J Food Sci Technol (Mar–Apr 2011) 48(2):150–158
DOI 10.1007/s13197-010-0160-8

ORIGINAL ARTICLE

Physico−chemical, respiratory and fungicide residue changes in wax coated mandarin fruit stored at chilling temperature with intermittent warming

Milind Shivratan Ladaniya

Revised: 26 July 2010 / Accepted: 29 July 2010 / Published online: 14 November 2010

Abstract Influence of chilling temperature, intermittent warming (IW) and fungicidal wax coating was evaluated during storage of 'Nagpur' mandarins (*Citrus reticulata* Blanco). Fruits were light green coloured with slight colour-break at the start of storage. Waxed and non-waxed fruits were stored at 3.5 °C (constant), 2 weeks at 3.5 °C followed by IW for 1 week at 19.5 °C (cycle) and at 6.5 °C (constant), and were evaluated immediately after 30, 45, 60, 75 days and also after 1 week holding at ambient condition (24±2 °C, 60–70% RH). There was no chilling injury to fruit under IW treatment irrespective of coating. At 3.5 °C (constant) chilling injury appeared after 45 days during 1 week holding and thereafter increased at each storage interval. Wax coated fruit had lower chilling injury. Fruit under IW treatment and at 6.5 °C (constant) developed yellow-orange colour while at 3.5 °C (constant) fruit remained green during storage. Juice content, titratable acidity and ascorbic acid contents were not affected by temperature regimes and waxing while total soluble solids content was higher with IW treatment. Reducing and total sugars were higher in fruits stored at IW treatment and at 6.5 °C (constant) than at 3.5 °C (constant). Total peel phenols content were not significantly affected by waxing and temperature regimes. However, loss of phenols content was higher at 3.5 °C (constant). Phenol content decreased during storage. At 3.5 °C (constant), chlorophyll ('a', 'b' and total) content in peel was maximum while total carotenoids were minimum with little colour development. Rapid colour development was recorded under IW and also at 1 week holding. Wax coating delayed colour development at 3.5 °C (constant). Initially carbendazim residues were higher in peel (4.0 ppm) and pulp (3.2 ppm) of waxed fruit than in non-waxed (3.2 ppm in peel and 3.1 ppm in pulp) fruit. Overall drop in residues till storage up to 75 days+ 1 week over the initial values was 80.2–85.6% in peel and 56.2–75.8% in pulp of waxed and non-waxed fruit, respectively. Respiration was lower in waxed fruit. Respiratory rate was lowest at 3.5 °C (constant) and it changed with IW. At 3.5 °C and 6.5 °C (constant), range of respiration was 4–6 mgCO_2/kg/h and 7–9 mgCO_2/kg/h, respectively in waxed and non-waxed fruit. Respiratory rate increased as the fruit was removed to warmer temperature. Chilling injury caused considerable rise in respiration rate of fruit. Present findings indicated that storage life of 'Nagpur' mandarin can be extended up to 75 days at 3.5 °C with IW.

Keywords Mandarin · *Citrus reticulata* Blanco · Chilling injury · Colour · Composition · Respiration · Carbendazim

M. S. Ladaniya (✉)
National Research Centre for Citrus,
P. B. 464, Shankar Nagar P.O.,
Nagpur 440010, India
e-mail: ladaniyams@yahoo.com

Introduction

Storage of chilling-sensitive tropical and subtropical fruits at chilling temperatures causes several physiological and biochemical changes leading to disorders. Chilling injury (CI) symptoms are often visible only after the chilled fruit is removed to warmer temperatures. External symptoms are manifested as pitting, discolouration, water soaking, breakdown and off-flavour. Chilling injury appeared to be directly related to length of exposure to chilling and inversely related to temperature (Ladaniya and Sonkar 1996). Changes in cell membrane lipids, physical phase and peroxidation are reported to cause chilling (Eaks and

Raison 1970; Raison et al. 1971; Shewfelt and Purvis 1995). However, the mechanism of chilling and factors and biochemical processes responsible for development of chilling injury are not yet fully known. With disruption of tissues, anomalous respiration pattern have also been reported in some citrus fruits (Eaks 1980). Chilling is also reported to affect ethylene production in the tissues and colour change (Wang and Adams 1982). High levels of sugars are correlated positively with resistance to CI in grapefruit (Purvis and Grierson 1982). Surface coatings and vegetable oils were reported to maintain water status of fruits and thus minimize development of chilling injury (McDonald 1986; McDonald et al. 1993). Intermittent warming (IW) at chilling temperature can prevent or reduce CI development and maintain fruit quality for longer period (Wang 1993). However, chilling and warming temperatures, frequency and duration would vary from cultivar to cultivar and growing conditions.

Fungicides like thiabendazole and benomyl besides reducing decay have also been reported to ensure physiological protection against chilling in grapefruit (Schiffman-Nadel et al. 1975). The residues of fungicide at chilling temperature with IW may vary during long-term storage. 'Nagpur' mandarin is normally stored at 6.5 °C and 90–95% RH with storage life of only up to 45 days (Ladaniya and Sonkar 1996). Chilling temperature of 3.5 °C (constant) reduces decay and deteriorative changes but causes chilling injury while 6.5 °C (constant) causes rapid ageing and decay during prolonged storage (Ladaniya et al. 2005). There is a need to prolong storage life of mandarins beyond 45 days.

Keeping in view these findings, present study was initiated to evaluate changes in colour, juice sugars (reducing and total), peel constituents (phenols and pigments), chilling injury, respiration, composition and fungicide residue of mandarin fruit in response to wax coating and temperature regimes followed by post-storage holding at ambient condition (24±2 °C, 60–70% RH). Study was also aimed at evaluating role of physiological and biochemical changes in chilling injury development and determining efficacy of waxing and IW at chilling temperature (3.5 °C) to extend storage life beyond 45 days.

