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CHILLING INJURY AND PHYSICO-CHEMICAL ATTRIBUTES OF MANGO FRUIT INFLUENCED BY LOW TEMPERATURE STORAGE

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ABSTRACT

Mango fruit can not be stored for longer time at ambient temperature due to its highly perishable nature. Conversely, low temperature storage may also lead to chilling injury. Therefore, the storage performance of mango cv. 'Langra' fruit at different temperatures was investigated to optimize the temperature for its storage. Mango fruits were stored at 5, 10 and 15 ± 1 ºC for 0, 5, 10, 15, 20, 25 and 30 days and evaluated for weight loss (%), fruit juice pH, ascorbic acid, chilling injury score and ion leakage (%) after completion of the respective storage period. Weight loss and pH increased with the increase in temperature and storage duration. The ascorbic acid exhibited an inverse relation with storage temperature and duration. Chilling injury score increased with the extension in storage duration but was lower at higher temperatures. Ion leakage (%) was the highest in fruits exposed to 15 ± 1 ºC followed by 5 ± 1 ºC whereas, it was the least at 10 ± 1 ºC and increased when storage duration was prolonged. It was concluded that chilling injury occurs after 10 days in mango cv. 'Langra' fruit at 5 ± 1 ºC and if no technology is available for alleviation of chilling injury, it may be stored at 10 ± 1 ºC instead of 5 ± 1 ºC.

Key words: Mango, storage, temperature, chilling injury, ion leakage.

INTRODUCTION

Mango (*Mangifera indica* L.) is an important member of family Anacardiaceae and is also known as the king of fruits. It is native to Indo-Burmese region (Subramanyam *et al.,* 1975; Islam and Rab, 2016a) and is widely cultivated in the areas with tropical and subtropical climatic conditions. Nutritionally, it is an important fruit (Wen *et al.,* 2006) and is a rich source of carbohydrates, vitamins, minerals, proteins and fats (Talcott *et al.,* 2005; Iqbal *et al.,* 2012). Pakistan stands fourth (Maqbool *et al.,* 2007) with a share of 7.8% in the world mango export (Sauco, 2004). Mango fruit is predominantly consumed as fresh but the production season is very short therefore, it is necessary to store it under appropriate conditions to ensure its availability for longer time (Pesis *et al.,* 2000). However, the increasing physiological processes with the extension in storage duration usually lead to the reduction in fruit quality (Herianus *et al.,* 2003; Islam and Rab, 2016b).

Mango is a climacteric fruit, characterized by high rates of respiration (Wongmetha *et al.,* 2016) and ethylene production (McCollum *et al.,* 1993). Hence it is a highly perishable fruit (Gil *et al.,* 2000) and susceptible to postharvest losses (Amin *et al.,* 2008). Generally, mature green mango fruit is harvested to extend the postharvest life as it takes 9 to 12 days for ripening process after harvest (Herianus *et al.,* 2003). Due to the highly perishable nature of mango fruit, the postharvest handling technologies have a significant influence on its quality and consumers' acceptance (Rathore, 2007). This high perishability also limits its international trade (Gil *et al.,* 2000), especially where long distance transport is through sea.

Various technologies such as controlled atmosphere storage (Ali *et al.,* 2016), modified atmosphere storage (Giuggioli *et al*., 2014), use of different coatings (Abbasi *et al.,* 2009) and application of different chemicals such as methyl jasmonate (Gonzalez-Aguilar *et al.,* 2000), 1-MCP (Manganaris, *et al.,* 2007), methyl salicylate (Junmatong *et al.,* 1212) etc. have been tested to reduce the physiological changes taking place in various fruits during storage. The quality of fruits and vegetables can be maintained by temperature management. Low temperature reduces the metabolic processes and prolongs the postharvest life of fruits and vegetables (Sivakumar *et al.,* 2011). The temperate fruits can easily be stored at low temperature (0-5°C). However, the tropical and subtropical fruits may undergo chilling injury at low temperatures (Patel *et al*., 2016). Mango is also sensitive to low temperature (below 10 ºC) but the sensitivity varies among different cultivars because some varieties may be stored at temperature as low as 7 ºC while others develop chilling injury at 13 ºC (Mitra and Baldwin, 1997). Lenticel browning, surface discoloration, internal break down, surface pitting and decay are the major symptoms of chilling injury in mango fruit (Kader, 1992: Mitra and Baldwin, 1997; Miguel *et al.*, 2016).

