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ASSESSMENT OF FRUIT QUALITY AND ANTIOXIDANT ACTIVITY OF THREE CITRUS SPECIES DURING RIPENING

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Abstract. *Physicochemical changes and antioxidant activity of pulp from three citrus varieties were investigated during ripening. Results showed, all varieties except for 'Page' had not significant differences ($p<0.05$) on total soluble solid (TSS) and titratable acidity (TA) after Oct. evaluation. Generally, all varieties had optimum peel colour in Nov. and Dec. harvesting time. Although, phenolic content was superior in Sep. and Dec. but total flavonoid varied depending the variety and ripening stage. 'Moro' showed high anthocyanin content (0.83 mg/l) in Nov. evaluation. Total carotenoid increased during Sep. and Oct. and then was constant after Nov., but ascorbic acid decreased during ripening. IC_{50} values of $DPPH^{\cdot}$ were high in all samples taken in November. Furthermore, antiradical efficiency (AE) of 'Moro' was higher (Oct.) than 'Thamson' and 'Siavaraz' (Dec.) species. According to antioxidant assay founded that antioxidant capacities at all varieties were in highest level at early of December harvesting date. In generals, due to optimum fruit quality and antioxidant capacity during late November and early December, we suggested that these times is suitable to harvest of studied varieties.*

Key words*: Citrus, Peel colour, Antioxidant activity, Carotenoid, Flavonoid, Ascorbic acid*

INTRODUCTION

Orange is the second largest fruit crop grown in Iran after apple with an annual production of 4022256 tones in 2009 (FAO, 2009). It is produced in large amounts in the North and South regions of Iran. Due to citrus antioxidant activity and beneficial health effects, strong interest in citrus production and consumption has recently been expressed. Major antioxidant components in citrus are tannins, antioxidant vitamins (ascorbic acid, tocopherol and ß-carotene), anthocyanin (only in juices from pigmented citrus varieties), and hydroxycinnamic acids, with potential

health-promoting properties (Jang et al. 2010). Citrus plants are also rich in naturally flavonoids, which are primarily found in fruit peel. Flavonoids identified in citrus fruits are of over 60 types, according to the five classes of flavones, flavanones, flavonols, flavans and anthocyanins (Tripoli et al. 2007). Blood colored genotypes have been found to contain cyanidin 3-glucoside as major anthocyanins and the same glycoside C of petunidin as minor anthocyanins (Fattahi et al. 2009; Kelebek et al. 2008). Several studies exist on the change of fruit quality and antioxidant component at the ripening stage of citrus fruits (Xu et al. 2008). However, during fruit ripening, several biochemical, physiological and structural modifications happen and these changes determine the fruit quality attributes. Different phenotypes of citrus have been shown to exhibit different antioxidant activities. Antioxidant capacities can be influenced, mainly by ripening time, genotype, cultivation techniques, climatic conditions that occur during fruit development period and also the operations carried out during the postharvest storage (Tavarini et al. 2008). Maturity stage is an important factor that influences the compositional quality of fruits and vegetables. Harvesting at the proper ripening stage is essential for optimum quality and often for the maintenance of this quality after harvest.

In plants, especially in citrus, ascorbic acid content plays a key role not only as a free radical scavenger but also as an electron donor for ascorbate peroxidase in the reduction of H_2O_2 to H_2O (Jimenez et al. 2002). Xu et al. (2008) calculated the contribution of ascorbic acid to total antioxidant capacity in fifteen citrus varieties. It was found that ascorbic acid contribution to total antioxidant capacity of citrus juices was more than 50% except ‘Wase’ Satsuma (48.12%). Several chlorometric assays have been frequently used to estimate antioxidant capacities in fresh fruit such DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity, TEAC assay using ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulphonic acid)) a peroxidase substrate, ferric reducing antioxidant power (FRAP), and the oxygen radical absorption capacity (ORAC) (Thaipong et al. 2006).

Several researchers have studied the relationship between total phenol and different methods of antioxidant capacity assay. They often found excellent linear correlations between the total phenolic profiles and the antioxidant activity (Karadag et al. 2009). However, Awika et al. (2004) observed high correlation between ABTS, DPPH, and ORAC among sorghum and its bioactive.

