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Fruit Antioxidant Activity, Ascorbic Acid, Total Phenol, Quercetin, and Carotene of Irwin Mango Fruits Stored at Low Temperature after High Electric Field Pretreatment

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Greenhouse-grown tree ripe (TR) and mature green (MG) mangoes (cv. Irwin) were exposed to high electric field treatment before 20 and 30 days of storage at 5 °C. MG fruits were allowed to ripen at room temperature after low-temperature storage. Fruit physical quality attributes, ascorbic acid, carotene, quercetin, total phenols, and antioxidant capacity were estimated before and after the storage period. Antioxidant capacity of fruit juice was estimated using the ferric reducing antioxidant power (FRAP) assay. Fruit firmness decreased significantly during storage. Titratable acidity decreased 20 days after storage. Total soluble solids did not change during storage. Antioxidant capacity of fruits remained unchanged up to 20 days of storage period and decreased thereafter. Total phenol and carotenes increased during storage. Antioxidant capacity of fruits was significantly correlated only to ascorbic acids. Peel color and carotenes were higher in TR fruits, whereas titratable acidity and firmness were higher in MG fruits. There was no significant difference in other parameters between the stages of picking. Electric field pretreatment affected the respiration and antioxidant capacity of TR fruits and did not have any significant affect on other parameters. TR mangoes of cv. Irwin are more suitable for low-temperature storage and can be successfully stored for up to 20 days at 5 °C without any significant losses in functional properties and quality attributes.

KEYWORDS: Mango; Irwin; antioxidant capacity; low-temperature storage; tree ripe; mature green; ascorbic acid; quercetin; carotene; total phenol

INTRODUCTION

Fruit and vegetable antioxidants play an important role in reducing the risk of degenerative diseases such as cardiovascular disease and various cancers and neurological diseases (*1*). Ascorbate is the most studied antioxidant vitamin for its role in reducing the risk of degenerative diseases (*2*). However, recent studies have shown that fruit and vegetable total phenolics and anthocyanins contribute more to the antioxidant capacity than ascorbate (*3*−*6*). The antioxidant capacity of fruits varies with genetic differences, stage of harvest, season of harvest, and postharvest storage conditions and processing (*7*). Studies on the antioxidant capacity of tropical fruits are very important due to their short postharvest shelf life.

Mango is one of the tropical fruits known for its characteristic aroma and taste. Mango has a short shelf life when held at ambient temperatures and is sensitive to low temperatures due to chilling injury. Many studies have been done on increasing the shelf life of mango using controlled atmospheres and low temperatures (*8*−*13*). Prestorage treatments such as high temperature, temperature conditioning, methyl jasmonate, and nitrogen gas exposure and also stage of harvest have been reported to reduce the chilling injury (*8*, *12*, *14*, *15*). Even though changes in carotenoids, ascorbic acid, and other quality attributes during low-temperature storage have been well studied, reports on the antioxidant capacity of mango fruits are scarce. The loss of firmness and occurrence of chilling injury during low-temperature storage highlight the importance of cell wall and cell membrane. An electric field is known to affect the cell membranes and enzymes (*16*, *17*). High electric field exposure for short periods was reported to suppress the respiration rate in some fruits and extended the freshness of sweet peppers (*18*). Postharvest treatment of strawberries with a high electric field has also been reported to increase the shelf life (*19*). In this study, therefore, a high electric field was used as a prestorage treatment, and its effect on the shelf life and quality of fruits was evaluated. In addition to quality attributes, changes in antioxidative capacity of tree-ripe (TR) and mature-green (MG) Irwin mangoes were also evaluated after 20 and 30 days of low-temperature storage.