Keeping in view the importance of low temperature application and its limitations for mango

fruit, this experiment was designed to examine the chilling sensitivity and quality of mango cv. 'Langra' fruit at various temperatures and storage durations.

MATERIALS AND METHODS

Experimental materials and design: Mango fruits of cv. 'Langra' were harvested at physiological maturity from mango orchard at Agricultural Extension Farm, Dera Ismail Khan during June, 2012. The fruits were transported in corrugated card board boxes on the same day to the laboratory at Swabi University. The fruits were washed thoroughly and dried with a gentle blow of air from an electric fan. Well graded fruits were grouped and allotted to all treatments of storage durations i.e. 0, 5, 10, 15, 20, 25 and 30 days (factor A) and temperatures i.e. 5, 10 and 15 ± 1 °C (factor B). As on day "0" no weight loss occurs so this treatment was not considered for weight loss.

The experiment was laid out in completely randomized design with three repeats. Ten fruits were included in each treatment per repeat. After completion of the respective storage duration fruits were analyzed for physical and chemical attributes.

Procedures of sample analysis and data collection: To determine the weight loss, 10 fruits of each treatment per repeat were marked separately and weighed with a digital balance. Then a final reading was taken on the completion of every storage interval for each fruit. The percent weight loss was calculated by the following formula.

$$Weight\ loss\ (\%) = \frac{Initial\ \ fruit\ weight - Final\ \ fruit\ weight}{Initial\ \ fruit\ weight} \times 100$$

Fruit juice was prepared separately for each treatment and a portable pH meter (Model, KL-097, China) was used for measurement of pH. The bulb of meter was dipped to the marked point in juice randomly at four different points in the juicer and reading was recorded. Then an average of all four readings for each treatment was calculated and recorded.

The standard method of AOAC (2000) was used for determination of ascorbic acid. The dye solution was prepared by dissolving 0.5 g 2, 6-dichlorophenol indophenol and 0.42 g sodium bicarbonate in 200 ml hot distilled water. The volume was made 250 ml through distilled water and kept at a cool and dark place. Similarly, 4 g oxalic acid was dissolved in 1 liter distilled water to get 0.4% oxalic acid solution. Then standard ascorbic acid solution was prepared by dissolving 50 mg standard ascorbic acid in 50 ml oxalic acid (0.4%). A solution of 1 ml standard ascorbic acid and 25 ml oxalic acid (0.4%) taken in a titration flask was titrated against the dye till the appearance of pink color. The dye factor was calculated as under.

$$\text{Dye factor} = \frac{1}{Volume\ \ of\ \ dye\ \ used\ \ \ from\ \ \ burrette}$$

Mango fruit juice of 20 g was dissolved in 100 ml oxalic acid (0.4%) and diluted to 200 ml with the same oxalic acid. A 10 ml aliquot taken in a titration flask from this solution was titrated against the dye till the appearance of pink color. The reading was recorded and ascorbic acid in the juice sample was calculated with the following formula.

$$\text{Ascorbic acid (mg/100g)} = \frac{Titration\ reading \times dye\ factor \times 200 \times 100}{Weight\ of\ sample \times 10}$$