Materials and methods

Treatment of mandarin fruit Mature 'Nagpur' mandarin (*Citrus reticulata* Blanco) fruits (size 6.5–7.5 cm diam) growing in Nagpur on rough lemon rootstock were harvested at little yellow colour-break on rind. Post-harvest treatment of Bavistin 50 wp (a.i.500 ppm carbendazim) was applied with fine spray (2.5 lit/ton fruit) to all fruits and then they were divided into two equal lots; one lot was retained without wax treatment while other lot was given wax coating by fine spray application on citrus packing line (Sta-fresh 921 in 1:3 ratio of wax: water + 2,000 ppm carbendazim with delivery rate of 1.25 l wax solution per ton fruit on horse–hair brushes). Sta-fresh 921 coating for citrus fruits contained food grade carnauba wax and resins with 18% total solids (FMC Corporation, Lake land, Florida, USA). Fruits were surface dried before packing.

Storage conditions Fruits were packed in vented (0.5% area punched with 4 mm diameter holes) polyethylene (25 μm thick) lined corrugated fibre board boxes (universal type, 5 ply, 30×23×9 cm size with 5% vented area on two sides). There were in all six treatment combinations of wax coating and temperature regimes. The treatments were T_1: 3.5 °C (constant); T_2: 3.5 °C (2 weeks) followed by 19.5 °C (1 week) cycle; T_3: 6.5 °C (constant); T_4: wax coating + 3.5 °C (constant); T_5: wax coating + 3.5 °C (2 weeks) followed by 19.5 °C (1 week) cycle; T_6: wax coating + 6.5 °C (constant). Temperature treatments with variation of ±0.5 °C were allocated to separate cold rooms (walk-in type) and RH maintained at about 90% using humidifiers. Treatments were replicated 4 times with 25 fruits per replicate. Fruit were analyzed after storage intervals of 30, 45, 60 and 75 days. Separate lots were stored for analysis at various storage intervals. At the end of storage intervals, fruits were held for a week at ambient condition (24±2 °C, 60–70% RH) to simulate marketing conditions and were again analyzed. Percent fruit with chilling injury (on weight basis) were recorded during storage. For calculation of percentage total chilling injury, all fruits with surface pitting/brown staining (slight to severe) were taken together as these fruits suffered objectionable chilling damage and would deter consumers, from acceptance.

Physico-chemical attributes and respiration Five fruits from each treatment (with four replicates) were analysed for rind colour, juice content, total soluble solids (TSS), titratable acidity and ascorbic acid content. Rind colour was recorded with colour difference meter (Gardener Color Gard Systems 1000/05 Pacific Scientific, USA). The measurements were according to CIE system as' a*' ('-a' * indicates bluish green while + a* indicates red) and 'b*' ('–b*' indicates blue while '+ b*' indicates yellow). Ratio of a*/b* along with –ve or + v sign indicates extent of green or yellow-orange colour. TSS were measured with a hand refractometer and titratable acidity and ascorbic acid contents were measured by titrimetric methods (AOAC 1985). Initial fruit quality was measured on 50 fruits for the physico-chemical quality attributes. Chlorophyll ('a', 'b' and total) in rind, reducing sugars in juice by Nelson-Somogy method, total phenols in rind using Folin-Ciocalteau reagent (Sadasivam and Manickam 1996) and total carotenoids (Ting and Rouseff 1986) in rind flavedo were determined by spectrophotometric methods using spectrophotometer (Model Helios Alpha S2, Thermo electron Scientific Corp, WI, USA). Phenol sulphuric acid method (Dubois et al. 1956) was followed to estimate total sugars in juice. During storage under various temperature regimes and after 1 week holding, respiration rate of whole fruits (without chilling injury) was measured with Infrared CO_2 analyser (Amtek model CD-3A, Thermox Instruments, Pittsburgh, PA, USA) by flushing fresh air (100 ml/min) over approximately 1,000 g fruit held in air tight glass jar to measure percent CO_2 and estimated as mgCO_2/kg/h. Healthy and chilling-injured fruits were separated and respiration of only chilling-injured fruit was measured separately.

Carbendazim residues Residues of carbendazim (methyl–2-benzimidazole carbamate or MBC) (actual ingredient of Bavistin) were determined in pulp and peel of mandarin fruits after 1 week holding at ambient condition at each interval following spectrophotometric method (Vaidya and Bannerjee 1984). Ten g pulp and peel were taken initially and after each interval of storage. Residues were extracted in dilute HCl (1:20) in mixer. Mixture was filtered and pH adjusted to 3.4. In separating funnel, 2 ml of extract was mixed with 5 ml solution of bromocresol blue indicator as well as phthalate buffer and pH adjusted to 3.4 with NaOH (0.1 N). Chloroform (10 ml) was added and mixture was shaken for 1 min. Two phases were allowed to separate for 10 min and chloroform phase was collected. Excess moisture removed with sodium sulphate and absorbance measured at 420 nm in spectrophotometer. The MBC residue was quantified from a standard curve (on the basis of 0.5, 1, 2, 3, 4, 5 and 10 ppm) prepared from pure (99.9%) carbendazim (Accustandard Inc. New Haven, CT, USA). Average values of two samples from each treatment at each interval were recorded. The recovery of the residue in fortified sample was 85–90%. Recovery study was performed by spiking chopped peel and pulp with analytical carbendazim solution at 1 and 2 ppm level. The spiked samples were extracted and analysed according to the procedure mentioned above.

Statistical analysis The experiment was laid out using a factorial design with six main treatments, four replicates and storage intervals as sub-treatments and the data were subjected to Analysis of Variance (ANOVA). The treatment means were compared with critical difference (CD) calculated at $p=0.05$.The CD of interaction (Main× sub treatment) can be used to compare effect of temperature regimes within a storage interval as well as effect of storage intervals on a temperature regime.

