

Determination of storage duration and temperature effects on fruit quality parameters of blood orange (*Citrus sinensis* cv. Tarocco)

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Abstract. Blood orange (*Citrus sinensis*) cv. Tarocco, grown in the north of Iran, was harvested at the mature stage and stored at 8, 15 or 22°C for various duration. During the storage period, weight loss, total soluble solids (TSS), total titratable acidity (TA), pH, ascorbic acid, total phenols, flavonoids, antioxidant capacity, anthocyanin and L-phenylalanin ammonia-lyase enzyme (PAL) activity were determined. The highest increase in pH, anthocyanins in the pulp and rind, flavonoids and PAL activity after 85 days of storage at 8°C were 3.6, 12.4 mg/L, 15.8 mg/L, 142.6 mg/L and 2.5 μMOL/g FW.min⁻¹, respectively, and TSS, TA, fruit weight and ascorbic acid content decreased for fruit stored at different temperatures. Antioxidant capacity and total phenols increased during the first 45 days of storage at 8°C by 38% and 631.6 mg/L respectively. Also the results indicated a significant correlation between antioxidant activity and total phenolic compounds ($P \leq 0.01$).

Key words: anthocyanins, flavonoids, phenols, antioxidant capacity, ascorbic acid.

Introduction

Blood orange (*Citrus sinensis* [L.] Osbeck, is one of the important varieties of the world and Iran, have valuable medicinal and nutritional compounds and are mainly cultivated in the Mediterranean climate (Fotouhi & Fattahi 2007). These fruits are characterized by their unique flesh and rind colour, due to red pigments belonging to the anthocyanins (Maccarone et al. 1983). Anthocyanins are the largest group of water-soluble pigments in the plant kingdom and have a wide range of antioxidant activity (Arena et al. 2001, Bonina et al. 1998, Rapisarda et al. 1999). The major antioxidant components of blood orange (cv. Tarocco) juices are ascorbic acid, flavonoids, and hydroxycinnamic acids (Arena et al. 2001, Gardner et al. 2000, Rapisarda et al. 2001). These compounds are considered as useful markers for identification and evaluation of nutritional value in fresh and processed products. Cyanidin-3-glucoside (Cy3G) and cyanidin 3-(6"-malonyl)-glucoside (Cy3MG) are the main anthocyanins in blood orange juice, and Cy3G has a higher antioxidant activity than other more common forms of anthocyanins (Maccarone et al. 1983, Wang et al. 1997). Important therapeutic properties include prevention of injury to blood vessel membranes, anti-cancer activity, beneficial effects on capillary fragility and arteriosclerosis and antiviral activity (Sajia 1994). Temperature is one of the most important factors influencing the accumulation of anthocyanins in flowers and fruit (Dela et al. 2003). Although citrus fruits are non-climacteric and have a relatively long shelf-life, post harvest management and marketing allow these fruits to be stored in a low temperature until the summer months (Rapisada et al. 2008). Blood oranges are highly susceptible to chilling injury when stored below 7-8°C and they can undergo internal metabolic changes during prolonged storage (Pratella et al. 1969). Previous research showed that high temperature reduces the accumulation of anthocyanin pigments (Spayd et al. 2002). The major effects of high temperatures on the physico-chemical properties of fruits include significant decrease in pigment synthesis, fruit weight and rind quality (Pompo-

dakis et al. 2005). Fruit quality and its appearance are very important from a consumer's viewpoint. Therefore, the purpose of this study was the evaluation of the physiological and biochemical changes to determine the optimal storage temperature to maintain product quality and to provide acceptable fruit quality of Tarocco oranges to consumers.

Material and Methods

Fruit treatment

In this study fruit Tarocco blood orange cultivar harvested at the ripe stage in Mazandaran (North of Iran) then transferred to the laboratory after fruit were treatment 1g/L of Benomil to minimize decay during storage. fruits were then kept dry at room temperature for about 3 hour before being placed randomly in boxes (6 fruit per box) and stored in temperature 8, 15 and 22°C (relative humidity 85-90%). Fruits sampling was carried out before storage (time 0) and at about 45 day interval, for a total storage period of 85 days.

