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The appraisal of qualitative parameters and antioxidant contents during postharvest peach fruit ripening underlines the genotype significance

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ABSTRACT

Several studies document that peach and nectarine ripening related parameters can be efficiently predicted in a non-destructive manner; however, such studies are being restricted in a relatively limited number of cultivars and parameters measured. In addition, the combined effect of genotype and postharvest ripening on phytochemical content of peach and nectarines has not been elucidated. In the present study, the I_{AD} maturity index, ripening-related parameters, phenolic and flavonoid contents and *in vitro* antioxidant capacity were determined in fruit from 26 commercially important peach and nectarine cultivars, grown in Greece. Analyses were carried out at harvest and after additional ripening at room temperature (~23 ± 2 °C) for 1, 3 and 5 days, to simulate shelf life conditions. Results indicated great variation in the I_{AD} index (variation coefficient = 32%); this index can be used as reference in future studies on a cultivar basis. Flesh firmness was the strongest predicted parameter from the I_{AD} index during off-tree ripening. Segregation of peach and nectarine cultivars revealed great differences on quality parameters and on their ripening behavior. Varietal differences were more pronounced regarding the polyphenolic content; indicatively, total phenol (TP) content ranged from 11.7 to 90.1 mg gallic acid equivalents (GAE) 100^{-1} g fresh weight (FW) at harvest. 'Sun Cloud' and 'Gladys' fruits among peach cultivars and 'Tasty Free' fruits among nectarine cultivars demonstrated high antioxidant contents. Interestingly, postharvest ripening of peach and nectarine cultivars did not seem to affect polyphenolic content and antioxidant capacity in a constant mode. Hence I_{AD} was not correlated with antioxidant contents and to our knowledge this is the first work examining this correlation. Furthermore, data underlines that peach cultivars in general were characterized by higher antioxidant contents compared to nectarine cultivars; this was also the case for late-harvested cultivars *versus* the early-harvested ones.

1. Introduction

Peach and nectarine have a relatively large variation in the on-tree maturity; due to this variability it is essential to apply successive harvests. Maturity at harvest is usually determined based on commercial size and diameter, background color and flesh firmness (Crisosto and Valero, 2008). However, color can be hardly distinguished in some cultivars as an intense blush is developed before the fruit is ripe for harvest, while firmness determination is carried out in a destructive manner, and may vary for a given cultivar in relation to fruit size, climatic conditions, and agronomical practices (Iglesias and Echeverria, 2009).

Nowadays, non-destructive techniques have been developed to precisely evaluate ripening stage and assess fruit internal quality attributes. Among these non-destructive approaches, visible/near infrared (vis/NIR) spectroscopy seems particularly promising since it provides fast and reliable information on internal characteristics of many fruit species (Nicolaï et al., 2007; Vanoli and Buccheri, 2012; Farneti et al., 2015). A vis/NIR device is the DA-meter, which measures the I_{AD} index which is the absorbance difference between 670 nm (the absorbance peak for chlorophyll in stone-fruit) and 720 nm (the minimum absorbance which does not change as chlorophyll is degraded in the peel) (Ziosi et al., 2008). The measurement of the fruit's chlorophyll index gives an

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indication of the ripening stage. The I_{AD} index has allowed to assess peach ripening stage in the field and during storage (Herrero-Langreo et al., 2011; Bonora et al., 2013; Shinya et al., 2013). Nevertheless, reports document the relationships among I_{AD} and specific ripening-related changes in a relatively small number of peach and nectarine cultivars. Therefore, it is an emerging need to set non-destructive index thresholds on a cultivar basis.