Results and discussion

Chilling injury There was no chilling injury in waxed and non-waxed 'Nagpur' mandarin fruit stored under IW treatment up to 75 days and also during 1 week post-storage holding (T_2 and T_5) (Table 1). Chilling temperature causes stress and accumulation of toxic metabolites resulting in injury but IW results in depletion of such metabolites before degenerative change occurs (Wang 1993). In fruits stored at 6.5 °C (constant) (T_3 and T_6) chilling injury developed late (at 75 days) and damage was low (less than 5%). At 3.5 °C (constant) (T_1), maximum chilling injury occurred in non–waxed fruit. Waxing reduced chilling injury (T_4 and T_6) (Table 1). There was no chilling up to 30 days under any temperature regime. Tolerance to lower temperature varies in various citrus fruit. In ripe yellow coloured 'Kagzi' acid limes, chilling injury symptoms appeared at 5.5–7 °C after 30 days (Ladaniya 2004a) while 'Mosambi' sweet orange developed chilling at 3–5 °C after 90 days. For 'Mosambi' orange, 5–7 °C is the optimum storage temperature (Ladaniya 2004b).

Waxed fruit had lower chilling damage up to 60 days than non-waxed fruit but this ameliorative effect of waxing on chilling injury was reduced as storage period extended up to 75 days. Wax coating reduced susceptibility of grapefruits to chilling injury (Chalutz et al. 1985). Shellac wax was better than carnauba wax in reducing chilling injury in grapefruit (Dou 2004). The mechanism of action of coatings in minimizing chilling could be by restricting gaseous exchange (McDonald et al. 1993) and increased CO_2 level and decreased O_2 level in the fruit tissue thereby restricting respiration. Complete prevention of chilling injury with IW at 3.5 °C as observed in the present study is an important step forward in extending storage life of 'Nagpur' mandarin since chilling injury renders fruit susceptible to secondary infection and rotting. Rotting caused by pre-harvest infection can be taken care of by fungicide sprays in field so that storage life can be extended with minimum losses (Ladaniya et al. 2005).

Juice, Total Soluble Solids (TSS), titratable acidity and ascorbic acid content Temperature regimes and wax coating had non-significant effect on juice content. Non-waxed fruits stored at 3.5 °C (constant) (T_1) recorded significant drop in juice content during storage between 30 days and 75d+ 1 week, however, waxed fruit stored at similar conditions recorded marginal decline. Juice content declined significantly during storage (Table 2). The TSS were significantly higher in non-waxed and waxed fruits stored at IW treatment (T_2 and T_5) than at 3.5 °C (constant) (T_1) and 6.5 °C (constant) (T_3). The TSS increased (0–8.35%) in various treatments between the interval of 30 days and 75d+ 1 week. This increase could be due to solubilization of compounds other than carbohydrates/sugars (Echeverria and Ismail 1990) or

Table 1 Effect of temperature (T), intermittent warming and wax coating on chilling injury (%) of 'Nagpur' mandarin during storage (S)

Treatment	Storage period, days					
	45 d+ 1 week	60 d	60 d+ 1 week	75 d	75 d+ 1 week	Mean
T_1	37.7	25.8	36.8	43.9	32.4	22.0
T_3	0.0	0.0	0.0	2.8	1.1	0.4
T_4	20.5	16.2	24.1	42.1	35.9	17.3
T_6	0.0	0.0	0.0	1.2	1.5	0.3
Mean	9.7	7.0	10.1	15.0	11.8	

T: CD=2.0, S: CD=3.3, T × S: CD=8.2

T_1=3.5 °C (const), T_2=3.5 °C with IW, T_3=6.5 °C (const), T_4 = Waxed + 3.5 °C (const), T_5 = Waxed + 3.5 °C with IW, T_6 = Waxed + 6.5 °C (const)

Chilling injury was nil in all treatments up to 45 days. No chilling observed in T_2 and T_5 throughout storage period

Table 2 Effect of temperature (T), intermittent warming and wax coating on juice, total soluble solids (TSS), titratable acidity and ascorbic acid content of 'Nagpur' mandarin during storage (S)

Treatment	Storage period, days									
	30 d	30 d+1 week	45 d	45 d+1 week	60 d	60 d+1 week	75 d	75 d+1 week	Mean	
	Juice,%									
T_1	43.0	43.3	42.2	41.9	40.3	39.6	38.6	39.3	41.0	T: CD = NS S: CD = 0.7 T × S: CD = 1.8
T_2	40.6	39.6	42.2	41.4	43.0	43.2	40.8	40.7	41.4	Initial value: 42.4% juice
T_3	40.5	40.3	40.9	41.0	41.5	40.2	42.7	42.1	41.1	
T_4	42.6	42.5	40.8	41.6	40.3	39.7	40.2	41.1	41.1	
T_5	42.0	45.8	41.4	40.4	41.2	40.9	40.8	41.8	41.7	
T_6	41.5	44.2	41.8	40.2	41.0	39.6	40.9	39.7	41.1	
Mean	41.7	42.6	41.5	41.0	41.2	40.5	40.6	40.7		
	TSS,%									
T_1	10.2	11.1	10.7	10.2	9.4	9.2	10.1	10.3	10.2	T: CD=0.3 S: CD=0.2 T × S: CD=0.6
T_2	10.6	10.9	10.7	10.9	10.3	11.1	10.3	10.7	10.7	Initial value: 10.0% TSS
T_3	9.9	10.3	10.4	10.7	9.7	9.5	10.3	9.9	10.1	
T_4	9.5	11.0	9.1	9.3	10.3	10.8	10.3	10.3	10.1	
T_5	10.4	10.9	9.7	9.8	10.2	11.2	10.2	10.7	10.4	
T_6	9.9	10.3	9.2	9.5	9.7	10.1	10.6	10.0	9.9	
Mean	10.1	10.8	10.0	10.1	10.0	10.3	10.3	10.3		
	Titratable acidity,%									
T_1	0.9	0.9	1.0	1.0	0.9	0.7	0.9	0.7	0.8	T: CD=NS S: CD=0.05 T × S: CD=0.1
T_2	1.0	0.9	1.0	1.1	1.1	1.0	0.8	0.7	0.9	Initial value: 1.0% acidity
T_3	1.1	0.9	1.0	1.0	0.8	0.9	0.8	0.7	0.9	
T_4	1.1	1.0	1.0	1.0	0.9	1.0	0.9	0.8	0.9	
T_5	1.0	0.9	1.0	1.0	1.0	0.9	1.0	0.8	0.9	
T_6	1.2	1.0	1.0	1.0	0.9	0.8	0.9	0.7	0.9	
Mean	1.0	0.9	1.0	1.0	0.9	0.8	0.8	0.7		
	Ascorbic acid, mg/100 ml									
T_1	33.0	29.9	33.7	31.3	31.6	30.1	28.0	25.1	30.3	T: CD=NS S: CD=0.8 T × S:CD=2.0
T_2	31.9	28.3	33.0	31.9	33.0	31.5	28.4	25.1	30.3	Initial value: 30.8 mg/100 ml
T_3	30.6	30.8	33.7	30.9	32.1	27.9	27.1	25.9	29.8	
T_4	30.8	30.9	33.9	31.0	30.4	30.6	28.6	27.4	30.4	
T_5	31.0	32.0	33.7	32.3	32.3	30.6	27.8	25.6	30.6	
T_6	29.9	31.3	34.0	32.1	32.5	27.4	26.3	26.4	29.9	
Mean	31.6	30.1	33.6	31.5	31.9	29.6	27.7	25.9		