Physicochemical analysis

Total soluble solids by refractometer, pH using pH meter, titratable acidity by titration with 0.1N NaOH and ascorbic acid was determined by titration with 2, 6-dichlorophenol-indophenol (Saini et al. 2001, Rana et al. 1992).

Total phenol analysis

The orange juices were analyzed for total phenolics by the Folin-Ciocalteu (FC) colorimetric method (Singleton et al., 1999). Appropriately diluted sample (0.5 mL) were mixed with 5 mL FC commercial reagent (previously diluted with water 0.5:10, v/v) and 4 mL of a 7.5% sodium carbonate solution. The mixture was stirred for 1 h at room temperature. The absorbance of the resulting blue solution was measured spectrophotometrically at 740 nm and the concentration of total phenolics was expressed as (±) gallic acid equivalents (mg/L).

Total flavonoid

Total flavonoids were determined according to Miliauskas et al. (2004). One ml of fruit extract in methanol (10 g/L) was mixed with 1 mL aluminium trichloride in ethanol (20 g/L) and diluted with ethanol to 25 mL. The absorption at 415 nm was read after 40 min at 20°C. Blank samples were prepared from 1 ml plant extract and 200 μL acetic acid, and diluted to 25 mL. The quercetin calibration curve was prepared in ethanolic solutions with same procedure. All de-

terminations were carried out in quadruplicate and the mean values were used.

Antioxidant activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity measurements were carried out according to the procedure of Sanchez-Moreno et al. (1998) with some modifications. Briefly, 30 μ L of juice sample (diluted with distilled water and centrifuged) was added to 970 μ L of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH \cdot ; 4mg in 100 mL methanol) and mixed by vortex for 2 min. The absorbance of the samples was measured at 515 nm every 1 min for 5 min using the spectrophotometer. For each sample, three separate determinations were carried out. The antioxidant activity (AA), calculated through the absorbance decline rate after 1 min in relation to the control, corresponding to the percentage of DPPH radical that was scavenged. The percentage of DPPH \cdot , which was scavenged (%DPPH \cdot _{sc}) according to the following formula:

$$\%DPPH\cdot_{sc} = (A_{cont} - A_{samp}) \times 100 / A_{cont}$$

A_{cont} is the absorbance of the control.

A_{samp} is the absorbance of the sample.

Total anthocyanin analysis

The extraction solvent was prepared as methanol:hydrogen chloride (85:15, v/v). Juice and solvent were mixed as 3:1 ratio and was hold in a refrigerator at 4°C overnight. Subsequently, the sample were centrifuged at 10000 rpm for 15 min. Spectrophotometric analysis of total anthocyanin content in fruit juice was determined using the pH differential method (Wroslstad, 1976).

Phenylalanin ammonia-lyase analysis

Phenylalanin ammonia-lyase analysis was performed according to the method Saunders et al (1974) with some modifications. The content of enzyme assay including: 50 μ L enzyme extract, 450 μ L borat sodium buffer 10 mM, 250 μ L distilled water and was 250 μ L Phenylalanin substrate buffer which initial and final absorption respectively before and after placed in water bath with temperature of 40°C at a wavelength of 290 nm be read. Enzyme activity expressed on μ MOL/g FW.min.

Statistical analysis

Experimental results are expressed as mean \pm SD. All measurements were replicated three periods. The data were analyzed by analysis of

variance ($p < 0.05$) and the mean separated by LSD's multiple range test.

Results

The variance analysis showed that interaction effects of storage time and temperature were significant ($P < 0.01$) on pH, total phenol, flavenoid, antioxidant capacity, anthocyanin and L-phenylalanin ammonia-lyase enzyme activity. No significant differences were observed on weight loss, TSS, titrable acidity and vitamin C by variance analysis and mean comparison. There was no significant difference in amount of vitamin C at different temperature but decreased in during storage.

The amount of anthocyanin is important for the attractiveness and maturity assessment of blood orange as can be observed in Table 2. The highest anthocyanin level was recorded after 85 days of storage at 8°C with amount 12.39 mg/L in tissue and 15.78 mg/L in peel. With increasing temperature from 8 to 15°C, the average treatment at the first 45 days is more, and when the temperature was 22 °C sharp reductions in anthocyanin occurred to the end storage (Table 2).