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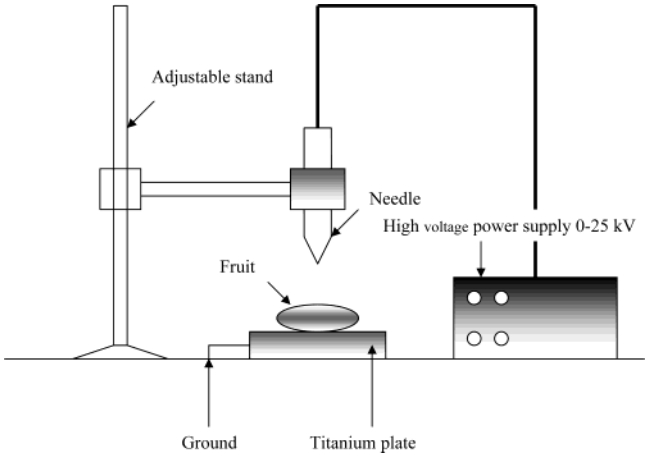

Figure 1. Electric field treatment system used in the experiments. Fruits were kept on the titanium plate and exposed to a high electric field. The high electric field was generated using the power supply unit.

Table 1. Statistical Significance (*F* Probability) of Changes in Total Soluble Solids (TSS), Titratable Acidity (TA), Firmness, Respiration, and Peel Color (*a** and *b**) in Fruits Harvested at Tree Ripe (TR) and Mature Green (MG) Stages, Pretreated with (EF) or without (Control) High Electric Field, and Stored at 5 °C for 20 or 30 Days

		F probability					
						peel color	
harvest stage	factor	TSS	TA	firmness	respiration	*a**	*b**
TF	EF vs control	NS	NS	NS	<0.05	NS	NS
	days	NS	<0.05	<0.01	<0.01	NS	NS
MG	EF vs control	NS	NS	NS	NS	NS	NS
	days	NS	<0.01	<0.01	<0.01	NS	NS
fields	TR vs MG	NS	<0.01	<0.01	NS	<0.01	<0.01
	days	NS	<0.01	<0.01	<0.01	NS	NS

MATERIALS AND METHODS

Plant Material. TR and MG mangoes (cv. Irwin) grown under greenhouse conditions on Okinawa Island, Japan, were procured in June 2002. Uniform fruits selected for the experiment were dipped in acidic electrolyzed water (pH 2.7 ORP 1100 mV, FAC 55 ppm) (*20*) and hot water (46−48 °C) for 10 min each to reduce fruit rot during the storage.

High Electric Field Treatment. After electrolyzed-water and hot-water treatments to avoid fungal diseases during storage, fruits were divided into two lots of 30 fruits each. One lot was used for electric field treatment and the other was used as control. Each fruit was exposed to 150 kV/m of electric field for 45 min as shown in **Figure 1** (*21*). Treated and control fruits were further divided into three lots of 10 fruits each for storage studies. Two lots were stored at a temperature of 5 °C and 75−80% relative humidity for 20 and 30 days, respectively. The third lot was stored at a room temperature of 25 °C. Fruit respiration, firmness, and color were measured at 10, 20, and 30 days of storage. Fruit quality attributes such as total soluble solids and titratable acidity were measured at the end of 20 and 30 days of storage. Mature-green fruits were ripened at room temperature (25 °C) after 20 and 30 days of low-temperature storage. Tree-ripe fruits were cut immediately, and the quality attributes such as ascorbic acid, total soluble solids (TSS), titratable acidity, color, and taste (personal observation) were recorded. After TSS measurement, the pulp from two fruits each was pooled, homogenized in a blender, and stored at −30 °C until other parameters were measured.

Respiration, Firmness, and Color Measurement. Respiration rate was estimated by measuring the changes in carbon dioxide and oxygen concentrations at 1, 2, and 3 h after fruits were enclosed in a gastight fruit chamber. Gas concentrations were measured using a Shimadzu gas chromatograph (model GC-8A, Shimadzu Corp., Kyoto, Japan) fitted with Porapack-N and molecular sieve-13X columns for simultaneous measurement of carbon dioxide and oxygen using helium as carrier gas. Column temperature was maintained at 60 °C; injector and detecor temperatures were at 80 °C. Gas concentrations were simultaneously measured using a thermal conductivity detector. Fruit firmness was measured using a portable firmness tester. This instrument measures the transmission velocity of sound waves through the fruit (*22*, *23*): the firmer the fruit, the higher is the velocity. Fruit color was measured by using a Minolta chroma meter (model CR-300). Color was recorded in *L** (brightness), *a** (red), and *b** (yellow) units. Values of *a** and *b** were used for comparing the treatments. All fruits were used for recording respiration, firmness, and color.