T_1=3.5 °C (const), T_2=3.5 °C with IW, T_3=6.5 °C (const), T_4=Waxed+3.5 °C (const), T_5=Waxed+3.5 °C with IW, T_6=Waxed+6.5 °C (const) to some extent concentration effect. Hydrolysis of cell-wall constituents could also possibly contribute to the increase in Brix (Burns 1990).Temperature regimes and wax coating had non-significant effect on titratable acidity, however, extended storage duration resulted in significant decline in acidity. Drop in acidity varied from 22.2 to 41% in various treatments between the interval of 30 days and 75d+ 1 week. Ascorbic acid content was not affected with temperature regimes and it declined during storage. There was a decline of 11.3–23.9% in ascorbic acid content in different treatments until interval of 75d+ 1 week. Maximum loss was recorded in non-waxed fruit stored at 3.5 °C (constant) (T_1) where maximum chilling injury was recorded. In lemon (Citrus limon) fruit, ascorbic acid losses were reported to be associated with symptom development (Eaks 1961). Ascorbic acid and acidity dropped considerably while TSS recorded little change in stored Kinnow fruit at 5 °C (Mahajan et al. 2006).

Sugars (juice) and phenol (peel) content Reducing sugars were lower in juice of waxed and non-waxed fruit stored at 3.5 °C (constant) (T_1 and T_4) (Table 3). Higher reducing sugars in fruit stored under IW treatment (T_2 and T_5) and at 6.5 °C (constant) (T_3 and T_6) could be due to higher activity of invertase enzyme (Purvis and Rice 1983). In grapefruit, reducing sugars in flavedo accumulated and sucrose decreased more rapidly in fruit stored under IW than at constant 5 °C (Purvis 1989). In peel of 'Marsh' grapefruit (*Citrus paradisi*) and 'Washington Navel' oranges (*Citrus sinensis*) reducing sugars significantly increased and chilling injury decreased significantly with proline sprays (Ezz 1999) indicating role of sugars in preventing chilling. Specific role of reducing sugars in chilling resistance need to be determined in further studies.

Lower total sugars were recorded at 3.5 °C (constant) in waxed and non-waxed fruit (T_1 and T_4) than at other temperature regimes irrespective of waxing. Sugars increased in juice at the end of 1 week holding after each storage interval except 75d+ 1 week. Considerably lower total sugars in waxed and non-waxed fruits stored at 3.5 °C (constant) were recorded at the interval of 75 days and also after 1 week. Concentration of total sugars in grapefruit peel did not change during storage at 5 °C although relative concentrations of non-reducing and reducing sugars did

Table 3 Effect of temperature (T), intermittent warming and wax coating on reducing sugar, total sugar, total phenol of 'Nagpur' mandarin during storage (S)

Treatment	Storage period, days									
	30 d	30 d+1 week	45 d	45 d+1 week	60 d	60 d+1 week	75 d	75 d+1 week	Mean	
	Reducing sugars,% in juice									
T_1	4.3	6.3	4.9	6.1	4.8	6.6	3.6	3.3	4.9	T: CD=0.5 S: CD=0.4 T × S:CD=1.1
T_2	3.9	5.1	4.7	5.3	6.7	7.5	3.8	3.3	5.0	Initial value: 3.7% sugar
T_3	4.2	6.1	4.7	6.2	6.1	7.3	3.5	3.0	5.1	
T_4	3.7	5.6	4.0	5.9	4.7	6.1	2.6	2.8	4.4	
T_5	4.2	6.1	4.3	6.3	6.6	6.5	3.4	3.1	5.0	
T_6	4.9	6.9	4.8	6.3	6.6	9.1	3.6	3.4	5.7	
Mean	4.2	6.0	4.5	6.0	5.9	7.1	3.4	3.1		
	Total sugars,% in juice									
T_1	10.0	10.5	9.7	10.3	10.0	10.0	8.4	5.4	9.2	T: CD=0.6 S: CD=0.6 T×S: CD=1.5
T_2	9.0	10.0	9.7	11.0	10.5	11.1	10.3	10.5	10.2	Initial value: 8.1% sugars
T_3	8.7	10.0	9.2	10.4	10.0	10.0	10.1	10.0	9.8	
T_4	7.0	10.9	8.0	9.3	10.0	11.0	7.7	4.7	8.4	
T_5	6.9	10.2	7.4	10.0	10.2	9.7	10.2	10.9	9.4	
T_6	6.7	10.6	7.2	9.2	10.0	10.6	10.6	10.0	9.3	
Mean	8.1	10.3	8.5	10.0	10.1	10.4	9.5	8.5		
	Total phenol, mg/100 g peel									
T_1	335.0	275.0	172.5	312.5	157.5	227.1	172.5	165.0	227.1	T: CD=NS S: CD=17.9 T × S: CD=43.9
T_2	350.0	222.5	195.0	190.0	215.0	215.0	205.0	230.0	227.8	Initial value: 297 mg/100 g peel
T_3	350.0	260.0	150.0	260.0	216.0	222.0	232.5	190.0	235.0	
T_4	292.5	295.0	157.0	267.0	172.0	237.0	192.0	202.0	226.8	
T_5	282.5	275.0	180.0	267.5	160.0	207.5	187.5	235.0	224.3	
T_6	285.0	250.0	197.5	277.5	197.5	197.5	222.5	200.0	228.4	
Mean	315.8	262.9	175.3	262.4	186.3	217.6	202.0	203.6		