Apart from ripening stage and qualitative properties, peach should be additionally evaluated in terms of phytochemical content (Cevallos-Casals et al., 2006; Vicente et al., 2011). Differences are mostly affected by the genotype; this issue is particularly important provided the fact that numerous cultivars exist, while new cultivars are being launched into the market on a yearly basis. Peach is widely consumed, being the second most important temperate fruit crop worldwide. Thus, due to its significant impact on human nutrition, it is important to define cultivars with the highest polyphenolic content (Tomas-Barberán et al., 2001; Gil et al., 2002; Di Vaio et al., 2008; Tavarini et al., 2008; Cantín et al., 2009). This initiative will additionally assist to consider particular genotypes for breeding purposes in order to select and promote cultivars with higher antioxidant content (Cevallos-Casals et al., 2006; Vizzotto et al., 2007; Drogoudi et al., 2008; Cantin et al., 2010; Reig et al., 2013).

Peach fruit physiology has been extensively studied both during on-tree maturation and postharvest ripening after harvest or after cold storage. Nevertheless, little is known on the combined effects of postharvest ripening and genotype on the fruit qualitative and antioxidant potency. The objectives of the current study were initially to evaluate the usefulness of non-destructive assessment of ripening related changes on an array of peach and nectarine cultivars and further to determine the genetic variation in antioxidant phenols and dissect potential correlations among the examined parameters.

2. Materials and methods

2.1. Fruit material and experimental design

Seventeen peach ('May Crest', 'Spring Belle', 'Royal Jem', 'June Gold', 'Royal Glory', 'Rich Lady', 'Maria Bianca', 'Red Haven', 'Sun Cloud', 'Kori', 'Sun Crest', 'Elegant lady', 'Symphonie', 'Fayette', 'Roubidoux', 'Gladys' and 'Opsimo Naoussas') and nine nectarine ('Andrianna', 'Big Bang', 'Rose Diamond', 'Rita Star', 'Big Top', 'Caldesi 2000', 'Red Gold', 'Venus' and 'Tasty Free') cultivars were used in the present study, evenly distributed during the harvest period [June 6–September 10] (Supplementary Fig. 1). Cultivar selection was carried out, mainly based on their commercial importance for Greece, a top-producing country for peaches and nectarines. In particular, the examined cultivars corresponded the 80.1% of peaches and 69.1% of nectarines distributed by the one of the biggest cooperatives in Greece (Agricultural Cooperative of Naoussa) (Supplementary Table 1).

For each cultivar, fruit at commercial maturity stage and of premium quality standards (relatively large size, without defects and on the basis of background skin color that is characteristic for each cultivar) were selected the day of harvest upon arrival to the Agricultural Cooperative of Naoussa. Subsequently, fruit were divided into four homogeneous 24-fruit lots, each analyzed at harvest and after 1, 3 and 5 days maintenance at room temperature ($23 \pm 2\,^{\circ}C$) respectively, to simulate shelf life conditions. Each lot was divided to three eight-fruit sub-lots, corresponding to the three biological replications, unless otherwise stated. The lots used for analysis after 5 days of shelf life were initially used for non-destructive measurements (I_{AD} index, weight loss, respiration rate, ethylene production) throughout the shelf life period.

2.2. Quality attributes

The I_{AD} index was measured with a DA-meter (TR, Sinteleia, Bologna, Italy) on the center of each fruit cheek taking the computer average value displayed on the instrument screen. Weight loss (WL)% was determined by following the formula: $100 \times (A–B)/A$, where A was the fruit weight at harvest and B was the fruit weight after the shelf life period. The color parameters CIE L* (brightness or lightness; 0 = black, 100 = white), a* (−a* = greenness, +a* = redness), b* (−b* = blueness, +b* = yellowness), hue angle (h°) (calculated as $\tan^{-1}b^*/a^*$; 0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue) and Chroma (calculated as $(a^{*2} + b^{*2})^{1/2}$; degree of departure from grey to pure chromatic color) were measured in the exocarp at both sides of each fruit, using a Minolta chromatometer (Minolta CR-300, Ramsey, NJ). Flesh firmness (FF) was determined on opposite sides of the equator of each fruit with a penetometer (Effegi, Ravenna, Italy) fitted with an 8 mm plunger; the two readings were averaged for each fruit, and results expressed in Newtons. The soluble solid content (SSC) of the juice was measured with a digital refractometer (model PR-1, Atago, Japan) and data were expressed as %. Titratable acidity (TA) was measured in juices using an automatic titrator (Titrometic 25, Crison Instruments S.A., Barcelona, Spain) and determined by titrating 5 mL of juice with 0.1 N NaOH to a pH end point of 8.2. Results were expressed as g malic acid per 100 g FW. Ripening index (RI) was calculated as the SSC/TA ratio.

