



Effect of CaCl_2 and controlled atmosphere storage on phytochemical attributes of Guava

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Abstract

Guava is very delicate and alluring fruit which is being ignored since very long time despite of highly nutritious fruit and rich source of Vitamin C. It contains Vitamin C 2-3 times more than orange. Naturally the guava fruit is enriched with vitamin C and polyphenoles. Guavas fruits after harvesting were dunked in solutions of CaCl_2 (1, 2 and 3%) at room temperature for 5 minutes and stored for 24 days in 5% CO_2 level at temperature of $10 \pm 1^\circ\text{C}$, while the humidity level of storage chamber were 80%. The stored fruits were analyzed at 6 days of interval for sugars (glucose, fructose and sucrose g/100g) total phenolic contents (mgGAE/100g), antioxidant activity ($\mu\text{molTE/g}$), and organic acids (citric, tartaric, ascorbic, and malic acids mg/100 g). The total phenolic content and antioxidant activity of guava fruits were declined during progression in storage but in fewer amounts as compared to room storage condition. Citric acid and ascorbic acid contents were reduced with the progression in storage, however tartaric and malic acid values were amplified at end of storage but the rate of changes were slower. The pretreatments in combination with modified atmosphere storage escalate the shelf life of guava and slow down nutritional degradation process.

Keywords: guava; CaCl_2 ; controlled atmosphere; sugars; organic acids; phytochemical.

Practical Application: Pretreatments in combination with controlled atmosphere storage to minimize postharvest losses of guava fruit.

1 Introduction

Calcium salts are exhibited a distinct role in sustaining cell wall integrity in fruits by interrelating with pectic acid of the cell wall to form calcium pectate which facilitate cross linkage of pectic compounds of the cell wall. CaCl_2 is being used as a firming agent and preservative in the fruit industry for fresh-cut and whole produces. CaCl_2 pretreatments of loquat fruit exhibited increased shelf life and firmness as compared to untreated fruits (Akhtar et al., 2010). Tissue firmness of whole peaches were increased by dipping in CaCl_2 solution (Manganaris et al., 2007). Manganaris et al. (2005) also described that fruits treated with calcium salts showed higher firmness (34.2-44.7%) as compared to the non-treated ones.

The demands of consumer for natural and minimally process foods are increasing day by day. Controlled atmosphere storage (CAS) a well known technology for maintaining the fresh food quality commodities in totaling to elongating storage of fruits. CAS is the most fruitful preservation systems appropriate for a vast range of food and agricultural commodities. The lifespan of food commodities is significantly prolonged by controlling the atmosphere adjacent to food, which lessens the respiration rate and microorganisms activity in food. CAS in combination with pretreatments minimize the post-harvest loss of guava fruit which will finally reduce the wastage of guava fruit (Kader, 2003).

Earlier typically guavas were cultivated for processed guava products like nectars, fruit paste, juices, jellies, jam, canned halves and whole in syrup. However, internationally fresh guavas market is very small. But now the international market has good potential for fresh guavas due to more consumer's awareness regarding the health benefits and alluring taste of fresh fruit.

2 Materials and methods

2.1 Sample procurement

Guavas (Safeda variety) samples were picked early in the morning at 6:30 a.m. from botanical garden of the university and transported to laboratory in cooled containers to retain the fresh samples.

Post-harvest treatment

Guavas were dunked in solutions of CaCl_2 for 5 min as depicted in Table 1 and stowed in climate chamber (ICH260 Memmert, Germany) for 24 days. The CO_2 level of the chamber was controlled at 5% during the whole storage period. Temperature and relative humidity were maintained at $10 \pm 1^\circ\text{C}$ and 80%, respectively.

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Total phenolic content (TPC)

The total phenolic contents were determined by Folin-Ciocalteu reagent method (Sun et al., 2006). UV-VIS spectrophotometer was used to was determined the absorbance of samples at 760 nm. Gallic acid was run as a standard and absorbance of gallic acid was taken at 725 nm as standard curve of gallic acid was depicted in Figure 1.