T_1–T_6: As in Table 2

change (Purvis 1989). Ezz et al. (2004) reported that total sugars in grapefruit peel increased accompanied by higher resistance to chilling following hot water dip and exposure to high CO_2. In the present study mandarin fruit under IW treatment had higher total sugars in juice.

Slightly higher phenols in the peel were recorded in fruit stored at 6.5 °C (constant) (T_3) than at 3.5 °C (constant) (T_1). Temperature regimes and IW had non-significant effect on phenol content. Phenol content declined during storage; the drop with reference to initial value being 31.1–44.0%, 32.6–36.0% and 20.2–22.6% in fruit stored at 3.5 °C (constant) (T_1), 6.5 °C (constant) (T_3) and under IW treatment (T_2 and T_5), respectively up to 75d+ 1 week. This indicated higher drop of phenolics in fruit which suffered chilling. Comparison of phenolic content in fruits stored under IW treatment and at 3.5 °C (constant) showed inconsistent trend and needs further studies. Decline of total phenolics in ber fruits was reported during storage at 3–5 °C up to 30 days (Jawandha et al. 2009).

Chlorophyll and carotenoid contents Chlorophyll contents ('a' 'b' and total) of peel were significantly higher in fruits stored at 3.5 °C (constant) (T_1 and T_4) than at other temperature regimes irrespective of wax coating (Table 4). In general chlorophyll content declined during storage. Initial total chlorophyll content was 114 μg/g peel which gradually declined to 6.3 μg/g at 75d+ 1 week in non-waxed fruit stored at 3.5 °C (constant) (T_1). Maximum total chlorophyll content (12.5 μg/g) was recorded in peel of waxed fruit stored at 3.5 °C (constant) (T_4) after 75d+ 1 week. During 1 week

Table 4 Effect of temperature (T), intermittent warming and wax coating on chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids of 'Nagpur' mandarin peel during storage (S)

Treatment	Storage period, days									
	30 d	30 d+1 week	45 d	45 d+1 week	60 d	60 d+1 week	75 d	75 d+1 week	Mean	
	Chlorophyll 'a', μg/g peel									
T_1	31.8	21.8	31.0	13.8	14.0	0.7	6.8	3.7	15.3	T:CD=1.5 S: CD=1.6 T × S: CD=3.9 Initial value: 44 μg/g peel
T_2	20.3	15.8	16.8	12.2	3.7	0.5	2.5	2.6	9.9	
T_3	20.0	3.1	18.8	12.5	4.0	0.9	2.4	3.5	8.1	
T_4	34.5	14.9	31.8	17.4	17.3	1.5	7.2	7.2	16.4	
T_5	21.5	3.9	18.8	6.2	3.2	0.9	4.0	1.9	7.5	
T_6	20.8	4.5	20.3	4.3	4.5	1.8	1.9	2.9	7.6	
Mean	25.8	10.6	19.9	11.0	7.7	0.8	4.1	3.6		
	Chlorophyll 'b', μg/g peel									
T_1	37.0	9.8	15.3	11.5	6.5	2.0	7.3	2.5	11.4	T: CD=1.8 S: CD=2.1 T × S: CD=5.2 Initial value: 70 μg/g peel
T_2	13.3	4.1	11.5	5.5	2.0	1.3	6.0	2.0	5.7	
T_3	12.5	0.6	9.5	4.8	3.6	2.4	7.2	3.9	5.5	
T_4	25.8	9.4	16.5	11.5	11.3	7.0	10.3	5.3	12.1	
T_5	18.7	1.7	4.0	4.8	3.8	3.9	3.5	2.8	5.4	
T_6	20.6	1.5	7.3	1.5	1.8	3.1	2.7	2.9	5.1	
Mean	21.3	4.5	10.6	6.6	4.8	3.2	6.1	3.2		
	Total chlorophyll, μg/g peel									
T_1	68.8	31.5	45.8	25.3	19.5	2.3	14.1	6.3	26.7	T: CD=2.6 S: CD=1.1 T × S: CD=2.7 Initial value: 114 μg/g peel
T_2	33.5	22.4	28.3	17.5	5.0	1.1	8.1	4.6	15.0	
T_3	32.5	1.6	26.8	16.8	6.4	4.5	10.8	7.2	13.3	
T_4	56.8	22.7	48.5	28.8	27.8	10.9	17.5	12.4	28.3	
T_5	40.1	5.5	23.5	10.5	6.0	4.8	7.5	4.7	12.8	
T_6	42.7	4.8	27.5	5.8	6.5	4.7	5.6	5.8	12.9	
Mean	45.7	14.7	33.4	17.4	11.8	4.8	17.7	6.8		
	Carotenoids, mg/100 g peel									
T_1	1.0	1.9	1.1	2.0	1.4	2.2	2.7	2.7	1.8	T: CD=0.4 S: CD=0.2 T × S: CD=0.3 Initial value: 1.1 mg/100 g peel
T_2	1.5	2.5	1.8	2.6	4.3	4.1	6.2	6.8	3.7	
T_3	1.7	3.6	2.4	3.1	4.2	4.6	6.2	6.6	4.0	
T_4	1.0	1.8	1.1	2.1	1.4	2.5	2.6	2.7	1.9	
T_5	1.6	2.2	2.0	2.7	3.6	3.9	3.3	3.6	2.8	
T_6	1.6	3.4	2.2	2.9	4.1	4.6	5.5	6.3	3.8	
Mean	1.4	2.5	1.7	2.5	3.1	3.6	4.4	4.7		