Quality Attributes. Total soluble solids and titratable acidity were measured from juice extracted from pulp. A small quantity (1−2 mL) of juice was extracted by gently squeezing the fruit slice for recording TSS. Soluble solids were measured using a hand-held refractometer (model N-20, Atago Co. Ltd.). Titratable acidity was determined by homogenizing thoroughly 10 g of pulp with distilled water and filtering under vacuum. The filtrate was made up to 30 mL and titrated against 0.1 N NaOH to pH 8.2 using phenolphthalein as an indicator and expressed as the units of citric acid (milligrams per gram of fresh weight) on a fresh weight basis. Ascorbic acid was estimated using the AOAC method (*24*).

Quercetin. Quercetin concentration was determined using HPLC. Quercetin was extracted using methanol (80% v/v). Fruit pulp (10 g) was mixed with 10 mL of methanol, sonicated for 30 min at 40 °C, and centrifuged at 5000 rpm for 10 min at room temperature. This was repeated three times, and the volume of pooled supernatant was made up to 50 mL. The extract was filtered through a 0.45 μm filter attached to a disposable 1 mL syringe, and 10 μL was used for quantification of quercetin using HPLC. The same extract was used for total phenol estimation according to the Folin−Ciocalteu method (*25*).

***β*-Carotene.** Homogenized fruit pulp (2 g) was mixed with 25 mL of methanol (100%) and 1 g of calcium carbonate and filtered. The residue was re-extracted using 50 mL of an acetone/hexane (80:20) mixture. The filtrates were pooled and transferred to a separating funnel and mixed with 75 mL of distilled water. The phases were allowed to separate, and the aqueous phase was drained off. The hexane fraction (nonaqueous) was made up to 10 mL and passed through a 0.45 μm filter attached to a 1 mL syringe. β-Carotene was quantified using HPLC (*26*).

Table 2. Statistical Significance (*F* Probability) of Pulp Color (*a** and *b**), β-Carotene, Ascorbic Acid, Quercetin, FRAP (Antioxidative Capacity), and Total Phenols in Fruits Harvested at Tree Ripe and Mature Green Stages and Stored at 5 °C for 20 or 30 Days

		F probability						
		pulp color			ascorbic			total
harvest stage	factor	*a**	*b**	β-carotene	acid	quercetin	FRAP	phenols
TR	EF vs control	NS	NS	NS	NS	NS	<0.01	NS
	days	<0.01	NS	<0.01	<0.01	<0.01	NS	<0.01
MG	EF vs control	NS	NS	NS	NS	NS	NS	NS
	days	NS	<0.01	<0.01	<0.05	<0.01	<0.01	NS
fields	TR vs MG	<0.01	NS	<0.01	NS	NS	<0.05	NS
	days	<0.01	<0.01	<0.01	<0.01	NS	<0.01	NS

