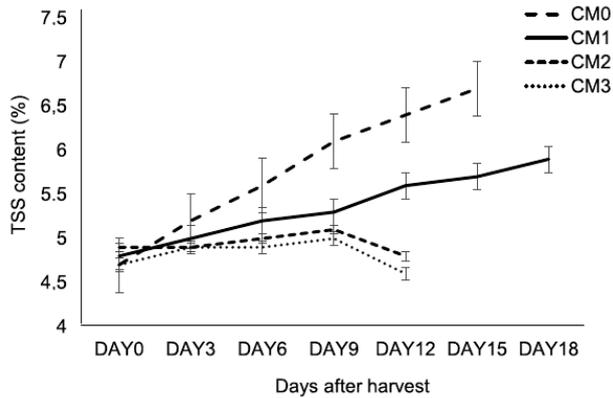


Fig. 3 - Effect of cactus mucilage coating on total soluble solids content of bell pepper. Error bars indicate \pm SE of the mean. CM0 is fruit coated with 0% cactus mucilage, CM1 is fruit coated with 1% cactus mucilage, CM2 is fruit coated with 2% cactus mucilage and CM3 is fruit coated with 3% cactus mucilage.

Effect of cactus mucilage coating on iron content of bell pepper fruit

Cactus mucilage coating also had a significant effect on iron content of bell pepper fruit from 4 DAH until the end of storage (Fig. 4). At 4 DAH, fruit coated with 1, 2 and 3% cactus mucilage recorded a significantly higher iron content as compared to a lower iron content recorded for 0% cactus mucilage treatment (Fig. 4). A similar trend was observed at 8 DAH (Fig. 4). At 12 DAH, a significantly higher iron content was recorded for fruits coated with 1% cactus mucilage as compared to a lower content recorded for 0, 2 and 3% cactus mucilage treatments (Fig. 4). Fruits coated with 1% cactus mucilage recorded a significantly higher iron content as compared to a lower content recorded under the control treatment (Fig. 4).

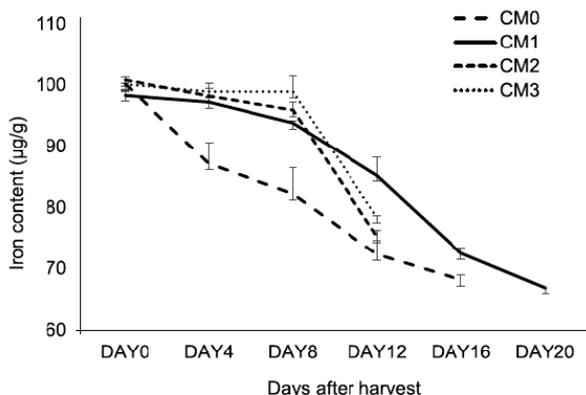


Fig. 4 - Effect of cactus mucilage coating on iron content of bell pepper. Error bars indicate \pm SE of the mean. CM0 is fruit coated with 0% cactus mucilage, CM1 is fruit coated with 1% cactus mucilage, CM2 is fruit coated with 2% cactus mucilage and CM3 is fruit coated with 3% cactus mucilage.

Effect of cactus mucilage coating on ascorbic acid content of bell pepper fruit

Ascorbic acid content in bell pepper fruit was influenced by cactus mucilage treatments during storage from 4 DAH through 16 DAH (Fig. 5). At 4 DAH, fruits coated with 1, 2 and 3% cactus mucilage recorded a significantly higher ascorbic acid content as compared to a lower ascorbic acid content recorded for the control treatment (Fig. 5). A similar trend was observed at 8 DAH (Fig. 5). A significantly higher ascorbic acid content was recorded for fruit coated with 1% cactus mucilage as compared to a lower ascorbic acid content recorded for 0, 2 and 3% cactus mucilage treatments at 12 DAH (Fig. 5). At 16 DAH, fruit coated with 1% cactus mucilage recorded a higher ascorbic acid content as compared to a lower ascorbic acid content recorded under the control treatment (Fig. 5).