T_1–T_6: As in Table 2

holding at ambient condition, drop in chlorophyll content was rapid. Total carotenoid content was significantly higher in fruits stored under IW treatment (T_2 and T_5) and at 6.5 °C (constant) (T_3 and T_6) than at 3.5 °C (constant) (T_1 and T_4) in waxed and non-waxed fruit indicating that chilling temperature (3.5 °C constant) had inhibitory effect on carotenoid synthesis. Wax coating also had inhibitory effect on carotenoid development. During 1 week holding under ambient condition, carotenoid content increased significantly at each interval. Temperatures of 15–20 °C were found optimum for production of maximum colour in citrus rind. Even small quantity of endogenous ethylene induced colour development (Wheaton and Stewart 1973) while low temperature stress induced ethylene production (Vines et al. 1968).

Rind colour At 3.5 °C (constant) (T_1), non-waxed fruits recorded very slow colour change as evident from a*/b* ratio (Table 5). Wax coating delayed colour change. Rind colour improved dramatically during 1 week holding after each storage interval. After 45d+ 1 week, non-waxed fruit stored at 3.5 °C (constant) (T_1) had +0.1 a*/b* ratio which indicated loss of green colour and substantial development of yellow-orange colour as compared to waxed fruit which had –0.1 a*/b* ratio under similar conditions (T_4). At 60d+ 1 week, both waxed and non-waxed fruits stored at 3.5 °C (constant) (T_1 and T_4) had + a*/b* ratio. At the interval of 75 days, a*/b* ratio was –0.2 and –0.1 in waxed and non-waxed fruit, respectively (T_1 and T_4), however, after 75d+ 1 week colour improved considerably. Fruit stored under IW treatment (T_2 and T_5) and at 6.5 °C (constant) (T_3 and T_6) had good colour development irrespective of waxing. These findings indicated that attractive orange colour development

Table 5 Effect of temperature (T), intermittent warming and fungicidal wax coating on rind colour (a*/b* ratio) of 'Nagpur' mandarin

Storage period, days	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
30 days	−0.4	−0.2	−0.2	−0.4	−0.3	−0.3
30 d+1 week	−0.1	0.1	0.2	−0.2	−0.1	0.1
45 days	−0.3	0.2	0.3	−0.4	0.1	0.2
45 d+1 week	0.1	0.3	0.3	−0.1	0.3	0.4
60 days	−0.2	0.3	0.4	−0.2	0.3	0.4
60d+1 week	0.2	0.3	0.4	0.1	0.3	0.4
70 days	−0.1	0.3	0.5	−0.2	0.3	0.3
75 d+1 week	0.3	0.4	0.5	0.2	0.4	0.4

T_1–T_6: As in Table 2

(n=4). Negative value of a*/b* ratio indicates green colour of rind and higher the negative value more the green colour. Positive value indicates yellow-orange colour and higher value indicates higher intensity of yellow-orange colour

Table 6 Respiration rate (mg CO_2/kg/h) of 'Nagpur' mandarin as affected by temperature, intermittent warming and fungicidal wax coating

Storage period, days	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
0	40.0	41.2	42.1	28.5	29.7	30.0
6	7.0	6.7	7.8	7.0	6.0	9.0
12	6.5	6.1	9.5	5.5	5.8	8.6
18	6.6	28.2	8.7	5.4	21.5	8.0
24	5.1	20.5	8.0	4.2	14.0	8.0
30	6.1	7.0	8.8	5.5	7.5	7.6
36	6.9	6.0	7.8	5.0	4.5	8.2
42	8.4	31.4	8.2	7.5	27.0	8.0
48	12.0	9.1	8.8	9.0	8.9	8.0
54	12.1	6.9	8.9	10.5	5.9	7.4
60	13.5	20.2	8.0	12.0	15.6	7.5
66	14.0	32.6	9.0	12.5	30.3	8.0
72	14.5	8.2	11.8	13.0	7.0	10.0
75	15.5	6.6	13.2	12.0	6.0	12.5
75+1w	45.5	40.1	40.0	42.5	35.6	37.8

T_1–T_6: As in Table 2

(n=2). Observations at every 6 days interval are given. Observation on 75th day is last observation under low temperature storage treatments. Observation recorded on 75+1 w day was after 1 week holding at ambient condition

with increased storage life at chilling temperature with IW is an added advantage for mandarins grown in tropical conditions of central and south India where fruit rind remains green although fruit is mature.

Respiration Before storage (at '0' days), respiration rate was higher in non-waxed fruit (40–42 mgCO_2/kg/h) than in waxed fruit (28–30 mgCO_2/kg/h) (Table 6). Respiration rate declined gradually with storage at low temperature. Respiration was generally higher at 6.5 °C (constant) (7–8 mgCO_2/kg/h) than at 3.5 °C (constant) (4–6 mgCO_2/kg/h). In IW treatment, gradual decline in temperature (chilling cycle) resulted in drop in respiration with rise again during warming cycle (T_2 and T_5).