Where,

200 = dilution of juice solution used

10 = aliquot of juice solution taken for titration

For chilling injury score, the method of McCollum *et al.* (1993) was used with slight modifications. Browning, surface pitting and lenticel discoloration of fruits were used as indicators for chilling injury. It was rated on a scale from 1-5 as, 1 = No chilling injury, 2 = 1-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100% chilling injury. The calculation of chilling injury score was carried out by the following formula.

$$Chilling\ Injury\ Score = \frac{Injury\ level\ \times\ Number\ of\ \ fruits\ at\ the\ level}{Total\ number\ of\ \ fruits\ in\ treatment}$$

For measurement of ion leakage the method as described by Saltveit *et al.* (2004) with slight modifications, was used. Skin discs of 5 mm diameter were removed from the fruit through a cork borer on a tissue paper. The pulp was removed through surgical blades and then 10 discs per treatment were placed in conical tubes having 25 ml of 0.3 M manitol solution. The tubes were shaken for 30 minutes over rotary shaker and initial conductivity was recorded using a conductivity meter (Sartorious Professional Meter pp-20). The final reading was taken after three freeze-thaw cycles and considered as the total ion leakage. The ion leakage from skin was calculated as under:

$$\text{Ion Leakage (\%)} = \frac{Ion\ \ leakage\ \ after\ \ 30\ \min}{Total\ \ ion\ leakage} \times 100$$

Statistical analysis: The data collected were subjected to Analysis of Variance (ANOVA) using statistix 8.1 software (Analytical Software, 2003). Upon significant differences, the LSD (least significant difference) was applied for separation of means (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Weight loss (%): The percent weight loss varied significantly with storage durations, storage temperatures and their interactions (Table 1). The weight loss increased with the extension in storage duration and the highest weight loss (8.02%) was observed in fruits stored for 30 days followed by 6.80 and 5.55% at 25 and 20 days storage durations, respectively. The least weight loss

(3.22%) was recorded in the fruits stored for 5 days. Similarly, the weight loss increased with the rise in storage temperature and the maximum weight loss (7.92 %) was in fruits stored at 15 ± 1 ºC followed by 10 ± 1 ºC (5.52%) whereas, weight loss was as low as 3.06 % in fruits stored at 5 ± 1 ºC. The interaction of storage durations and temperatures (Fig.1) revealed an increase in weight loss with the increase in both factors. Fruits stored at 15 ± 1 ºC for 30 days had the highest percent weight loss (11.11 %) followed by storage at the same temperature for 25 days (9.65%) whereas, the least weight loss (1.98%) was recorded in fruits stored for 5 days at 5 ± 1 ºC followed by storage on the same temperature for 10 days (2.26%). The percent weight loss during 30 days study ranged from 1.98 to 4.21% at 5 ± 1 ºC, 3.07 to 8.74% at 10 ± 1 ºC and 4.60 to 11.11% at 15 ± 1 ºC.

The weight loss in fruit during storage is due to moisture loss (Doreyappa-Gowda and Huddar, 2001). Weight loss occurs due to evapotranspiration and respiration (Lebibet *et al*., 1995; Ahmad *et al.,* 2001). It is a biological activity and depends on storage duration (Rathore, 2007) as well as temperature (Perez *et al.,* 2004). Therefore, the weight loss increased with increasing storage duration. The increase in weight loss with the increasing storage time is common in stored fruits (Al- Obeed and Horhash, 2006). While working on sweet orange cv. Blood red, Haq *et al*., (2018) also reported an increase in the weight loss with the increasing storage duration. Similarly, increasing storage temperature also increased the weight loss. By contrast, weight loss was the least at the lowest temperature under study (5±1°C) due to less moisture loss (Tembo *et al*., 2008). Since, the weight loss depends both on storage duration and temperature (Arjona *et al*., 1992), it is likely to observe greater weight loss with increasing storage duration (30 days) at higher (15±1°C) than lower (5±1°C) temperature. The results are in line with the findings of Arendse *et al*. (2014) who reported increase in weight loss with the increasing storage time and temperature in pomegranate fruits.