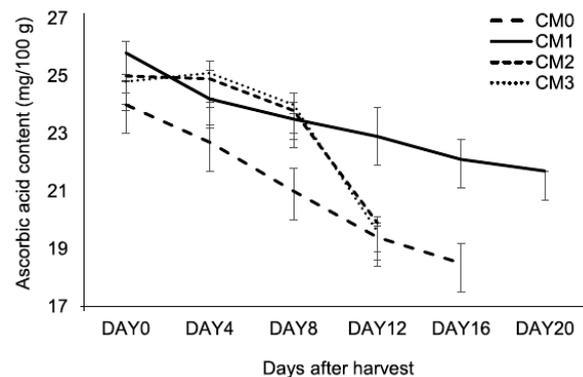


Fig. 5 - Effect of cactus mucilage coating on ascorbic acid content of bell pepper. Error bars indicate \pm SE of the mean. CM0 is fruit coated with 0% cactus mucilage, CM1 is fruit coated with 1% cactus mucilage, CM2 is fruit coated with 2% cactus mucilage and CM3 is fruit coated with 3% cactus mucilage.

4. Discussion and Conclusions

Effect of cactus mucilage coating on fresh weight loss and shelf life of bell pepper fruit

Fresh weight loss is an important index in determining postharvest quality and shelf life of pepper. Weight loss in harvested fruit is normally caused by continuous loss of water and stored starch as a result of respiration and evaporation leading to increase in weight loss as storage duration progresses. Cactus mucilage coating forms a film on the fruit's skin/cuticle which acts as a semi-permeable barrier against moisture, oxygen, carbon (IV) oxide, and solute

movement in the produce or between the produce and its environment. This leads to reduced rate of respiration, reduced water loss, starch or sugar loss, weight loss and extended shelf life. This could explain the reduced weight loss and extended shelf life recorded for fruit coated with 1% cactus mucilage compared to control fruit in the current study. Many studies have also reported reduced weight loss and extended shelf life in fruits and vegetables as a result of polysaccharide-based edible coatings (Menezes and Athmaselvi, 2016; Vishwasrao and Ananthanarayan, 2016). A thick layer of fruit coating blocks pores on the fruit's skin, decrease oxygen concentration in the fruit's tissues since oxygen in the fruit's environment cannot get inside and the respiration products cannot also get outside the fruit's tissues. Anoxic conditions initiated leads to ethanol fermentation in which stored carbohydrates and sugars are broken down to lactic acid, ethanol, acetaldehyde and carbon (IV) oxide which explains reduced fresh weight loss observed for fruits coated with 2 and 3% cactus mucilage during storage in the current study. Increased weight loss caused by anaerobic conditions led to a shorter shelf life of fruits. Fermentation bacteria and yeast proliferate the bell pepper tissues and breaks down stored carbohydrate, sugar, water and minerals for their growth and other metabolic activities. This explains the increased rate of fresh weight loss observed after 9 DAH of storage for fruits coated with 2 and 3% cactus mucilage. According to Kareem *et al.* (2017), anaerobic respiration leads to a decrease in stored carbohydrate content in fruits due to the utilization of some of the sugars by the fermenting organisms such as lactic acid bacteria for their growth and other metabolic activities.

Effect of cactus mucilage coating on total soluble solids of bell pepper fruit

Fruit TSS content tends to increase during storage due to biosynthesis of polysaccharides and accumulation of sugars during ripening (Ullah *et al.*, 2017) and volatilization of soluble compounds and water. At advanced stages of ripening, disassociation of some molecules and structural enzymes in soluble compounds results in increased levels of TSS. A slower rate of increase in TSS content in cactus mucilage coated fruit observed in the current study could be attributed to the role of fruit coatings acting as a barrier against oxygen, carbon IV oxide and ethylene, slowing down the rate of respiration and ripening leading to reduction in accumulated sugars and polysaccharides. Increased TSS content in con-

trol (uncoated) bell pepper fruit could be due to volatility of soluble compounds and water at a faster rate due to lack of a protective barrier on the surface of such fruit. In addition, possible accumulation of sugars and polysaccharides as a result of increased rate of hydrolysis could have led to increased TSS content in control fruits. These results are consistent with that of Menezes and Athmaselvi (2016) in sapota (*Manilkara zapota*). Ethanol fermentation lowers TSS content in fruits due to development of off-flavours as observed for fruits coated with 2 and 3% cactus mucilage. Ethanol fermentation is a two-step process in which pyruvate is first carboxylated to acetaldehyde by Pyruvate Decarboxylase and acetaldehyde is subsequently converted to ethanol by Alcohol Dehydrogenase. This explains the lower TSS content observed for fruits coated with 2% and 3% cactus mucilage as compared to those coated with 1% and dipped in distilled water during storage.