At 3.5 °C (constant), respiration rate was lower up to 36 days and started increasing after 42 days (Table 6). At 6.5 °C (constant), fruit recorded steady but low respiration up to 60 days with increase in respiratory activity thereafter up to 75 days in non-waxed (T_3) and waxed fruit (T_6). This indicated metabolic changes during storage and rise in respiratory activity which could be beginning of chilling-injury development as evident at 3.5 °C (constant) at 45 days and at 6.5 °C (constant) at 75 days. It indicated that at relatively higher storage temperature chilling injury development coupled with respiratory rise occurred later. Removal of fruit from cool chamber at the interval of 75 days resulted in

Table 7 Respiratory rate (mg CO_2/kg/h) of 'Nagpur' mandarin fruit showing chilling injury symptoms

Storage period, days	Treatments			
	T_1	T_3	T_4	T_6
45 d+1 week	91.0	–	78.2	–
60 days	100.0	–	98.4	–
60 d+1 week	131.0	–	128.0	–
75 days	141.2	78.5	132.0	63.3
75 d+1 week	131.5	61.2	145.5	98.8

T_1–T_6: As in Table 2

(n=2). In T_3 and T_6 chilling injury was observed after 75 days. In T_2 and T_5 no chilling injury

rise in respiration as recorded on 82nd day (i.e. after 1 week); rise being higher at 3.5 °C (constant) (T_1 and T_4).

Respiratory activity of fruit showing chilling injury was measured separately and presented in Table 7. Fruit with chilling injury symptoms recorded very high respiration rate immediately after storage and also after 1 week holding. At the interval of 45 days, after 1 week holding, respiratory rate was 91.0 mgCO_2/kg/h and 78.2 mgCO_2/kg/h in non-waxed (T_1) and waxed fruit (T_4), respectively at 3.5 °C (constant). Maximum respiratory rate at 75 days and at 75d+ 1 week was observed in fruit stored at 3.5 °C (constant). There was no chilling–related rise in respiration in fruits under IW treatment (T_2 and T_5). At 6.5 °C (constant) (T_3 and T_6) chilling injury symptoms with consequent rise in respiration was observed at 75 days. Citrus fruits stored at chilling temperatures recorded considerable rise in respiration after removal to warmer temperatures (Eaks 1960). Fruits stored at 3.5 °C (constant) (T_1) showed sharp drop in reducing and total sugars at 75 days and 1 week holding when respiration was highest. Grapefruits with chilling damage had higher respiration rate (McCollum and McDonald 1991). It appears that sugars formed substrate for increased respiratory activity. Accumulation of ethanol and acetaldehyde in chilling-injured tissues indicated metabolic aberrations leading to anomalous respiratory behaviour (Eaks 1980).

Carbendazim residues Initial deposits of carbendazim in non-waxed fruit were 3.2 ppm in peel and 3.1 ppm in pulp while in waxed fruits the residues were 4.0 ppm in peel and 3.2 ppm in pulp (Table 8). Higher residues in waxed fruit (T_4, T_5 and T_6) were attributed to fungicidal wax application which included carbendazim (2,000 ppm). At 30d+ 1 week, residues declined in peel and pulp; peel recording relatively higher residues. Residues declined at each interval with very little difference in residues at 60d+ 1 week and at 75d+ 1 week. Temperature regimes did not have any marked effect on dissipation of carbendazim residues. In mandarins, peel is discarded during fruit consumption and hence hazard of fungicide residue is less as compared with grape and apple which are consumed without peeling. In modified atmosphere packaged 'Nagpur' mandarin, carbendazim residues declined considerably during storage (Ladaniya 2007). Carbendazim residues of whole mango fruit was 2.8 mg/kg at 0 day after hot water dip for 10 min with 500 ppm carbendazim and declined to 1.7 mg/kg at 4 days and 0.9 mg/kg at 15 days under ambient conditions (Bhattacharjee et al. 2009). In the present study, carbendazim residues were 0.5 and 0.2 ppm in fruit pulp at 75d+ 1 week under IW treatment with and without wax coating, respectively. Carbendazim is in use in many countries including India. Residues levels were below maximum limit of 5 ppm approved by Codex Alimentarius commission's (FAO/WHO) pesticide residue standards in fruit.

Conclusion

'Nagpur' mandarin fruit storage at 3.5 °C with intermittent warming cycle (2 weeks at 3.5 °C followed by 1 week at

Table 8 Carbendazim residues (mg/kg) of 'Nagpur' mandarin during storage

Treatment	Storage period, days 30 d+1 w Peel	45 d+1 w	60 d+1 w	75 d+1 w	30 d+1 w Pulp	45 d+1 w	60 d+1 w	75 d+1 w
T_1	1.7	0.6	0.7	0.6	1.0	0.6	0.5	0.3
T_2	1.6	1.1	0.6	0.7	1.0	0.6	0.4	0.2
T_3	1.5	0.8	0.6	0.6	1.0	0.5	0.4	0.2
T_4	1.9	1.0	0.8	0.6	1.6	0.5	0.5	0.5
T_5	2.2	1.5	0.7	0.7	1.6	0.6	0.6	0.5
T_6	2.1	1.4	0.7	0.7	1.6	0.5	0.6	0.4

T_1–T_6: As in Table 2

(n=2). Initial residues (2 days after treatment): Non-waxed fruit peel = 3.2 ppm, Pulp = 3.1 ppm; Waxed fruit peel = 4.0 ppm; Pulp = 3.2 ppm

1 w: Holding for 1 week at ambient condition

19.5 °C) with wax coating resulted in orange colour development, complete alleviation of chilling injury and loss of fungicide residue (carbendazim) during storage up to 75 days. Respiration rate recorded rise and fall during IW cycle at chilling temperature. Difference was non-significant in wax coated and non-coated fruit under IW with respect to chilling, reducing sugars, phenols and composition but carotenoid content was low in waxed fruit than in non-waxed fruit. Constant chilling temperature resulted in lower reducing and total sugars, TSS and carotenoids with higher chilling injury and chlorophyll content in peel while IW at 3.5 °C resulted in higher sugars, juice and TSS contents and carotenoids. Fruit stored at 3.5 °C (constant) had very high chilling injury and it appeared at 45 days while at 6.5 °C (constant) chilling appeared at 75 days. This study clearly indicated that 'Nagpur' mandarin storage life can be extended up to 75 days at 3.5 °C with IW cycle with or without wax coating.