Visual fruit quality has been correlated with various physical characteristics like peel colour, shape and size of fruit. Colour can be quantitatively defined into three dimensions of hue, chroma, and lightness. In general, consumers prefer a deep orange-coloured fruits. As citrus fruit

matures, changes in rind colour occur that are mainly due to decreased chlorophyll and increased carotenoid concentrations (Roux & Barry 2006).

There has been no detailed research on the physicochemical and antioxidant capacity of different citrus varieties grown in northern Iran. Therefore, in this investigation we have determined the changes of phenolics, vitamin C and the antioxidant capacity of two blond oranges 'Thamson' and 'Siavaraz' (a local variety), and a blood variety namely 'Moro' during fruit ripening.

MATERIALS AND METHODS

Plant material

In this study, three citrus genotypes including two blond oranges (*Citrus sinensis* cv 'Thamson' and *Citrus sinensis* cv 'Siavaraz' a local variety), a blood variety namely 'Moro' (*Citrus sinensis* (L.) Osbeck) were selected from the northern of Iran. The fruit samples were harvested one month interval as beginning of Sept. 7th and continued at the same time in October, November and December 2009 from experimental orchard of Iran Citrus Research Institute. Then, three independent samples of each variety harvested in three separate containers (60 fruits), transferred to the laboratory for physical and chemical analysis.

Extraction of plant materials

Extraction solvent was methanol:acetic acid (85:15, v/v) for blood orange and methanol for blond varieties. Fruit juice and solvent were mixed as 3:1 ratio and then hold in a refrigerator at 4 ℃ overnight. After that, the samples were centrifuged at 6,000 rpm for 15 min (Hettich, Germany). Finally, extracted citrus pulp samples were stored at -80 ˚C until experiments were performed.

Quality parameters

For each sampling date, Fruit volume was measured using water volume displacement method (Baumann & Henze, 1983). Fruit peel colour parameters (*L**, *a**, *b**, hue angle and chroma) in three points of each fruit were measured using Colourimeter CR-400 (Minolta, Japan) in the CIELAB System. The colourimeter was calibrated with standard black and white calibrations tiles.

Percentage of juice, TSS and TA measurement

Each fruit was cut in two, squeezed and then juice weighted to provide the percentage of juice. Total soluble solids (TSS) of fruit juice for each genotype were determined by a hand type refractometer (ATAGO ATC-1, Tokyo, Japan). Titratable acidity (TA) was determined using an acid base titration method. Fruit juice (10 ml) and distilled water (20 ml) with two drops of phenolphthalein (1%) as indicator mixed and then titrated with 0.1M NaOH to an endpoint pink (pH 8.2). Total acid contents were determined as citric acid equivalents.

Determination of ascorbic acid
The ascorbic acid concentration was measured based on the reduction of the dye 2,6-dichlorophenolindophenol (DCIP) by ascorbic acid. Pulp samples (1 g) were mixed in 3 ml metaphosphoric acid (1%) and after centrifuging, DCIP (0.5 ml) was added to the supernatant, and then measured at 520 nm versus the blank by an UV/vis spectrophotometer. Ascorbic acid amounts were calculated using standard curve prepared from ascorbic acid.

Determination of total phenolic content
The total phenolic content has been analyzed spectrophotometrically using the Folin-Ciocalteu method with different modifications (Meyers et al. 2003). Briefly, the fruit juice extracts (50 µl) was mixed with 125 µl Folin–Ciocalteu reagent (10 %) which was earlier diluted with 112.5 µl deionized water. After 5 min, 100 µl of bicarbonate solution (7 %) was added at 25 ^{o}C. The mixed solution was allowed to stand for 120 min in the absent of light. Absorbance was measured at 765 nm using the spectronic Nano Drop (NanoDrop Model ND-1000; USA). Total phenolic was quantified according to a standard curve prepared using gallic acid. The total phenolic content was expressed as mg Gallic acid equivalents (GAE) per ml of fresh sample.