Antioxidant activity of guava: (1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity)

The antioxidant activity of guava fruit extracts was determined by spectrophotometer at 517 nm (Conforti et al., 2006). The sample absorbance was determined at 517 nm by spectrophotometer. Validation of assay was done by using Trolox.

Table 1. Treatment plan.

Treatment	CaCl ₂ concentration
T ₀	0%
T ₁	1%
T ₂	2%
T ₃	3%

Determination of Organic acids and Sugars

Organic acids (citric, ascorbic, malic and tartaric acid) and the sugars (fructose, glucose and sucrose) were measured by using high performance liquid chromatography (Javed et al., 2016).

2.2 Statistical analysis

Statistical analysis of the data was done to check the level of significance as described by Steel et al. (1997).

3 Results and discussion

3.1 Results

The results of mean squares regarding total phenolic content of stored guava that momentous differences were observed for the effect of storage and treatments. The interaction of days and treatment was also found significant for this trait as depicted in Figure 2.

All treatments showing a sturdy decline in total phenolic content during storage. Decrease in total phenolic content was maximum in T₀ which varied from 131.67, 116.67, 110.33 and 104.67 at 0 to 6th, 12th and 18th day, respectively.

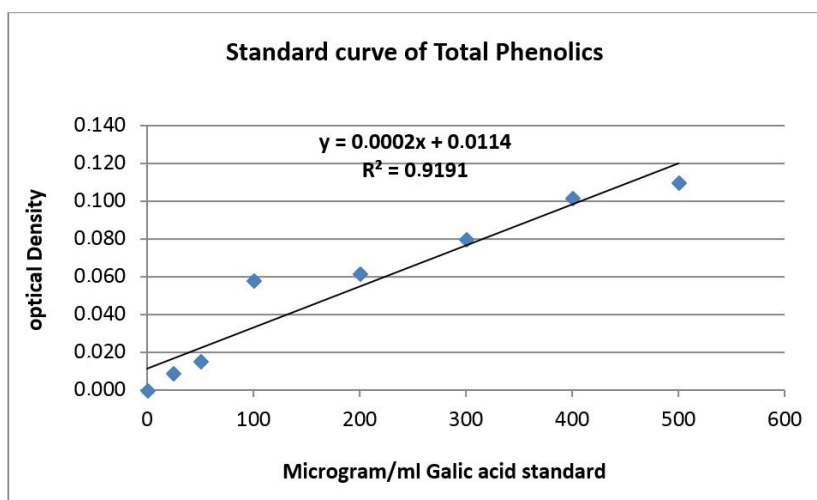


Figure 1. Standard curve of total phenolics.

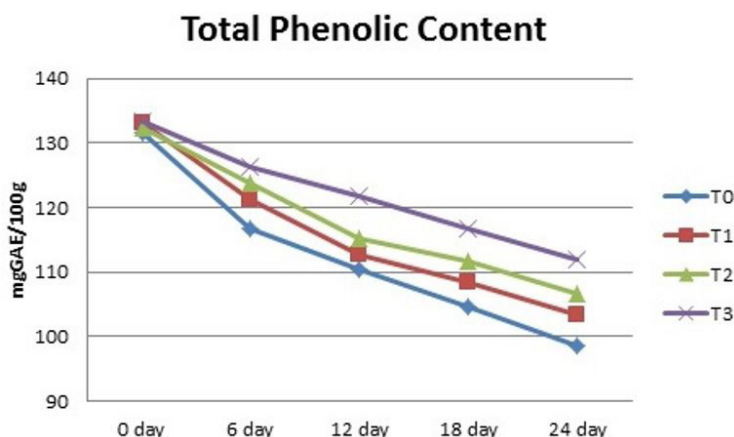


Figure 2. Effect of chemical treatment and controlled atmosphere storage on total phenolic content.

Furthermore, observed value for the trait at 24th day was 98.67. Minimum decrease in total phenolic content was observed for T₃ that varied from 133.33, 126.33, 121.67, 116.67 and 112.00 at 0, 6th, 12th, 18th, and 24 days of storage, respectively.