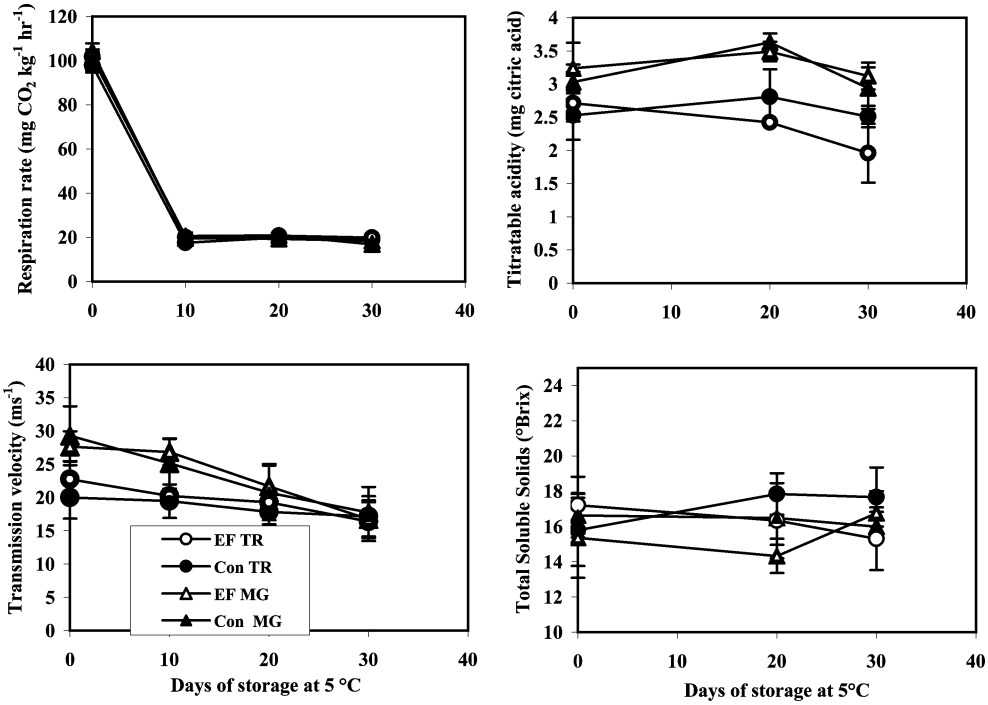

Figure 2. Fruit respiration, titratable acidity, firmness, and total soluble solids in tree ripe (TR) (○, ●) and mature green (MG) (▲, Δ) fruits after 0, 20, and 30 days of storage at 5 °C. EF = electric field treated (open symbols); control = not treated with electric field (solid symbols).