2.3. Ethylene and CO_2 production rate

Five two-fruit lots per cultivar were enclosed in 2 L airtight jars and left at room temperature for 2 h. An 1 mL gas sample was taken from the exit air flow of the jars and injected into a gas chromatograph (model Varian 3300, Varian Instruments, Walnut Cree, CA) equipped with a flame ionization detector and a stainless column to determine ethylene. Another 1 mL gas sample was directed to an infrared CO_2 analyzer (model Combo 280, David Bishop Instruments, UK) for the CO_2 measurement. Results were converted into $\mu L\ C_2H_4\ kg^{-1}\ h^{-1}$ and $mL\ CO_2\ kg^{-1}\ h^{-1}$ for the ethylene production and respiration rates, respectively.

2.4. Extraction for polyphenol determinations and antioxidant capacities

Sampling for the antioxidant measurements was carried out after the firmness measurements. Two wedged-shaped slices from the intact peach fruit were dissected, exocarp was removed, immediately frozen into liquid nitrogen and stored at $-20\,^{\circ}C$ until needed.

Five grams of frozen flesh tissue was homogenized in a Polytron with 10 mL extraction buffer comprising water-methanol (2:8, v/v) and 2 mM NaF to inactivate polyphenol oxidases and prevent phenolic degradation due to browning. Homogenates were kept on ice until centrifuged at 11,500 rpm for 15 min at 4 °C. The supernatant was carefully recovered to prevent contamination from the pellet, as elsewhere described (Tomas-Barberán et al., 2001).

2.5. Total phenolics (TPs)

The TPs content was measured using a modified Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965). The reaction mixture consisted of 0.5 mL of diluted extract, 5 mL of distilled water and 0.5 mL of the Folin-Ciocalteu reagent. The tube was vortexed and then allowed to stand at room temperature for 3 min when one mL of saturated sodium carbonate solution was added.

The solution was diluted to 10 mL and after 1 h at room temperature the absorbance was measured at 725 nm against a blank solution. Each measurement was repeated in triplicate and total phenolic content was expressed as mg gallic acid equivalents (GAE) 100 g^{-1} fresh weigh (FW).

2.6. Total flavanols (TFs)

The content of total flavanols was determined using the colorimetric assay described by Tabart et al. (2010). Briefly, 1 mL of diluted extract was mixed with 2.5 mL of vanillin (1% w/v in methanol) and 2.5 mL of 9 mol L^{-1} hydrochloric acid. The mixture was incubated for 20 min at 35 °C and the absorbance was measured at 500 nm. Each measurement was repeated in triplicate and total flavanol content was expressed as mg catechin equivalents 100 g^{-1} FW.

2.7. Total antioxidant capacity

Total antioxidant capacity (TAC) was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulfonic acid) ($ABTS^{*+}$) and ferric reducing antioxidant power (FRAP) assays.

DPPH assay was conducted as elsewhere described (Goulas and Manganaris, 2012). Briefly, 2 mL of diluted extract were mixed with one 1 mL of 0.3 mmol L^{-1} solution of DPPH in methanol, incubated in the dark for 30 min and the absorbance of the mixture was monitored at 517 nm.

The $ABTS^{*+}$ assay was performed according to the procedure described in Shanmgum et al. (2010). The ABTS radical cation ($ABTS^{*+}$) solution was prepared by the reaction of 5 mL of 14 mM ABTS and 5 mL of 4.9 mM potassium persulphate, after incubation at room temperature in the dark for 16 h. The $ABTS^{*+}$ solution was then diluted with distilled water to obtain an absorbance of 0.700 ± 0.005 at 734 nm. $ABTS^{*+}$ solution (0.9 mL; absorbance of 0.700 ± 0.005) was added to 100 μL of the diluted extract and mixed thoroughly. The reaction mixture was allowed to stand at room temperature for 6 min and the absorbance at 734 nm was immediately recorded. The samples were diluted so as to give 20–80% reduction of the blank absorbance with 100 μL of sample.