The highest L-phenylalanin ammonia-lyase enzyme activity and protein was 2.45 μ MOL/g FW.min and 16.40 mg/gFW, respectively, after 85 days of storage at 8°C. Increasing temperature and storage time significantly reduced amount of L-phenylalanin ammonia-lyase enzyme activity and protein (Table 3).

The total flavonoid content were higher in 8°C with amount 142.56 mg/L after 85 days of storage and 122.63 mg/L in 15°C at the first 45 day storage (Table 1).

According to Table 1, time and temperature of storage significantly affected the total phenol content. During storage, there was a significant initial increase in 8 and 15°C at

Table 1. Mean comparison of fruit pH, total phenol and flavenoid in blood orange juices during 85 days of storage at 8, 15 and 22°C.

Storage (days)	pH			Phenol(mg/L)			Flavenoid(mg/L)		
	8°C	15°C	22°C	8°C	15°C	22°C	8°C	15°C	22°C
0	3.42bc	3.42bc	3.42bc	521.6d	521.6d	521.6d	115.9d	115.9d	115.9d
45	3.52ab	3.47ab	3.32c	631.6a	553.7b	441.6e	129.8b	122.6c	102.7e
85	3.57a	3.28cd	3.18d	537.0c	418.6f	361.5g	142.6a	99.5e	88.6f

Mean separation within columns by LSD's multiple rang test at $P = 0.01$

Table 2. Mean comparison of fruit DPPH, anthocyanin in peel and tissue in blood orange juices during 85 days of storage at 8, 15 and 22°C.

Storage (days)	DPPH(%)			anthocyanin in peel (mg/L)			anthocyanin in tissue(mg/L)		
	8°C	15°C	22°C	8°C	15°C	22°C	8°C	15°C	22°C
0	37.5a	37.5a	37.5a	8.9d	8.9d	8.9d	7.1d	7.1d	7.1d
45	38.0a	37.8a	34.7b	13.3b	9.9c	7.3e	10.4b	8.1c	5.6f
85	36.0b	35.5b	33.1c	15.8a	8.3d	5.2f	13.0a	6.8e	3.6g

Mean separation within columns by LSD's multiple rang test at $P = 0.01$

Table 3. Mean comparison of fruit peel/tissue, PAL and protein in blood orange juices during 85 days of storage at 8, 15 and 22°C.

Storage (days)	peel/tissue			PAL μ MOL/g FW.min			Pr μ MOL/g		
	8°C	15°C	22°C	8°C	15°C	22°C	8°C	15°C	22°C
0	1.24d	1.24d	1.24d	1.81c	1.81c	1.81c	14.0c	14.0c	14.0c
45	1.28c	1.22e	1.30b	2.10b	2.01b	1.58d	15.5b	15.1b	12.4d
85	1.27c	1.28c	1.44a	2.45a	1.70cd	1.21e	16.4a	13.7c	10.7e

Mean separation within columns by LSD's multiple rang test at $P = 0.01$

Table 4. Correlation coefficients between Phenol falvonoid, DPPH, anthocyanin in peel, anthocyanin in tissue, peel/tissue, PAL and protein in blood orange juices during 85 days of storage at 8, 15 and 22°C.

	Phenol	falvonoid	DPPH	anthocyanin in peel	anthocyanin in tissue	peel/tissue	PAL	protein
Phenol	1							
falvonoid	-0.28	1						
DPPH	0.12	0.30	1					
anthocyanin in peel	0.002	0.60**	0.81**	1				
anthocyanin in tissue	0.2	0.41**	0.88**	0.85**	1			
peel/tissue	-0.34	0.65**	0.66**	0.82**	0.63**	1		
PAL	0.51**	0.22	0.79**	0.74**	0.93**	0.46*	1	
protein	0.47**	0.27	0.82**	0.77**	0.94**	0.52**	0.99**	1

Significant at* $P \leq 0.05$ and ** $P \leq 0.01$

the 45 days with amount 631.58 mg/Land 553.65 mg/L, respectively, subsequent decrease in total phenol in oranges juices at 85 day storage. At the end storage at 8, 15 and 22°C the content of these compounds decreased (Table 1).