Determination of total flavonoid content
The total flavonoid contents were determined according to the aluminium chloride colourimetric method Deccribed by Bor et al. (2006). Briefly, 50 µl of fruit pulp extract were mixed with 10 µl aluminum chloride (10%), 10 µl potassium acetate (1 M), and deionized water (280 µl). Samples vortexes vigorously and then incubated at room temperature for 40 min at 25°C. The absorbance of the reaction mixture was measured at 415 nm against a deionized water blank using a spectrophotometer (NanoDrop Model ND-1000; USA). The total flavonoid content was quantified according to the standard curve using a six point concentration (0.016-0.5 mg ml^{-1}) of quercetin. The data were expressed as mg quercetin equivalents (QE) per ml of fresh fruit matter.

Determination of total anthocyanin and carotenoid content
Spectrophotometric analysis of total anthocyanin content in fruit juice was determined using of the pH differential method (Wrolstad 1976). Briefly, absorbance of each extracted sample was measured at 520 and 700 nm by two buffers at pH 1.0 and 4.5. Then calculated using A = [(A_{520}−A_{700}) $pH_{1.0}$ − (A_{520}−A_{700}) $pH_{4.5}$] formula by the molar extinction coefficient of cyanidin 3-glucoside (26900) for citrus fruit juice. The total anthocyanin calculated using [(mg/L) = (A/26900) (10^{3}) (445.2) (5)] formula. Pulp carotenoid was determined colourimetrically operating as Deccribed by Lichtenthaler (1987).

Determination of antioxidant activity using the DPPH$^{\cdot}$ method
The radical scavenging activities of the plant extracts against 2,2-diphenyl-1-picrylhydrazyl radical (Sigma-Aldrich) were determined by UV spectrophotometry at

517 nm by a slightly modified method Deccribed by Brand-William et al. (1995). In short, 23.5 mg of DPPH$^{\bullet}$ were dissolved on 100 ml methanol. Different ratios of sample to DPPH volume of 6/44, 12/38, 18/32, and 25/25 µl were prepared and the mixture was then vortexed vigorously. The reaction was completed after 30 min at room temperature and in the absence of light. The DPPH radical scavenging activity was calculated as follows:

DPPH radical scavenging activity (%) = 100 ($1-A_S/A_C$), where A_C is the absorbance of the DPPH radical without any antioxidant as control, A_S is the absorbance reading of DPPH$^{\bullet}$ added to sample at 517 nm. Methanol was used as a blank. The antioxidant capacity of each sample was expressed as the amount of sample necessary to inhibit the initial DPPH$^{\bullet}$ concentration by 50% (IC_{50}) that was calculated graphically. The antiradical efficiency ($AE = 1/ IC_{50}$) was also calculated.

Determination of antioxidant activity using the ABTS$^{\bullet+}$ method

The antioxidant activity of different citrus varieties were measured using the capacity of the extract to scavenge ABTS$^{\bullet+}$ radicals (Liyana-Pathirana & Shahidi 2006). In short, a 7 mM solution of ABTS in water was prepared and ABTS$^{\bullet+}$ was formed after the addition of potassium persulphate (2.45 mM) to the solution. after 16 h incubation in darkness at room temperature, the stock solution was diluted with ethanol until the absorbance reached 0.7 ± 0.02 at 734 nm. After mixing of 10 µl sample to 200 µl of diluted ABTS$^{\bullet+}$ solution, the reaction mixture was incubated for 5 min at 30 ^{o}C. The decrease in the absorbance reflected the ABTS$^{\bullet+}$ radical scavenging capacity of the antioxidant. The absorbance of ABTS$^{\bullet+}$ without sample was measured as the control. The inhibition percent was calculated using the following formulae: Inhibition % = $[(A_C - A_S)/A_C]\times100$, where A_C is the absorbance of the control and A_S is the absorbance of the sample plus ABTS radical at t=5 min.

Statistical analysis

Physicochemical data were analyzed with MSTAT–C statistical software (Michigan State University, USA). Treatments were arranged in complete randomized block design, and Tukey's test ($p< 0.5$) was used to reveal any differences for each orange variety.