Fruit juice pH: The influence of storage durations, temperatures and their interaction on the fruit juice pH was significant (Table 1). The highest pH (5.17) was recorded in 30 days storage duration followed by 4.87 in fruits stored for 25 days while it was the least (3.94) at day 0 of storage. Similarly, the pH of fruit juice increased with the rise in temperature and was maximum (4.85) at 15 ± 1 ºC followed by 4.58 at 10 ± 1 ºC whereas, the minimum pH (4.15) was recorded at 5 ± 1 ºC. The interaction of storage durations and temperatures indicated that the fruit juice pH increased with the increase in both storage temperature and duration. The least increase was observed at 5 ± 1 ºC which ranged from 3.93 at 0 day to 4.33 at 30 day while the maximum at 15 ± 1 ºC (Fig. 2).

The pH depends on the organic acids in the fruit (Sahari *et al.*, 2004). The organic acids are progressively converted to other compounds with increasing storage duration, thus, increasing the pH of fruit juice (Rab *et al.,* 2011). The results are in agreement with the findings of Nadeem *et al*. (2017) who reported an increase in pH of persimmon fruits with the increasing storage time. Furthermore, at higher temperature, more organic acids are used as substrate in respiration (Pesis *et al.,* 1999) thus resulting in higher pH. The interaction of storage durations and temperatures revealed a much slower increase in the pH with storage at 5 ± 1 ºC as compared to 10 and 15 ± 1 ºC which could be attributed to retention of more organic acids due to slow conversion into respiratory substrate (Ezz and Awad, 2011). As the fruit juice pH increased with the increase in storage duration and temperature hence, the highest pH was at 15 ± 1 ºC on day 30.

Ascorbic acid (mg/100 g): Ascorbic acid content was significantly influenced by storage durations, storage temperatures and their interactions (Table 1). The ascorbic acid content decreased with the increasing storage duration. The maximum (33.85 mg/100g) ascorbic acid content at day 0 decreased to 29.17mg/100g after 5 days and finally to the minimum (19.81mg/100g) in fruits stored for 30 days. Similarly, an inverse relation was found between temperature and ascorbic acid content of fruits with the highest ascorbic acid (29.59 mg/100g) in fruits stored at 5 ± 1 ºC and the least (24.51 mg/100g) at 15 ± 1 ºC. The interaction of storage durations and storage temperatures showed a declining trend in the ascorbic acid content with the increase in both storage temperature and duration. Hence, the lowest ascorbic acid content was with 30 days storage at 15 ± 1 ºC (Fig. 3).

The increase in pH and enzymatic activities causes the conversion of L-ascorbic acid to dihydroascorbic acid (Bashir and Abu-Goukh, 2002), thus resulting in the decline of the ascorbic acid content during storage (Teruel *et al.,* 2000). The relative high ascorbic acid at lower temperature may be attributed to its stability in acidic environment (Wechtersbach and Cigic, 2007). Since the fruits stored at 5 ± 1 ºC maintained higher acidity as compared to 10 and 15 ± 1 ºC therefore, the ascorbic acid content of these fruits was also higher. Furthermore, the higher metabolic processes at 15 ± 1 ºC might have added to the decline in ascorbic acid content (Ezz and Awad, 2011). The ascorbic acid decreased with the extended storage. The results are in line with the findings of Jan *et al*. (2012) who reported a decrease in the ascorbic acid content of different apple cultivars with the increase in storage time. A declining trend in ascorbic acid was observed at all temperatures with the passage of time. However, the greatest reduction

with extended storage at 15 ± 1 ºC could be due to the accelerated metabolic processes (Tembo *et al.,* 2008).