Effect of cactus mucilage coating on iron content of bell pepper fruit

Results of this study indicate that coating bell pepper fruit with cactus mucilage may preserve the fruit's iron content. Iron is stored in fruit's tissues in the chloroplast where 80% of the iron is located as iron-protein complexes known as Fe-phytoferritin, Fe-citrate, Fe-phytosiderophore and Fe-nicotianamine (Maathuis and Diatloff, 2013). Iron is necessary for the synthesis of many proteins (ferredoxin and cytochromes) that carry electrons during respiration in which most iron ions are used to biosynthesize proteins that carry electrons (Bhatla and Lal, 2018). Cactus mucilage coating acts as a barrier against O₂ and CO₂ inside and out of the fruit, thus reducing the rate of respiration and therefore reducing the amount of iron ions used to synthesize proteins that carry electrons during respiration. This could explain the high amount of iron in fruit coated with cactus mucilage during the current study. Rapid decline of iron content in fruit that were dipped in distilled water could possibly have been due to increased respiration rate as a result of increase in O₂ and decreased CO₂ inside and out of the fruit. Results of the current study are consistent with those of Amirthaveni and Daga (2016) who recorded higher iron content in bell pepper coated with *Aloe vera* gel and gum Arabic. Ethanol fermentation causes decline in minerals such as iron in the fruit. The nutrients are utilised by yeasts and lactic acid bacteria as they carry out their metabolism and fermentation activity. In

addition, their growth is supported by the existence of basic compounds such as fermentable minerals. This could offer an explanation for the result observed on fruits coated with 2 and 3% cactus mucilage during storage. These findings are supported by Kareem *et al.* (2017) and Maicas (2020) who reported utilization of minerals by lactic acid bacteria for growth and other metabolic activities during fermentation of fruits.

Effect of cactus mucilage coating on ascorbic acid content of bell pepper fruit

Ascorbic acid is commonly used as a quality indicator of fruits and vegetables since it is very sensitive to degradation due to its oxidation compared to other nutrients during food processing and storage. Plants biosynthesize ascorbic acid mainly through the Smirnoff-Wheeler pathway. In the final step of ascorbic acid synthesis, galactono-1,4-lactone is oxidized by galactono-1,4-lactone dehydrogenase (GLDH) to produce ascorbic acid. Ascorbic acid produced reduces during storage due to degradation mainly through the direct oxidation of dehydroascorbate (DHA) or 4-O-oxalyl-L-threonic acid to produce both oxalic acid and L-threonic acid. In the current study, cactus mucilage coating could have acted as a barrier against oxygen gas that enters the fruit thereby reducing oxidation of dehydroascorbate (DHA) or 4-O-oxalyl-L-threonic acid resulting in higher amounts of ascorbic acid in the fruit during storage. A rapid decrease in ascorbic acid in uncoated fruits could be attributed to increased oxidation of dehydroascorbate (DHA) or 4-O-oxalyl-L-threonic acid due to increased oxygen concentration in the fruit tissues. Results of this study are in agreement with those of a number of scholars who also reported oxidation reactions in the presence of oxygen in uncoated fruits during storage leading to reduction of ascorbic acid (Menezes and Athmaselvi, 2016; Ullah *et al.*, 2017). A rapid decrease in ascorbic acid content observed for fruits coated with 2 and 3% cactus mucilage during storage was attributed to ethanol fermentation of fruits as a result of low oxygen concentration in the fruit tissues. During fermentation, microorganisms such as yeasts and lactic acid bacteria uses nutrients such as minerals like ascorbic acid for their growth, reproduction and other metabolic activities leading to a decrease in ascorbic acid content in fermenting fruits.

Based on the objective and findings of this study, it can be concluded that cactus mucilage coating significantly influenced postharvest quality and shelf life

of bell pepper. One % cactus mucilage coating was the best treatment in terms of fresh weight loss reduction, maintenance of total soluble solids, iron, ascorbic acid content, and extension of shelf life of bell pepper fruit.

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