Acknowledgement Author is highly thankful to ICAR, New Delhi for providing funds under NATP project "Reduction in post-harvest losses in fruits and vegetables" for the present work. Author also expresses deep appreciation of assistance provided by Bipin Mahalle and Khan NA in this work.

References

AOAC (1985) Official methods of analysis, 14th edn. Association of Official Analytical Chemists, Washington

Bhattacharjee AK, Pandey BK, Prakash O (2009) Persistence and dissipation of carbendazim residues in mango after pre and post–harvest applications. J Food Sci Technol 46:347–349

Burns JK (1990) α- and β galactosidase activity in juice of Valencia orange. Phytochemistry 29:2425–2429

Chalutz E, Waks J, Sciffman-Nadel M (1985) Reducing susceptibility of grapefruit to chilling injury during cold treatment. HortScience 20:226–228

Dou H (2004) Effect of coating application on chilling injury of grapefruit cultivars. HortScience 39:558–561

Dubois M, Gillies KA, Hamilton JK, Reber FA, Smith F (1956) Colorimetric methods of determination of sugars and related substances. Anal Chem 28:330–335

Eaks IL (1960) Physiological studies of chilling injury in citrus fruits. Pl Physiol 35:632–635

Eaks IL (1961) Effect of temperature and holding period on some physical and chemical characteristics of lemon fruits. J Food Sci 26:593–599

Eaks IL (1980) Effect of chilling on respiration and volatiles of California lemon fruit. J Am Soc Hortic Sci 105:865–869

Eaks IL, Raison JK (1970) Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. Pl Physiol 45:386–389

Echeverria E, Ismail M (1990) Sugars unrelated to Brix changes in stored citrus fruits. HortScience 25:710–711

Ezz TM (1999) Eliminating chilling injury of citrus fruit by pre-harvest proline foliar sprays. Alexandria J Agric Res 44:213–225

Ezz TM, Ritenour MA, Brecht JK (2004) Hot water and elevated CO_2 effects on proline and other compositional changes in relation to post-harvest chilling injury of Marsh grapefruit. J Am Soc Hortic Sci 129:578–582

Jawandha SK, Randhawa JS, Mahajan BVC, Gill PPS (2009) Effect of post harvest treatment on cellulase activity and quality of ber fruits under cold storage. J Food Sci Technol 46:87–88

Ladaniya MS (2004a) Response of 'Kagzi'acid lime to low temperature regimes during storage. J Food Sci Technol 41:284–288

Ladaniya MS (2004b) Standardization of temperature for long term refrigerated storage of 'Mosambi' sweet orange (C*itrus sinensis* Osbeck). J Food Sci Technol 41:692–695

Ladaniya MS (2007) Quality and carbendazim residues of 'Nagpur' mandarin fruit in modified atmosphere package. J Food Sci Technol 44:85–89

Ladaniya MS, Sonkar RK (1996) Influence of temperature and fruit maturity on 'Nagpur' mandarin (*Citrus reticulata* Blanco) in storage. Indian J Agric Sci 66:109–113

Ladaniya MS, Singh S, Mahalle B (2005) Sub-optimum low temperature storage of 'Nagpur' mandarin as influenced by wax coating and intermittent warming. Indian J Hortic 62:1–7

Mahajan BVC, Dhatt AS, Kumar S, Manohar L (2006) Effect of pre-storage treatments and packaging on storage behaviour and quality of Kinnow mandarin. J Food Sci Technol 43:589–593

McCollum TG, McDonald RE (1991) Electrolyte leakage, respiration and ethylene production as indices of chilling injury in grapefruit. HortScience 26:1191–1192

McDonald RE (1986) Effects of vegetable oils, CO_2 and film wrapping on chilling injury and decay of lemon. HortScience 21:261–266

McDonald RE, McCollum TG, Nordby HE (1993) Temperature conditioning and surface treatments of grapefruits affect expression of chilling injury and gas diffusion. J Am Soc Hortic Sci 118:490–496

Purvis AC (1989) Soluble sugars and respiration of flavedo tissue of grapefruit stored at low temperatures. HortScience 24:320–322

Purvis AC, Grierson W (1982) Accumulation of reducing sugars and resistance of grapefruit peel to chilling injury as related to winter temperatures. J Am Soc Hortic Sci 107:137–142

Purvis AC, Rice JD (1983) Low temperature induction of invertase activity in grapefruit flavedo tissue. Phytochem 22:831–834

Raison JK, Lyons JM, Mehlhorn RJ, Keith AD (1971) Temperature induced changes in mitochondrial membranes detected by spin labeling. J Biol Chem 246:4036–4040

Sadasivam S, Manickam A (1996) Biochemical methods. 2nd edn, New Age International Pvt. Ltd and Tamilnadu Agricultural University, Coimbatore, pp 190–191

Schiffman-Nadel M, Chalutz E, Waks Y, Dagan M (1975) Reduction of chilling injury in grapefruit by thiabendazole and benomyl during long term storage. J Am Soc Hortic Sci 100:270–272

Shewfelt RC, Purvis AC (1995) Towards a comprehensive model for lipid peroxidation in plant tissue disorders. HortScience 30:213–218

Ting SV, Rouseff RL (1986) Citrus fruits and their products: analysis and technology. Marcel Dekker Inc, New York, p 91

Vaidya UM, Bannerjee SC (1984) Colorimetric determination of carbendazim. Pesticides 18(3):17–18

Vines HM, Grierson W, Edwards GJ (1968) Respiration, internal atmosphere and ethylene evolution of citrus fruits. J Am Soc Hortic Sci 92:227–234

Wang CY (1993) Approaches to reduce chilling injury of fruits and vegetables. Hortic Rev 15:63–95

Wang CY, Adams DO (1982) Chilling induced ethylene production in cucumbers. Pl Physiol 89:424–427

Wheaton TA, Stewart I (1973) Optimum temperature and ethylene concentrations for post-harvest development of carotene pigments in citrus. J Am Soc Hortic Sci 98:337–348