Mean squares regarding antioxidant activity of guava indicated momentous variations for the effect of storage and treatments. Treatments and interaction of days were also found significant for this trait as depicted in Figure 3.

Continuous drop in antioxidant activity in samples during storage was observed. T₀ samples showed maximum decline in antioxidant activity that varied from 34.00, 24.67, 15.67 and 8.00 at 0 to 6th, 12th and 18th day, respectively. Furthermore, with progression in in storage period, observed value for trait at 24th day was 3.33. Similarly, T₃ indicated minimum decline in antioxidant activity that varied from 34.33, 32.33, 25.67, 21.67 and 15.67 at initiation to termination, respectively.

It is evident from the mean sum of squares regarding glucose content of guava indicated momentous variations for effect of storage and treatments. Furthermore, significant variations were recorded for their interaction as shown in Table 2.

Steady increase in the glucose content during storage was observed in all treatments. The treatment T₀ indicated maximum escalation in glucose content that varied from 2.73 to 2.92 and 3.13 at 0 to 6th and 12th day, respectively. Likewise, with progression in storage, recorded values for the trait were 3.28 and 3.22 at 18th and 24th day, respectively. Similarly, For T₁, indicated variations from 2.71 to 3.09 at 0 to 12th days, respectively. Moreover, the observed value of T₁ was 3.25 at the end of 24th days. The minimum escalation in the glucose value was observed for T₃ that varied from 2.73 to 3.22 at beginning to end.

Mean squares of fructose content of treated guava showed a momentous variation for the effect of storage and treatments. Furthermore, the interaction of storage and treatments were also found significant as depicted in Table 2.

The sturdy rise in fructose during the course of storage was observed. The T₀ showed the highest rise in the fructose content was observed that varied to 3.44 and 3.57 at 6th and 12th day from 3.30 at 0 day. Moreover, with progression in storage, recorded values for the trait were 3.66 and 3.62 at 18th and 24th day,

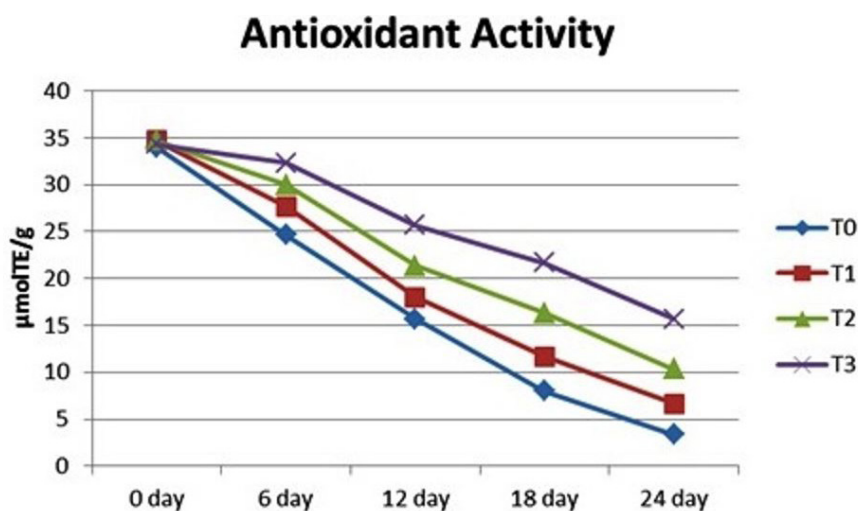


Figure 3. Effect of chemical treatment and controlled atmosphere storage on antioxidant activity.

Table 2. Effect of chemical treatment and controlled atmosphere storage on sugars.