RESULTS AND DISCUSSION

Change in qualitative parameters on the orchard during ripening

According to Table 1, all varieties showed an increasing fruit size, percentage of juice, TSS and TSS/TA during ripening. Titratable acidity increased gradually but then decreased at the full-ripe stage. In three varieties, no significant differences ($p<0.05$) were observed in the fruit size and juice percentage between November and December sampling time. Also, the percentage of juice was always high in large diameter fruits. After November, the fruit juice accumulation was steady and it is thought that

Table 1. Change in qualitative parameters on the orchard of different citrus genotypes during ripening.

Citrus variety	Sampling date (First week)	Fruit size (mm2)		Percentage of juice (%)	
'Thamson'	September	119.30	c*	22.39	d
	October	263.30	ab	29.03	c
	November	263.30	ab	31.19	bc
	December	307.50	a	34.52	a
'Siavaraz'	September	118.10	c	24.91	b
	October	151.20	bc	27.59	b
	November	178.30	ab	33.40	a
	December	194.00	ab	33.51	a
'Moro'	September	85.18	b	37.79	b
	October	95.50	b	38.29	b
	November	156.70	a	41.38	a
	December	157.70	a	41.60	a

Citrus variety	Sampling date (First week)	TSS (%)		TA (%)		TSS/TA	
'Thamson'	September	9.53	b	2.36	a	4.52	b
	October	10.67	ab	2.03	ab	4.69	b
	November	11.20	ab	1.66	c	6.75	a
	December	11.83	a	1.66	c	7.13	a
'Siavaraz'	September	9.70	b	4.48	a	2.17	a
	October	10.47	ab	3.41	b	3.07	ab
	November	11.00	ab	3.09	bc	3.56	a
	December	11.23	a	2.93	b	3.83	a
'Moro'	September	10.00	ab	2.52	a	3.97	c
	October	10.03	ab	2.28	ab	5.67	a
	November	10.80	ab	2.25	ab	4.74	ab
	December	11.47	a	1.77	c	5.10	a

*Values in the same column for each orange variety having different letters are significantly different ($p \leq 0.05$).

more changes occur later related to internal pulp composition. Fattahi et al. (2009) observed a gradual increase in TSS and total sugars during citrus fruit ripening. Similar findings were reported for other fruits such kiwifruit (Fisk et al. 2008). Biale (1960) attributed that increase in TSS and total sugars during fruit ripening were due to the hydrolysis of starch to sugars and also decline in the rate of sugar breakdown by respiration. The ratio of TSS/TA is an important factor for determining of fruit taste and suitable harvesting time. We observed that TSS/TA ratio was increased during fruit development only in 'Thamson' and 'Moro' varieties. Generally, fruit size had a positive relationship with TSS and TSS/TA ratio and conversely with TA at every analysis date. In fact, the increase in TSS/TA, is mainly due to the TA decreasing since TSS remained quite stable. For this reason, TA value was reported to influence fruit taste quality in strawberry (Kafkas et al. 2007) and kiwifruit (Fisk et al. 2008). The TSS/TA ratio about 7 is a good index to determine of suitable harvesting time for the North conditions of Iran.

Peel colour changes

In general, all skin colour values except in hue angle for all varieties showed progressive enhance during ripening. The time of harvesting had significant effect (p<0.05) on fruit peel colour. For all varieties, the L^*, a^* and b^* values were higher in Nov. and Dec. than Sep. and Oct. sampling time. In 'Thamson' and 'Siavaraz' varieties, L^* value was increased up to Nov. and then remained constant to end of evaluation period. The peak of L^* value occurred rapidly (Oct.) in 'Moro' among studied varieties. In fact, L^* value measures luminosity that varies from zero (black) to 100 (pure white). With investigation of a^* value revealed that fruits picked in Sep. and Oct. were greenish (a^* negative) and significantly differed (p < 0.05) from those in Nov. and Dec. with reddish peel colour (a^* positive). Moreover, the yellow colour of fruit peel (b^* value) and chroma was higher in Nov. and Dec. sampling date than that harvested in Sep. and Oct. (Table 2). Although hue angle of all varieties decreased throughout the ripening period but hue angle mean in Nov. and Dec. evaluation date was significantly (p<0.05) lower than that analyzed in Sep. and Oct.