Chilling injury score: The results revealed that storage durations, storage temperatures and their interaction significantly affected the incidence of chilling injury in mango fruit (Table 1). With the increase in storage duration, the chilling injury intensified. The chilling injury score was maximum (2.27) in fruits stored for 30 days followed by 1.98 after 25 days whereas, the lowest chilling injury score (1.00) was recorded at 0 and 5 days storage where no chilling injury was observed. Based on response to temperature treatments the chilling injury score was the highest (2.06) when the fruits were stored at 5 ± 1 ºC while at 15 ± 1 ºC it was the least (1.12). The interaction of storage durations and temperatures indicated delay in chilling injury with the increase in storage temperature. The earliest chilling injury symptoms appeared at 5 ± 1 ºC after 10 days of storage followed by 10 ± 1 ºC where the onset of chilling injury happened after 20 days. After 30 days storage, the highest chilling injury score (3.47) was measured at 5 ± 1 ºC whereas, the lowest results (1.57) were recorded at 15 ± 1 ºC (Fig. 4).

Initially the mango fruits were free of physiological chilling injury which appeared on day 10th and increased with the increase in storage duration. Chilling injury is a physiological disorder and occurs due to oxidative stress at low temperature (Scandalios, 1993). Low temperature increases the synthesis of active oxygen species (AOS) and reduces efficiency of antioxidants (Bartosz, 1997; Sala and Lafuente, 2000) that affect the cell membrane integrity and flexibility (Bartosz, 1997; Purvis and Shewfelt, 1993). The reduced flexibility of cell membrane causes chilling injury symptoms (Leshem, 1992). The membrane damage increases with increasing storage duration (Junmatong, *et al* 2012). The rapid increase in chilling injury with the prolong storage at 5 ± 1 ºC and negligible chilling injury at 15 ± 1 ºC after 25 days indicated that decreasing temperature added to the AOS synthesis that increased chilling injury. These results confirmed the findings of Nair and Singh (2009) who after studying Kensington pride mango under 0, 5, 10, 15 and 20 °C for 1, 3, 7, 14, 21 and 28 days, reported that chilling injury development is a function of storage time and temperature.

Ion leakage (%): The ion leakage was significantly influenced by storage durations, temperatures and their interaction (Table 1). The ion leakage increased with the increase in storage duration, reaching a maximum value of 35.43% for fruits of 30 days. The ion leakage was minimum (11.77%) at 0 day of storage. In terms of temperature response, the highest ion leakage (27.04%) was recorded in fruits stored at 15 ± 1 ºC followed by 21.35% at 5 ± 1 ºC whereas, ion leakage was the least (20.95%) at 10 ± 1 ºC. The interaction of storage durations and temperatures revealed that ion leakage increased with increase in storage duration and temperature initially. While ion leakage was the highest at 15 ± 1 ºC but the increase was higher in fruits stored at 5 ± 1 ºC than 10 ± 1 ºC after 20 days storage.

Table 1. Influence of storage temperatures and durations on mean weight loss (%), titratable acidity (%), ascorbic acid (mg/100g), chilling injury score and ion leakage (%) during storage

Treatments	Weight loss (%)	Fruit juice Ph	Ascorbic acid (mg/100 g)	Chilling injury score	Ion leakage (%)
Temperature (T , °C)					
5	3.06 c	4.15 c	29.59 a	2.06 a	21.35 b
10	5.52 b	4.58 b	26.34 b	1.22 b	20.95 c
15	7.92 a	4.85 a	24.51 c	1.12 c	27.04 a
LSD at α = 0.05	0.37	0.03	0.71	0.07	0.37
Storage duration (SD, days)					
0	-	3.94 g	33.85 a	1.00 f	11.77 g
5	3.22 e	4.23 f	29.17 b	1.00 f	15.51 f
10	4.31 d	4.38 e	28.32 bc	1.12 e	18.75 e
15	5.12 c	4.48 d	27.41 c	1.24 d	21.92 d
20	5.55 c	4.60 c	25.88 d	1.64 c	27.60 c
25	6.80 b	4.87 b	23.28 e	1.98 b	31.01 b
30	8.02 a	5.17 a	19.81 f	2.27 a	35.43 a
LSD at α = 0.05	0.53	0.52	1.10	0.10	0.57
Interaction					
T x SD	**	**	**	**	**

** = Significant at p≤0.01; In each category, means followed by different letter (s) are significantly different from each other at 5% level of probability using LSD test.