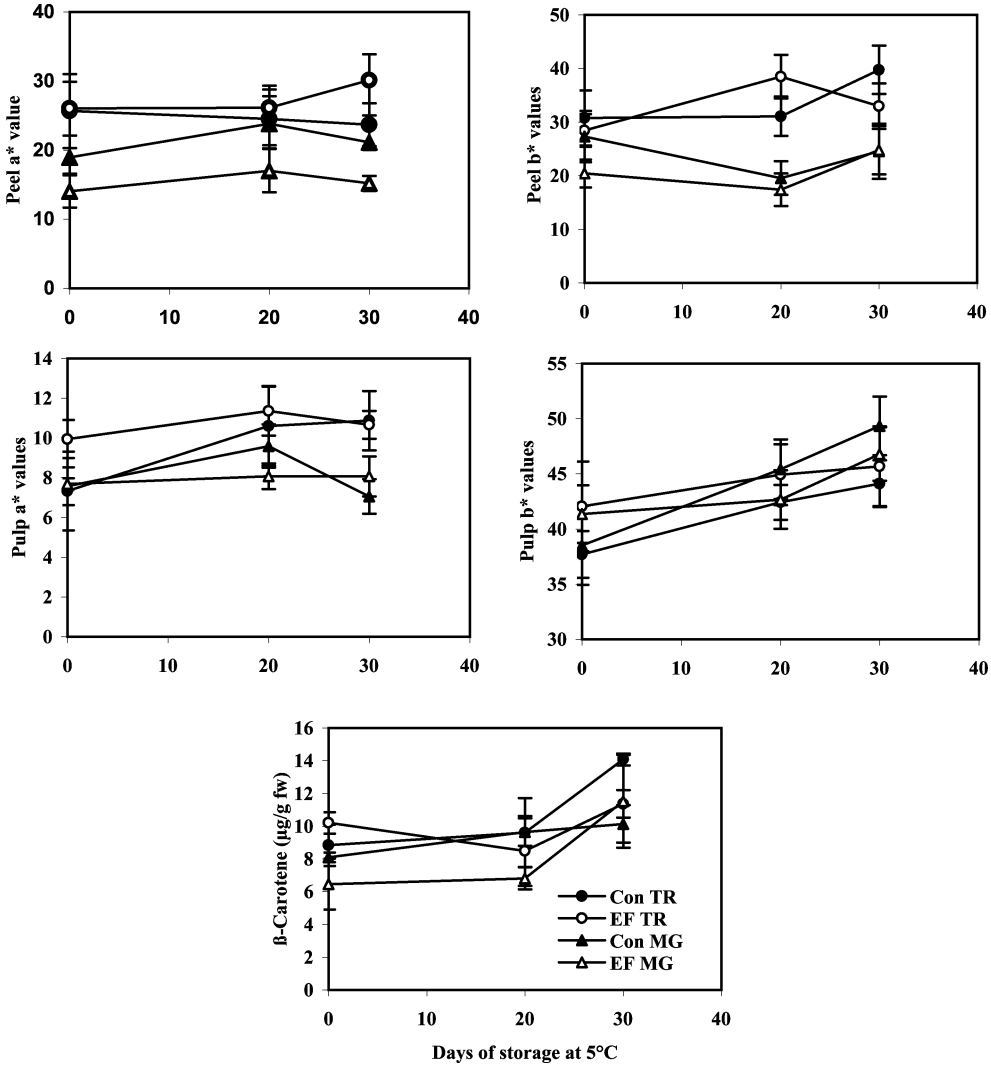

Figure 3. Peel and pulp *a** and *b** values and β-carotene in TR and MG fruits after 0, 20, and 30 days of storage at 5 °C.

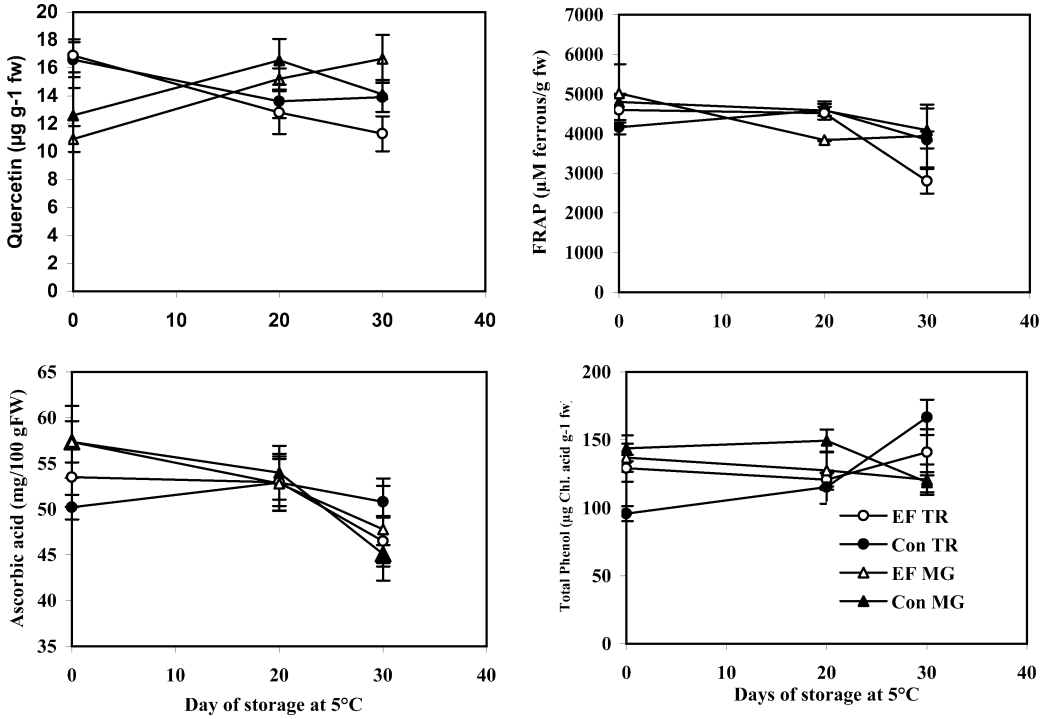

Figure 4. Fruit antioxidant capacity, ascorbic acid, quercetin, and total phenol (micrograms of chlorogenic acid per gram of fresh weight) content in TR and MG fruits after 0, 20, and 30 days of storage at 5 °C.

HPLC. Gradient HPLC was used with mobile phases of 10 mM phosphoric acid/acetonitrile (90:10) as A and acetonitrile (100%) as B using a C18 column. Quercetin was estimated at 280 nm. Carotene was estimated using methanol (A) and ethyl acetate (B) as mobile phases on a C18 column at 473 nm. Concentrations of quercetin and β-carotene were calculated using standards.