An increasing correlation was noted between the yellow colour (b^*), reddish (a^*) of fruit peel and declining of the mean daily temperature during the autumn season. Peel greenness was decreased slightly in fruits that were picked in Nov. and Dec. times. It seems that decreasing of daily temperature during fruit development activates chlorophyllase enzyme which leads to degradation of peel chlorophylls and appearance of carotenoid (Alquezar et al. 2008). Low temperatures also caused increa-

Table 2. Change in peel color of different orange genotypes during ripening.

Citrus variety	Sampling date (First week)	*L**		*a**		*b**	
'Thamson'	September	44.44	bc*	-19.78	b	40.57	b
	October	41.59	c	-18.37	b	39.94	b
	November	68.31	a	6.88	a	74.10	a
	December	67.97	a	8.46	a	75.41	a
'Siavaraz'	September	43.49	c	-18.71	b	38.25	b
	October	46.19	bc	-19.66	b	42.93	b
	November	64.07	a	4.23	a	71.22	a
	December	65.03	a	4.80	a	71.74	a
'Moro'	September	54.16	c	-18.41	d	49.52	b
	October	66.51	ab	-5.61	c	70.46	a
	November	68.07	ab	8.24	b	73.75	a
	December	70.15	a	11.65	a	73.64	a

Citrus variety	Sampling date (First week)	hue (*h*°)		chroma (*C*°)	
'Thamson'	September	116.00	a	45.13	b
	October	114.20	a	44.76	b
	November	74.53	b	84.75	a
	December	77.25	b	84.02	a
'Siavaraz'	September	115.90	a	37.64	c
	October	115.00	a	47.27	b
	November	72.11	b	87.00	a
	December	72.62	b	86.99	a
'Moro'	September	110.50	a	52.97	c
	October	90.79	b	70.74	b
	November	74.37	c	83.83	a
	December	74.81	c	80.94	a

*Values in the same column for each orange variety having different letters are significantly different ($p \leq 0.05$).

sing of carotenoid concentrations in 'Redblush' grapefruit. According to peel carotenoid (data not shown), we found that peel carotenoid accumulation increased during seasonal growth, in agreement with pervious studies (Alquezar et al. 2008).

Changes in hue angle constituted the major variation in fruit colour coordinates. A hue angle (*h*°), with 90 and 180 values shows a fully yellow colour and an entirely green colour, respectively. The decline in hue angle reflected the change from green to yellow. In other hand, the increase in chroma value represented increasing intensity of yellow colour. Therefor, as seen in Table 2, hue and chroma values confirmed that peel colour trend to yellowness during ripening. Generally, in orange coloured citrus fruits, a lightness between 65 and 70, a chroma >60 and a hue angle <80º are considered to be acceptable in most markets (Roux & Barry 2006). In this study, the L*, Hue and chroma values were in agreement with Roux and Barry (2006) report that uncured after the seventh of November.

Content of total phenol, flavonoid, carotenoid and anthocyanin

Total phenolic and flavonoid contents varied in each ripeness stage and variety. Fruits picked in Sep. and Dec. had high level of total phenolic compounds. The 'Siavaraz' variety showed lower values (0.08 mg/ml) of phenol at Oct. sampling date than others (Table 3). In other hand, investigation of total flavonoids were clear that 'Thamson' and 'Moro' varieties had high level of flavonoid contents during Dec. but for 'Siavaraz' was Sep. (0.64 mg/l).

Among studied varieties, just 'Moro' belong to blood orange group. With evaluation of total anthocyanin it was clear that fruits harvested in Nov. had high level (0.83 mg/l) of total anthocyanin (Table 3). Cyanidin-3-glucoside (the most abundant anthocyanin present in red orange juice) was the main antioxidant in comparison to other anthocyanins (Fattahi et al. 2009).