The ion leakage is a common measure of senescence (Ahmad *et al.*, 2001) or chilling injury (Saltveit, 2002). It increased with extension in storage duration that may be due to the increasing chilling injury and ripening (Sfakiotakis *et al.,* 2005; Suwapanich and Haewsungcharoen, 2007). The increased ion leakage at 15 ± 1 ºC may be due to natural ripening. However, higher ion leakage at 5 ± 1 ºC than 10 ± 1 ºC is because of structural changes and disruption of plasmalemma (Saltveit, 2002; Suwapanich and Haewsungcharoen, 2007). The bilayers are made up of several types of lipids and proteins. Unsaturated fatty acids increase the flexibility of cell membrane thus imparting resistance to low temperature injury but the onset of chilling injury causes an imbalance in the unsaturated to saturated fatty acids ratio. This disturbance in the unsaturated to saturated fatty acids ratio converts the cell membrane from a liquid crystalline state to a solid gel state which is prone to crakes, that in turn results in more ion leakage (Sfakiotakis *et al.,* 2005). The interaction of storage temperature and duration resulted an increase in ion leakage with the increasing storage temperature and duration. However, fruits stored at 5 ± 1 ºC showed an abrupt rise in the ion leakage on day 20th which may be explained by the higher chilling injury at the same temperature and storage duration (Antunes and Sfakiotakis, 2008).

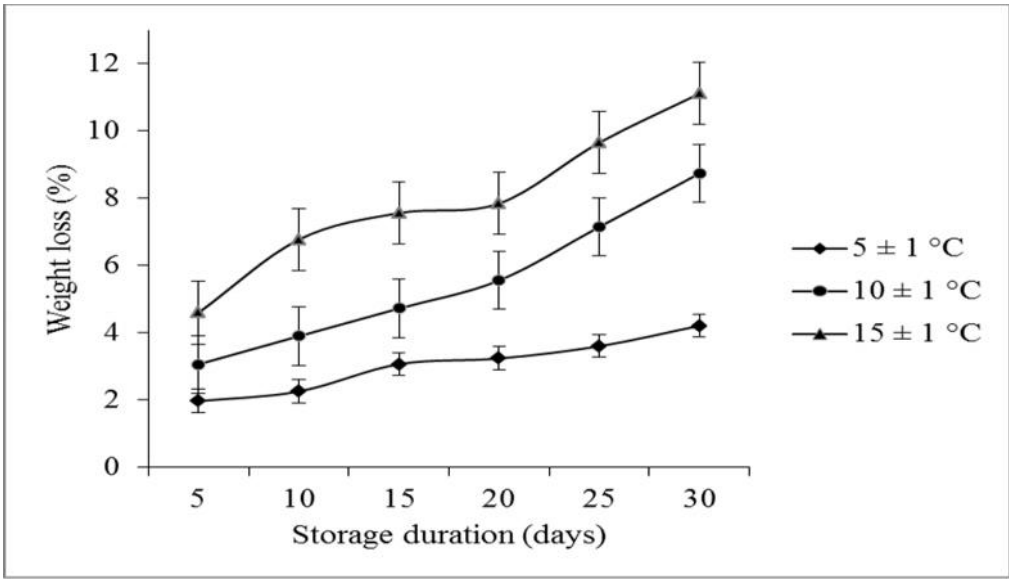

Fig. 1. The interaction of temperature and storage duration for weight loss (%). Vertical bars are standard errors of means.