Parameter	Treatments	0 day	6 day	12 day	18 day	24 day
Glucose (g/100g)	T ₀	2.73 ± 0.58K	2.92 ± 0.45 G	3.13 ± 0.75 CD	3.28 ± 0.92 A	3.22 ± 0.76 B
	T ₁	2.71 ± 0.58 L	2.88 ± 0.63 H	3.09 ± 0.38 D	3.21 ± 0.56 B	3.25 ± 0.43 AB
	T ₂	2.72 ± 0.58 L	2.85 ± 0.24 I	3.04 ± 0.51 E	3.16 ± 0.61C	3.27 ± 0.43AB
	T ₃	2.73 ± 0.53 K	2.81 ± 0.29 J	3.00 ± 0.46 F	3.11 ± 0.72 CD	3.22 ± 0.58 B
Fructose (g/100g)	T ₀	3.30 ± 0.77 G	3.44 ± 0.59 DE	3.57 ± 0.63 BC	3.66 ± 0.84 A	3.62 ± 1.03 AB
	T ₁	3.32 ± 0.32 FG	3.41 ± 0.67 E	3.52 ± 0.73 C	3.62 ± 0.75 AB	3.63 ± 1.10 AB
	T ₂	3.31 ± 0.46 FG	3.39 ± 0.49 EF	3.50 ± 0.78 CD	3.58 ± 0.48 B	3.63 ± 0.75 AB
	T ₃	3.32 ± 0.58 FG	3.34 ± 0.74 F	3.47 ± 0.97 D	3.56 ± 0.54BC	3.62 ± 0.58 AB
Sucrose (g/100g)	T ₀	1.66 ± 0.33IJ	1.79 ± 0.25 FG	1.94 ± 0.48 DE	2.08 ± 0.59 A	2.04 ± 0.58AB
	T ₁	1.63 ± 0.31 J	1.77 ± 0.29 FG	1.88 ± 0.43 E	2.04 ± 0.67 AB	2.05 ± 0.52 AB
	T ₂	1.67 ± 0.31 I	1.75 ± 0.33 G	1.84 ± 0.53 EF	2.00 ± 0.28 BC	2.03 ± 0.43 B
	T ₃	1.66 ± 0.33 IJ	1.72 ± 0.45 H	1.82 ± 0.58 F	1.96 ± 0.39 D	1.99 ± 0.38 C

respectively. Likewise, For T_1 the fructose content varied from 3.32 to 3.52 at 0 to 12th day. Likewise, the observed value for T_1 was 3.63 at 24th day. The minimum intensification in the fructose content was observed for T_3 which varied from 3.32 to 3.62 at beginning to end, respectively.

Mean squares of sucrose content indicated a significant variation for the effect of storage and treatments. Likewise, the interaction of storage and treatments were also showed momentous variation as depicted in Table 2.

Sucrose content of guava fruit showed continuous escalation with the progression in storage period. The T_0 showed highest escalation in sucrose content that differed from 1.66 to 1.79 and 1.94 at 0 to 6th and 12th day, correspondingly. Additionally, with progression in storage, recorded values for the trait were 2.08 and 2.04 at 18th and 24th day, correspondingly. Likewise, for T_1 and T_2 variations in sucrose content recorded were differed from 1.63 to 1.88 and 1.67 to 1.84 at 0 to 12th days, correspondingly. Additionally, the observed value for T_1 and T_2 were 2.05 and 2.03 at the 24th day of study. The minimum rise in sucrose content was observed for T_3 that varied from 1.66 to 1.99 from beginning to cessation, correspondingly.

Momentous variations were recorded for effect storage and treatments from mean squares of citric acid content of stored guava fruits. The interaction of days and treatment was also found significant for this trait as depicted in Table 3.

Citric acid content showed a continuous decreasing trend with progression in storage. The highest decline in citric acid content was observed for T_0 that differed to 356.00 and 341.67 at 6th and 12th day from 374.00 at 0 day, correspondingly. Additionally, with progression in storage, observed values for trait were 328.67 and 318.67 at 18th and 24th day, respectively. The lowest decline in citric acid content was observed for T_3 that varied from 375.00 to 338.00 at start to cessation of study period, respectively.

The ascorbic acid content of guava depicted momentous variations for effect of storage and treatments. The interface of

days and treatment was also found significant for this trait as depicted in Table 3.

Steady decline in the ascorbic acid content was observed in all treatments with the progression in the storage period. The maximum drop in ascorbic acid content was noticed for T_0 which varied to 157.00 and 137.67 from 178.00 at 6th and 12th from 0 day, correspondingly. Additionally, with further progression in storage, observed values for trait were 124.00 and 111.67 at 18th and 24th day, respectively. The lowest drop in ascorbic acid content was detected for T_3 that varied from 177.33, 167.33, 155.00, 145.33 and 129.67 at initiation to termination, respectively.