The ferric reducing antioxidant power (FRAP) assay was done according to Goulas and Manganaris (2012). A sample containing 3 mL of freshly prepared FRAP solution (0.3 mol L^{-1} acetate buffer (pH 3.6) containing 10 mmol L^{-1} 2,4,6-Tripyridyl-s-Triazine (TPTZ) and 40 mmol L^{-1} $FeCl_3$ $10H_2O$) and 100 μL of peach extract was incubated at 37 °C for 4 min and the absorbance was measured at 593 nm.

For all three assays, a standard curve was obtained by using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standard solution, and accordingly results were expressed as μmol Trolox equivalents 100 g^{-1} FW.

2.8. Statistical analyses

Data were subjected to two-way ANOVA analyses, using the cultivar and shelf life period as treatments and least significant differences (LSD) were calculated. Pearson's correlation analysis

Table 1
I_{AD} index, flesh firmness (N), ethylene (μl kg^{-1} h^{-1}) and % fruit weight loss, in 17 peach and 9 nectarine cultivars, at harvest. CV% = variation coefficient; LSD = least significant difference.

	I_{AD}				Flesh firmness (N)				Ethylene				% Fruit weight loss		
Peach cultivars	d0	d1	d3	d5	d0	d1	d3	d5	d0	d1	d3	d5	d1	d3	d5
May Crest	0.91	0.82	0.52	0.30	44.0	36.0	13.2	5.3	6.0	24.5	37.1	62.7	1.5	8.3	13.7
Spring Belle	0.74	0.56	0.37	0.21	45.6	47.1	15.4	4.4	3.6	4.2	19.0	40.2	1.1	3.9	6.5
Royal Gem	0.92	0.92	0.79	0.58	53.5	57.5	26.5	27.9	0.4	0.5	3.3	22.0	1.6	4.2	6.6
June Gold	0.49	0.49	0.32	0.25	59.4	42.0	8.7	6.4	1.6	3.7	5.7	7.0	1.7	4.5	5.8
Royal Glory	1.20	1.20	0.92	0.62	55.1	43.6	34.4	15.0	1.6	1.8	12.8	25.7	1.5	4.7	5.9
Rich Lady	0.79	0.79	0.51	0.37	47.1	48.8	15.7	6.2	2.1	8.4	9.1	13.1	1.5	4.0	6.6
Maria Bianca	1.11	1.11	0.57	0.26	46.2	39.5	12.2	5.2	1.1	2.7	3.8	9.1	1.3	2.4	3.9
Red Haven	0.33	0.33	0.11	0.04	34.9	21.4	4.1	4.1	2.0	3.5	11.0	10.2	0.9	2.2	3.5
Sun Cloud	0.69	0.69	0.34	0.17	39.1	26.9	13.6	5.2	2.7	3.8	10.4	10.4	0.6	2.5	4.0
Kori	0.56	0.56	0.35	0.22	65.3	64.6	29.5	11.0	0.8	1.4	2.3	2.6	1.6	3.2	4.8
Sun Crest	1.03	1.03	0.62	0.36	58.9	51.0	21.7	14.6	0.2	0.5	0.9	2.3	1.2	2.3	3.6
Elegant Lady	1.00	1.00	0.70	0.38	71.5	70.2	42.1	18.8	0.6	0.3	1.5	5.0	1.3	2.7	4.2
Symphonie	0.69	0.69	0.35	0.21	54.0	56.2	18.3	10.9	0.5	1.6	2.2	5.9	0.9	2.8	4.2
Fayette	0.57	0.57	0.39	0.27	47.1	46.1	17.4	12.6	0.3	0.4	0.6	0.8	1.0	2.4	3.6
Rubidoux	0.57	0.57	0.34	0.20	48.5	33.5	29.3	9.7	1.2	2.6	3.6	6.5	1.1	2.4	3.9
Gladys	0.70	0.70	0.43	0.25	61.3	45.7	18.1	9.9	87.2	29.7	33.3	51.6	0.9	2.2	3.6