The differences in yellow pulp colour between fruits were probably associated with variations in fruit carotenoid contents. Total carotenoid extracted from fruit pulp ranged from 0.02 to 0.36, 0.04 to 0.35 and 0.01 to 0.26 mg/ml in 'Thamson', 'Siavaraz' and 'Moro', respectively (Table 3). As shown in Table 3, carotenoid accumulation enhanced during fruit ripening with contemporary of temperature decline during autumn season. In other hand, no significant differences (p<0.05) were observed in carotenoid content in Nov. and Dec. harvesting time. Coggins et al. (1981) reported that when 'Frost Valencia' orange was exposed to low (20/15 ºC) and high (30/15 ºC) day/night air temperatures, the low temperatures induced higher carotenoid concentrations compared with the high temperatures. We also found an enhanced carotenoid accumulation in pulp when temperature

Table 3. Change in antioxidant components in juice of different orange genotypes during ripening.

Citrus variety	Sampling date (First week)	Total phenolics (mg/ml)	Total flavonoid (mg/ml)
'Thamson'	September	1.04* a	0.28 b
	October	0.62 b	0.33 a
	November	0.62 b	0.27 b
	December	1.03 a	0.36 a
'Siavaraz'	September	1.16 a	0.37 b
	October	0.08 c	0.23 c
	November	0.53 b	0.64 a
	December	0.96 ab	0.38 b
'Moro'	September	0.17 a	0.24 c
	October	0.06 b	0.24 c
	November	0.09 ab	0.34 b
	December	0.19 a	0.39 a

Citrus variety	Sampling date (First week)	Total carotenoid (mg/ml)	Total anthocyanin (mg/l)
'Thamson'	September	0.02 c	
	October	0.11 b	
	November	0.36 a	
	December	0.36 a	
'Siavaraz'	September	0.09 b	
	October	0.04 b	
	November	0.34 a	
	December	0.35 a	
'Moro'	September	0.01 bc	0.08 c
	October	0.07 b	0.08 c
	November	0.23 a	0.83 a
	December	0.26 a	0.17 b

*Values in the same column for each orange variety having different letters are significantly different ($p \leq 0.05$).

declined during seasonal growth. According to our results, three orange varieties contained significant total phenolics that increase antioxidant intake in human diet (Romero et al. 2009). In totally, the comparison of total phenol, flavonoid, carotenoid and anthocyanin content of different citrus fruits revealed useful hints about the varieties quality and amount of these important natural antioxidants.

Ascorbic acid content and antioxidant activity

Ascorbic acid was also quantified using DCIP method. The amount of ascorbic acid in all varieties was similar. Generally, all varieties followed a similar pattern to accumulate ascorbic acid during ripening. Ascorbic acid content was high in Sep. and Oct. and then significantly decreased during full ripening progressively. The ascorbic acid values ranged from 41.68 to 66.24 mg/100 g FW. The amount of ascorbic acid in ripening stage was in agreement with Idso et al. (2002), who found that ascorbic acid content ranged from 30.98 to 40.28 mg per 100 ml of citrus juce. Also, Long et al. (1957) observed that ascorbic acid concentrations in two different varieties of grapefruit increased as fruit size decreased. Our results was in agreement with thos of Long et al. (1957). Arena et al. (2001) evaluated the ascorbic acid of blood orange juices in comparison with a blond variety. They reported that all the blood juices have higher amounts of ascorbic acid than blond ones. Inversely, according to the data obtained in the present study, the amount of ascorbic acid in 'Moro' was similar to 'Thamson' and 'Siavaraz' varieties.

The results of antioxidant activity were summarized in Table 4. As seen, fruit juices showed a noticeable antioxidant potential (expressed as IC_{50}) which ranged from 0.25 to 10.42, 1.54 to 8.06 and 0.3 to 11.54 mg juice/mg DPPH in 'Thamson', 'Siavaraz' and 'Moro', respectively. Overall, 'Siavaraz' variety exhibited higher antioxidant activity than as reflected the lowest range of IC_{50}. The best antiradical of the unripe 'Moro' (3.33) who for samples obtained in Oct. whereas it occurred in Dec. for 'Thamson' and 'Siavaraz' fruits with 4.02 and 0.65 values, respectively. IC_{50} is inversely associated with antioxidant activity of compounds. Therefore, the lower of IC_{50} value expressed higher antioxidant activity (Kelebek et al. 2008). The antiradical efficiency (AE) is a parameter for the measurement the free radical scavenging of samples. Kelebek et al. (2008) by investigation of antioxidant capacities of orange juices obtained from cvs. 'Moro' and Sanguinello suggested that AE value of 'Moro' juice (0.05) was found to be higher than Sanguinello juice (0.03) at seasonal harvesting time. In this experiment, high AE value (3.33) of 'Moro' pulp was observed in Oct. Fur-