Noteworthy variations were logged for effect of storage and treatments from mean square of malic acid content of stored guava. The interaction of days and treatment was also found significant for this trait as depicted in Table 3.

During progression in the storage period malic acid content of all treatments tended to escalate. T_0 treatment showed the maximum escalation in the malic acid content of the stored guava which varied from 106.00, 120.67 and 135 at 0 day to 6th day and 12th day, correspondingly. Likewise, with progression in storage, observed values for the malic acid content were 138.33 and 143.67 at 18th and 24th day, respectively. The lowest rise in malic acid content was seen for T_3 that varied to 131.33 from 106.33 at termination from initiation, respectively.

Notable variations were noted for effect of storage and treatments regarding tartaric acid content of guava. The interaction of days and treatment was also found significant for this trait as depicted in Table 3.

The tartaric acid content of guava depicted a significant increase with the progression in the storage. The maximum escalation in tartaric acid content was observed for T_0 that varied from 0.787, 0.826 and 0.847 at 0 day to 6th day and 12th day, respectively. Additionally, with progression in storage, noted values for attribute were 0.858 and 0.875 at 18th and 24th day, respectively. The lowest rise in tartaric acid content was seen for

Table 3. Effect of chemical treatment and controlled atmosphere storage on organic acids.

Parameter	Treatments	0 day	6 day	12 day	18 day	24 day
Citric Acid (mg/100g)	T_0	374.00 ± 4.00 B	356.00 ± 5.00 E	341.67 ± 4.56 H	328.67 ± 3.64 JK	318.67 ± 4.17 M
	T_1	374.67 ± 5.33 AB	359.67 ± 5.43 DE	351.00 ± 5.53 F	332.33 ± 3.66 J	322.33 ± 4.53 L
	T_2	376.00 ± 6.16A	362.33 ± 6.02 D	354.67 ± 3.39 EF	336.67 ± 4.33 IJ	327.67 ± 3.43 K
	T_3	375.00 ± 3.00 AB	366.33 ± 7.45 C	359.67 ± 5.33 DE	348.00 ± 6.13G	338.00 ± 3.53 I
Ascorbic Acid (mg/100g)	T_0	178.00 ± 7.00 A	157.00 ± 4.00D	137.67 ± 3.58F	124.00 ± 5.00 H	111.67 ± 5.47 J
	T_1	176.33 ± 6.00 AB	160.67 ± 5.68 CD	143.67 ± 14.27EF	131.00 ± 6.33 G	115.33 ± 4.25 I
	T_2	175.00 ± 8.00 B	162.33 ± 4.53CD	148.00 ± 4.00E	134.67 ± 3.33 FG	120.00 ± 6.00 HI
	T_3	177.33 ± 6.00 AB	167.33 ± 6.58 C	155.00 ± 3.58DE	145.33 ± 5.67EF	129.67 ± 4.33GH
Malic Acid (mg/100g)	T_0	106.00 ± 4.00 H	120.67 ± 2.74 E	135.00 ± 4.00BC	138.33 ± 1.89B	143.67 ± 4.56 A
	T_1	104.33 ± 4.67 HI	116.67 ± 4.66 F	131.67 ± 6.66 C	134.67 ± 2.87 BC	140.33 ± 3.43AB
	T_2	108.67 ± 3.66 GH	112.67 ± 6.66 FG	129.33 ± 2.33 CD	129.33 ± 5.66 CD	137.67 ± 5.43B
	T_3	106.33 ± 5.67 H	109.67 ± 3.59 G	123.67 ± 4.66 DE	124.67 ± 2.74 D	131.33 ± 1.28C
Tartaric acid (mg/100g)	T_0	0.787 ± 0.008 L	0.826 ± 0.004 GH	0.847 ± 0.007 DE	0.858 ± 0.006 C	0.875 ± 0.008 A
	T_1	0.785 ± 0.005 LM	0.816 ± 0.008 I	0.838 ± 0.010 F	0.844 ± 0.004 E	0.864 ± 0.006 B
	T_2	0.786 ± 0.004 LM	0.806 ± 0.004 J	0.828 ± 0.008 G	0.835 ± 0.005 FG	0.857 ± 0.005 CD
	T_3	0.783 ± 0.009 M	0.800 ± 0.006 K	0.822 ± 0.005 H	0.828 ± 0.005 G	0.848 ± 0.010 D

T_3 which varied from 0.783 to 0.848 at start to end of storage period, respectively.