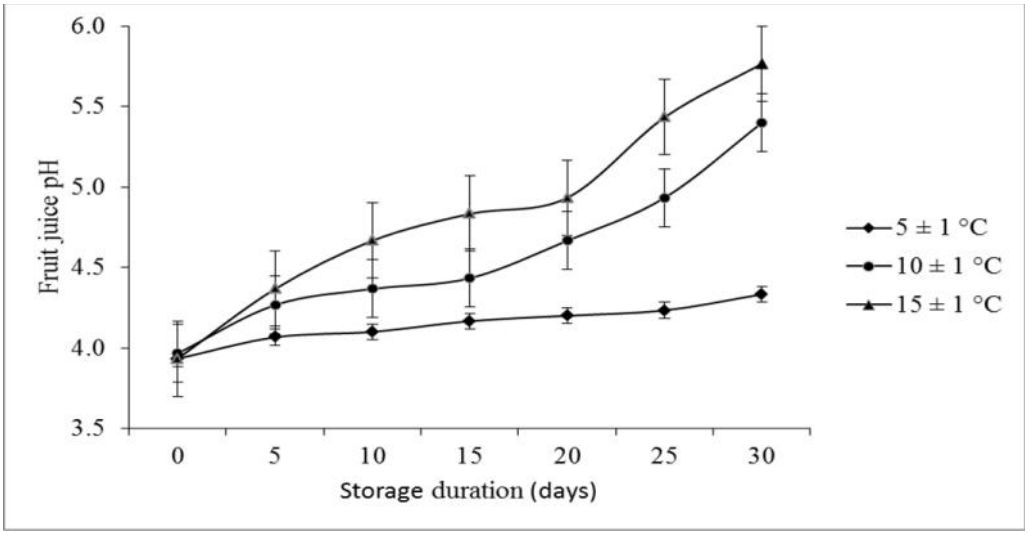

Fig. 2. The interaction of temperature and storage duration for fruit juice pH. Vertical bars are standard errors of means.

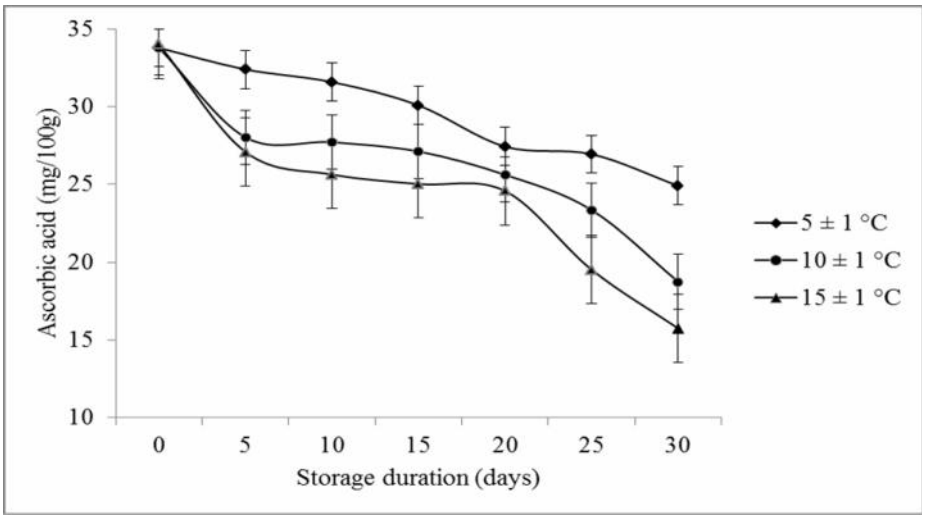

Fig. 3. The interaction of temperature and storage duration for ascorbic acid (mg/100 g). Vertical bars are standard errors of means.