Antioxidant Capacity (FRAP Method). The antioxidant capacity of fruit juice was estimated using the FRAP assay (5). The antioxidant capacity of fruit juice was determined by its ability to reduce ferric iron to ferrous in a solution of 2,4,6-tripyridyl-2-triazine (TPTZ) prepared in sodium acetate at pH 3.6. The reduction of iron in the TPTZ−ferric chloride solution (FRAP reagent) results in the formation of a blue product (ferrous tripyridyltriazine complex), the absorbance of which is read at 593 nm at 4 min after the addition of 2% aqueous dilution of fruit pulp. The antioxidant standard curve was developed using ferrous ammonium sulfate. The 2% (w/v) homogenized aqueous dilution of the pulp was vacuum filtered before it was used in the final reaction with FRAP reagent. The results are expressed as micromoles of ferrous equivalents per gram of fresh weight.

Statistical Analyses. Two-way analyses of variance were performed over pretreatment and stage of harvest in TR and MG fruits separately using MS-Excel. Pooled data were also analyzed over stages of harvest (TR and MG) to find the statistical significance between the TR and MG stages of harvest. Treatment significance was tested using the *F* probability test. The relationship of antioxidant capacity with other parameters was estimated using correlation coefficients. Error bars were drawn in graphs using standard deviation values.

RESULTS

Storage Duration Effects. Titratable acidity, firmness, respiration, β-carotene, ascorbic acid, quercetin, and total phenols were significantly ($P < 0.01$) affected by the storage duration in both TR and MG fruits (**Tables 1** and **2**). Pulp a^* and pulp b^* values were affected in TR and MG fruits, respectively. Fruit peel color was not affected by the storage duration. Antioxidant capacity was affected by the storage duration only in MG fruits. Pooled data indicate that all of the parameters except peel color, TSS, quercetin, and total phenols were affected by the storage duration ($P < 0.01$). Generation of off-flavors was noticed in TR and MG fruits stored for 20 and 30 days, respectively (personal observation).

Fruit firmness, as indicated by the transmission velocity, decreased continuously during the storage. The titratable acidity of TR and MG fruits decreased after 20 days of storage (**Figure 2**). In general, pulp a^* and b^* values significantly increased during the storage (**Figure 3**). Carotene increased significantly after 20 days of storage in both TR and MG fruits. Reduction in ascorbic acid and antioxidative capacity was seen after 20 days of storage (**Figure 4**). An increase in total phenol content was observed at the end of 30 days in TR fruits. Storage duration had the opposite effect on quercetin content of TR and MG fruits. Quercetin content increased in MG fruits but decreased in TR fruits.

Pretreatment Effects. Pretreatment with high electric field affected only respiration ($P < 0.05$), and antioxidative capacity ($P < 0.01$) in TR did not have any effect on MG fruits (**Tables 1** and **2**). Respiration was increased and antioxidative capacity was decreased by the electric field pretreatment.

Effect of Stage of Harvest. Fruits were harvested at TR and MG stages. Mature green fruits were allowed to ripen at room temperature after the low-temperature storage treatments. All of the quality attributes were estimated in ripened fruits. The probability table indicates that stages of ripening significantly affected titratable acidity, firmness, peel a^* and b^* values, pulp a^* values, β-carotene ($P < 0.01$, respectively), and FRAP ($P < 0.05$) (**Tables 1** and **2**).

Titratable acidity and firmness were higher, whereas peel a^* and b^* values and β-carotene were lower, in MG fruits when compared to TR fruits (**Figures 2** and **3**). Fruit color development was therefore dependent mainly on the stage of ripening at harvest. The higher firmness of MG fruits was maintained only up to 10 days of storage duration. Antioxidative capacity was also slightly higher in MG fruits (**Figure 4**).

Correlation coefficients were run between the FRAP and other antioxidative parameters. A significant positive relationship was

seen between FRAP and ascorbic acid (0.776, $P < 0.03$). Other parameters did not show any significant relationship with FRAP.