3.2 Discussion

The antioxidant activity and total phenolic content of current work dwindled all over the storage period; any how the rate of decay was reliant on the concentration of salt used and storage environment. Greater amount of polyphenols are present in unripe fruits but with the progression in ripening the polyphenols contents decreased. Reducing levels of polyphenolic compounds during ripening were also determined in mango (Abu-Goukh & Abu-Sarra, 1993) and banana (Ibrahim et al., 1994)

Phenolic content of guava fruits peel and pulp gradually lessened with drop in firmness of flesh. The astringency of guava fruit was decreased which result in the amplified polymerization of leucoanthocyanidins which cause hydrolysis of the astringent arabinose ester of hexahydrodiphenic acid and the increased polymerization of leucoanthocyanidins are related with decline in astringency of guava during ripening (Javed et al., 2016).

Environmental factors were also effective in the fluctuations of polyphenolic compounds. These circumstances may be climatic and agronomic. In agronomic factors; greenhouse, biological culture, and yield of fruits are intricate. Type of soil, rainfall amount and exposure to sunlight were the main climatic factors involved that effect the polyphenolic contents.

The findings of our present investigation were in the corroboration with the previous results of Patthamakanokporn et al. (2008) who conclude that the guava fruit antioxidant activity dwindled during storage. The findings of current work were also in line with the findings of Kulkarni & Aradhya (2005) who claimed that pomegranate arils antioxidant activity declined by 13% with in twenty to sixty days of fruit development. The decay in antioxidant activity was might be due to the diminution in phenolic contents, rapid consumption of anthocyanin's and compositional changes due to fruit development. Antioxidant activity of guava extract was found at different maturity stages. It was found that at un-ripe stage guava showed maximum DPPH scavenging capacity (40-45%), while the minimum value (38%) was observed at the fully-matured phase. Lim et al. (2006) found that more DPPH activity at the green phase of development of fruit may be associated with its greater levels of total phenolic contents. Free radicals play main functions in different types of chronic diseases such as cancer and heart diseases (Valko et al., 2004; Nakabeppu et al., 2006).

The present investigation revealed that sugars (fructose, sucrose and glucose) content exist in guava (climacteric) fruit amplified during storage. The escalation in mentioned traits might be due to formation of sugar molecules from starch molecules. The water loss from the fruits during storage may also be a reason for this increase. The described parameters were observed to be amplified during storage but after attainment a climacteric peak they tend to drop. The change of above parameters mentioned in present study were concentration depended of calcium salts, the high amount of CaCl_2 the minimum was the changing in traits. The development of calcium pectate might be responsible for delay in ripening of treated guava fruits, which result in

diminutions the respiration rate of fruits by lessening the ethylene gas production. Calcium chloride played a momentous role in assuring the constant values of sugars in guava during storage (Mahajan et al., 2011).

The findings of current investigation were in corroboration with the findings of Rodriguez et al. (1971) who described that the fructose and glucose contents of fruits amplified during storage and further it declined with the onset of senescence. The rise in reducing sugar during storage was due to degradation of starches to fructose and glucose by the activities of maltase and amylase (Wills & Trimazi, 1982). The fructose content amplified in fruits during ripening (Tandon et al., 1985). Joshi & Roy (1988) claimed that reducing sugars amplified during cold storage till 25 days and after that it dropped abruptly because of senescence.