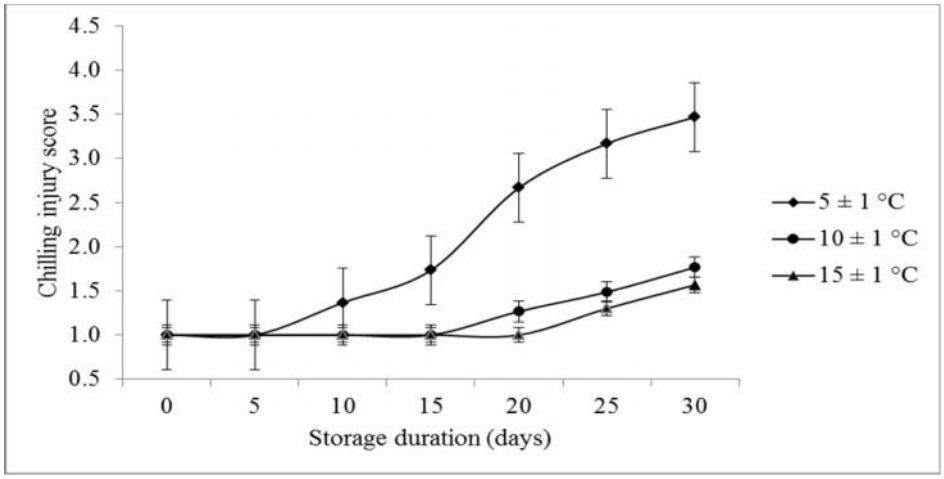

Fig. 4. The interaction of temperature and storage duration for chilling injury score. Vertical bars are standard errors of means.

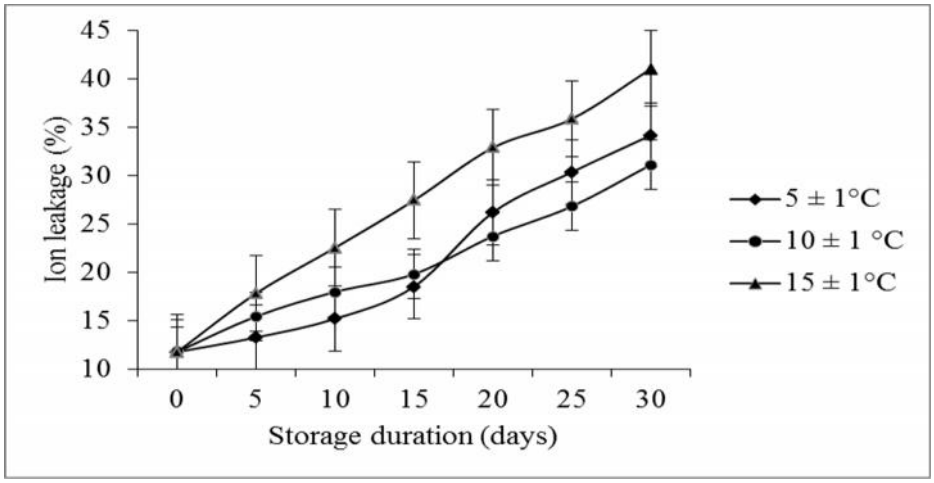

Fig. 5. The interaction of temperature and storage duration for ion leakage (%).Vertical bars are standard errors of means.

Conclusion and Recommendations: It can be concluded from this study that storage of mango cv. 'Langra' fruit at 5 ± 1 °C showed better results for quality attributes but suffered chilling injury as evident from chilling injury score and ion leakage. Chilling injury developed after 10 days in mango cv. "Langra" fruit when stored at 5 ± 1 °C. The symptoms of chilling injury deteriorated its visual quality and will certainly affect consumer's acceptability. On the other hand, storage at 15 ± 1 °C resulted in rapid loss of quality. Both loss of quality and chilling injury was modest at 10 ± 1 °C. Therefore, storage at 10 ± 1 °C is recommended. Furthermore, attempts should be made to explore different measures to avoid chilling injury by inducing chilling tolerance so that mango fruits can be stored at 5 ± 1 °C for longer time.

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