Table 2 showed the changes in the antioxidant activity of orange juice sample measured by DPPH assay. In the test with DPPH• radical, there was a slight increase in antioxidant capacity during the first 45 days of storage at 8 and 15°C with a mount 37.98 and 37.83%, respectively. Therefore, increasing of temperature and storage period reduced amount of antioxidant capacity.

According to Table 4 positive correlations were observed between anthocyanin (tissue and peel), Phenylalanin ammonia-lyase and total protein in orange juice, with the highest anthocyanin content in 8°C after the 85 day storage. Total phenol was negatively correlated with flavonoid but showing a significant relationship between Phenylalanin ammonia-lyase and total protein. DPPH showed a significant correlation with anthocyanin in tissue and peel, Phenylalanin ammonia-lyase and protein in blood orange juices. Results summarized in Table 4.

Discussion

The screening of the blood orange juices indicates that the temperature affected, on the acceleration of anthocyanins synthesis and the restricted amount of pigments. These findings are according with the previous study on blood orange juice (e.g. Rapisarda et al. 2008). The results also indicated that the anthocyanin compounds accumulated more in the fruit peel than in the tissue. Low temperature positively affected vitamin C content accumulation during storage of fruits and vegetables, (Lee & Kader 2000, Wills et al. 1984). In this study there is no mention of ascorbic acid synthesis during cold storage. During investigation period, increase temperature and storage time significantly reduced amount of L-phenylalanin ammonia-lyase enzyme activity and protein. Recently, It has been reported that effect of low temperature of storage on anthocyanin production, enzymes involved: phenylpropanoid metabolism such as Phenylalanin ammonia-lyase (PAL), chalcon synthase (CHS), dihydroflavonol 4-reduced (DFR), and UDP-glucose flavonoid glucosyl transfer (UGT) (Lo Piero et al. 2005). Similarly Shaked-sachray et al. (2002) showed that in several plant species, such as corn seedlings, Arabidopsis and satiny, temperature has a significant effect on anthocyanins gene expression. Also, high tem-

perature caused to decrease the total phenol. Folin-ciocalteu reagent is nonspecific for phenolic compounds since it measures sample reduction capacity through electron transfer (ET)-based antioxidant capacity, thus it can be reduced by many non-phenolic compounds such as vitamin C (Huang et al. 2005). The amount of total phenolics in blood orange depends on high anthocyanin concentration and antioxidant capacity. In our trials, measurements of antioxidant activity showed, slight increase in antioxidant capacity during the first 45 days of storage at 8 and 15°C. Arena et al. (2001) and piga et al. (2002), have been shown that the antioxidant activity in orange juices after 2 months of low storage increased. According to Piga et al (2002), DPPH antioxidant activity in mandarin juices increased during 15 days at 4°C of the cold storage. If the decrease in the antioxidant activity may be major part to lower content of phenolic compounds and vitamin C in stored juice as compared to fresh, the increase in the antioxidant activity is usually ascribed to Maillard's reaction products (Anese et al. 1999). Other studies have shown that the antioxidant efficiency of orange juice may be attributed to a large part because total phenolic content (Rapisarda et al. 1999). However, according to Kähkönen et al. (2001), ascorbic acid and antioxidant activity could show clearly the synergistic effect with phenolic components. The relationship between antioxidant activity and total phenol in natural foods is known such as fruit and vegetables have been studied (Klimczak et al. 2007, Kieselova et al. 2006, Kedage et al. 2007).

In conclusion, the results of the present study showed that the temperature factors, including 8, 15 and 22°C have the greatest impacts on blood orange ripening and fruit color. The temperature and cold storage parameters are taken into greater consideration when assessing the development of fruit quality. The different temperatures in cold storage are responsible for the physicochemical differences of blood orange during storage. Also, the data indicate the highest fruit quality at 8°C after 85 days storage. In other words, with an increase in storage temperature, physiological and biochemical changes of fruits significantly reduced.

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