Non reducing sugars of banana fruits (climacteric fruit) were very small primarily but after five days of harvesting they tend to increase but again declined drastically (Hakim et al., 2012). Sucrose, glucose and fructose were main sugars in pink and white fleshed guavas, the sugar content amplified during ripening in guava and reduced in over-ripe fruits (Mowlah & Ito 1982). Mitra (1997) described that sugars rise in the skin and flesh during the ripening of guava fruit. Rodriguez et al. (1971) claimed that amount of sucrose in fruits first amplified during storage then started to decline.

The findings of the present investigation revealed that ascorbic and citric acid content were decreased but tartaric acid and malic acid content amplified during storage, however change rate depended on pretreatments and storage condition. The flavors of the fruits were affected by amount of organic acids present in that. The sweetness of the fruits was perceived by the concentration of organic acids (Passam et al., 2011). Citric acid content was present in higher amount in guava fruits while the tartaric acid, malic acid and ascorbic acid, content are in lower amounts, respectively. In matured or ripened fruits the citric acid content were dropped as compared to unripe fruits. The current findings of our investigation are in corroboration with the previous findings of the Gibson et al. (2013) who observed the changes in citric acid content in lowbush blueberry with progression in fruit maturity. They observed that citric acid content escalate as the fruits turn red from green but the citric acid content declined as the fruit became over-matured. The investigations of Randhawa et al. (2014) were also in line with our investigation who described that in citrus juice the citric acid content (non-climacteric) decreased during storage.

The variation in malic acid and citric acid content in peach fruit at different fruit maturity stages were observed by Wu et al. (2005). The rate of change in concentration at different maturity and fruit development stage were observed by them. They described that in peach fruit citric acid content amplified during fruit development but after that citric acid content start to decrease when fruits began to ripe and rise in the sweetness, but malic acid content was minimum and declined in peach during fruit development stage; however with development in maturation the malic acid content augmented.

The tartaric acid and malic acid content of lowbush blueberry (climacteric fruit) tend to increase with fruit ripening

and escalate more in over-ripe fruits (Gibson et al., 2013). Similarly Randhawa et al. (2014) described that the tartaric acid and malic acid content amplified during storage, our results were also in line with these findings.

The ascorbic acid content tend to decrease during storage in guava fruits. Enzyme (Polyphenol oxidase, ascorbic acid oxidase, catalase and peroxidase) reduce ascorbic acid content of guava fruits during storage (Singh et al., 2005).

The results of our study are in corroboration with previous findings of Mahajan et al. (2011) who found that during storage the ascorbic acid content changes momentarily and further demonstrated that higher amount of ascorbic acid were present in fruits received calcium pretreatments. Calcium treated fruits showed slow and steadier degradation of ascorbic acid contents as determined by Laufmann & Sams (1989) and found pretreated with CaCl_2 fruits had high amount of ascorbic acid as compared to control.

The findings of Akhtar et al. (2010) were also in the line as of ours who described that CaCl_2 treated loquat contained higher amount of ascorbic acid as compared to non-treated fruits. The loss of ascorbic was 10.9% and 8.4% in 1% and 2% CaCl_2 treated fruits was as compared to 19% loss in non-treated ones, respectively. Gradual decrease in ascorbic acid content was observed during storage period. Ruoyi et al. (2005) described that post-harvest application of CaCl_2 @ 0.5% the amount ascorbic acid in peaches was maintained till fifty days.

4 Conclusion

Guava is also known as apple of poor people because of its very low prices and easily accessible to common man. In Pakistan the production of guava fruit is 552 million ton annually but unfortunately 30-40% of guava fruit is spoiled after its harvesting due to inappropriate guava fruit handling and storage. The fruit is very delicious and imperative which is a highly nutritious and rich source of polyphenols and antioxidants. The antioxidant activity and total phenolic content of guava declines with storage. Organic acids and sugars ratio present in guava played important role in fruit taste and shelf life. The ascorbic acid and citric acid present in fruits reduced while tartaric acid and malic acid present in fruit tend to increase. Sugars present in guava augmented in the beginning but after attainment maximum value they tend to decay. The results of our investigation indicated that calcium chloride in combination with controlled atmosphere storage were effective to escalation of shelf life and delay in degradation of phytochemicals of fruits.

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