Table 4. Change in antioxidant activity of different orange genotypes during ripening.

Citrus variety	Sampling date (First week)	Ascorbic acid (mg/100g FW)		IC_{50} (mg DPPH)	
'Thamson'	September	66.24	a*	2.20	b
	October	66.08	a	1.06	c
	November	44.64	b	10.42	a
	December	41.68	b	0.25	d
'Siavaraz'	September	63.28	a	2.35	b
	October	61.68	a	1.92	c
	November	43.04	c	8.06	a
	December	48.88	b	1.54	d
'Moro'	September	61.44	a	3.87	c
	October	55.4	b	0.30	d
	November	42.76	c	11.54	a
	December	43.84	c	6.06	b

Citrus variety	Sampling date (First week)	AE value		ABTS radical scavenging (%)	
'Thamson'	September	0.45	c	54.12	ab
	October	0.94	b	49.92	b
	November	0.10	d	40.67	c
	December	4.02	a	60.00	a
'Siavaraz'	September	0.43	c	67.06	a
	October	0.52	b	48.24	c
	November	0.12	d	66.89	a
	December	0.65	a	58.82	b
'Moro'	September	0.26	b	47.56	b
	October	3.33	a	31.43	d
	November	0.09	c	41.18	c
	December	0.17	b	55.29	a

*Values in the same column for each orange variety having different letters are significantly different ($p \leq 0.05$).

thermore, in seasonal harvesting (Dec.), AE value of 'Moro' and other varieties were higher than values reported by Kelebek et al.

For ABTS assay, the highest value of inhibitory (60 %) for 'Thamson' sample observed in Dec. which had not significant differences (p<0.05) with Sep. sampling date. About 'Siavaraz', it happened in Sep. (67.06 %) and Nov. (66.89 %), while 'Moro' occurred (55.29 %) in December. In this experiment, the antioxidant activity of 'Moro' juice was increased with evaluated of blood pigment in fruit pulp. It was in agreement with the suggest of Rapisarda et al. 1999, who reported the orange juices with higher anthocyanin levels had, in each test employed, better antioxidant activity than those with lower anthocyanin content. Also, Kelbelk et al. (2008) according to DPPH assay determined, that the antioxidant capacity of 'Moro' juice was higher than that of Sanguinello juice (due to late accumulation of anthocyanin pigment).

There is little information about the influence of the stage of citrus fruits ripening on bioactive components and antioxidant capacities, as well as their relationship with the overall antioxidant capacity. In this study, results indicated the different effects of ripening stage on the antioxidant capacity of citrus fruits. Rapisarda et al. (1999) found a direct correlation between the antioxidant effectiveness of orange juices and their total phenol content. However, in our experiment the total phenolic and flavonoid contents did not correlate well with the results from the DPPH and ABTS assay. This may indicate a specific mechanism of antiradical activity of the extracts, probably due to the physicochemical and structural characteristics of the components (Termentzi et al. 2006).

CONCLUSIONS

The results suggested that growth parameters and external colour indices are useful trait, for determining citrus fruit quality and suitable harvesting time. The quantitative composition of phenolics, carotenoid and anthocyanins of orange juices represent good parameters for characterization of the product, content of dietary phytochemicals and health-promotion value of selected citrus fruit. This may have implications for both the nutritional value and oxidative damage inhibition during post-harvest of fruits. In generals, due to optimum fruit quality and antioxidant capacity during late November and early December, we suggested that these times is suitable to harvest of studied varieties.

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