DISCUSSION

Storage Duration Effects. Fruit firmness was decreased during the storage in both TR and MG fruits, but the reduction was steeper in MG fruits. Peel color was not affected during storage in any of the treatments. Pulp a^* values were affected in both TR and MG fruits during the storage period. Titratable acidity decreased after 20 days of storage irrespective of the stage of harvest. Total soluble solids did not change significantly during the storage. β-Carotene content remained unchanged up to 20 days of storage and increased later. Off-flavors were noticed in TR and MG fruits after 20 and 30 days of storage, respectively (personal observation). Reductions in firmness, suppressed flesh color, and higher acidity were reported in cold-stored mangoes (*9*, *11*, *27*). Skin browning was reported when ambient temperature ripened mangoes were stored at 5, 10, and 15 °C (*8*). Reduction in total acidity, increase in ascorbic acid, and no change in TSS and carotenes up to 14 days of storage were also reported in the same study (*8*). However, skin browning during low-temperature storage was not observed when fruits were ripened at 20 °C before subsequent storage at 5 °C (*8*). Our results indicated that low-temperature storage did not reduce β-carotene or peel and pulp colors of TR fruits.

Antioxidant capacity of fruits was maintained during the low-temperature storage up to 20 days. The variation in antioxidant capacity during the storage period was related only to ascorbic acid (0.776) and did not show any significant relationship with total phenol, quercetin, or β-carotene content. This is in contrast to the earlier reports that the contribution of ascorbic acid to the antioxidant capacity of fruits is less than that of the total phenols (*3*, *5*, *7*). In blueberry no reduction in antioxidant capacity was observed during the storage at 5 °C for 3−5 weeks and was found to be related to total phenols and anthocyanins (*3*, *5*). No reduction in antioxidant capacity was observed in blueberry despite a 27% loss of ascorbate at 20 and 30 °C (*1*). The reduction in antioxidant capacity after 20 days of storage in our study was mainly due to its strong relationship with ascorbic acid rather than total phenols in mango. An increase in antioxidant capacity during low-temperature storage may be possible in fruits in which the contribution of total phenolics is greater than that of the ascorbic acid.

Stage of Harvest. The probability table indicates that titratable acidity and firmness were higher in MG fruits, whereas peel a^* and b^* and carotenes were higher in TR mangoes. The antioxidant capacity of fruits was slightly better in MG fruits. Higher β-carotene and color of TR fruits indicate that TR fruits are better for low-temperature storage. Development of fruit color after low-temperature storage was reported to be dependent on the stage of maturity at harvest (*15*). Tree ripe fruits were reported to tolerate low-temperature better than nonripened fruits (*13*). Pretreatment at 38 °C for 3 days before storage at 4 °C was also reported to improve the skin color and reduce the acidity (*10*). This pretreatment might have initiated the ripening before transfer to low-temperature storage, indicating that the storage of ripened fruits is better to retain the quality attributes. Our study also indicated that the storage of ripened fruits will maintain the quality for up to 20 days at 5 °C. Storage at still lower temperatures may also help in maintaining the firmness of ripened fruits because the softening was related to the storage temperature (*9*).

Pretreatment Effects. Use of high electric field treatment to increase the shelf life of strawberries has been reported (*16*). However, a significant effect of electric field in improving the shelf life of mangoes was not seen in our study. Electric field exposure pretreatment for 45 min affected respiration and antioxidant capacity only in TR fruits and not in MG fruits. Kharel and Hasinaga (*19*) have reported a significant reduction in rotting of fruits exposed to continuous electric field. Continuous exposure to high electric field resulted in excessive water loss from strawberries. The study of Kharel and Hasinaga (*19*) and the greater effect of electric field exposure on TR fruits in this study indicate that the electric field effect may be greater on soft fruits rather than on firmer fruits due to its effect on water loss from the fruits. Failure of electric field exposure to increase the shelf life might be due to the shorter duration of exposure of fruits in our study. To increase the water loss and to improve the shelf life in bigger fruits such as mango, longer electric field exposures are needed.

Results indicate that TR fruits can be successfully stored at 5 °C for 20 days without significant changes in fruit color, acidity, TSS, and flavor. Fruits also maintain the ascorbic acid, carotene, antioxidant capacity, and taste for up to 20 days of storage. Fruit color development after low-temperature storage was less in MG fruits, and they also developed off-flavors. Electric field pretreatment affected the respiration and antioxidant capacity in TR fruits and did not have any significant effect on MG fruits. Therefore, TR fruits are better suited for low-temperature storage.

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