Opsimo Naoussas	0.98	0.98	0.68	0.66	60.2	54.6	53.4	36.0	0.3	0.5	0.6	0.7	1.3	3.6	4.9
min	0.33	0.33	0.11	0.04	34.9	21.4	4.1	4.1	0.2	0.3	0.6	0.7	0.6	2.2	3.5
max	1.20	1.20	0.92	0.66	71.5	70.2	53.4	36.0	87.2	29.7	37.1	62.7	1.7	8.3	13.7
CV%	31	32	42	53	18	27	57	73	97.8	160.4	119.6	114.4	24.0	44.2	46.7
LSD	0.08				3.3				4.3				0.4		
Nectarine cultivars															
Andrianna	0.64	0.64	0.30	0.13	42.1	44.8	15.3	6.9	1.9	2.3	7.0	10.1	0.9	2.5	5.1
Big Bang	0.68	0.68	0.50	0.29	46.2	40.4	25.5	8.3	0.5	0.7	10.4	10.4	1.0	3.3	5.7
Rose Diamond	0.40	0.40	0.25	0.08	46.8	39.9	10.4	6.5	2.7	4.7	4.9	21.5	1.2	4.0	6.5
Rita Star	0.61	0.61	0.37	0.23	47.1	51.6	9.0	4.6	2.8	3.2	7.1	5.2	1.6	4.2	7.0
Big Top	0.58	0.58	0.46	0.37	60.0	52.4	48.3	46.0	0.2	0.2	1.1	2.0	1.7	4.6	6.1
Caldesi 2000	0.65	0.65	0.39	0.29	42.1	34.1	9.9	5.1	1.3	3.0	3.2	5.9	0.9	2.5	4.0
Red Gold	1.13	1.13	0.78	0.45	73.9	72.5	38.0	21.7	2.6	3.2	1.5	1.7	1.0	2.0	3.1
Venus	0.58	0.58	0.24	0.14	58.9	59.2	20.1	8.7	0.4	2.3	2.5	2.2	0.7	2.1	3.3
Tasty Free	0.51	0.51	0.31	0.28	54.9	46.6	45.7	18.5	0.4	0.9	1.1	1.4	1.1	3.0	4.5
min	0.40	0.40	0.24	0.08	42.1	34.1	9.0	4.6	0.2	0.2	1.1	1.4	0.7	2.0	3.1
max	1.13	1.13	0.78	0.45	73.9	72.5	48.3	46.0	2.8	4.7	10.4	21.5	1.7	4.6	7.0
CV%	31	32	42	53	20	24.0	63.0	96.0	98	160	120	114	24.0	44.2	46.7
LSD	0.06				2.6				0.87				0.49		

was performed between ripening related traits and antioxidant parameters. The percentage changes in all studied parameters were calculated between day 5 and 0 (% D5-0). Principal component analysis (PCA) was applied to mean values of each measurement trait during harvest, and %D5-0 of each measured parameter. Single linkage cluster analyses were performed using the method of Euclidean distance on factors produced after PCA analyses (uncorrelated factor scores). Statistical analyses were performed using SPSS (SPSS Inc., Chicago, Illinois, USA)

3. Results and discussion

3.1. Physical and chemical characteristics at harvest

Flesh firmness was highly variable among the studied peach and nectarine cultivars, ranged between 34.9 N ('Red Haven') and 73.9 N ('Red Gold') at harvest (Table 1). Crisosto et al. (2001) postulated that FF values between 18 and 35 N for peaches and nectarines can be considered as 'ready to eat', while maximum levels of fruit firmness for marketing flesh peaches and nectarines are set by the EU at 63.7 N with an 8 mm diameter probe [Commission Regulation (EC) No. 1861/2004].

Soluble solid content at commercial harvest ranged from 9.5% ('Adrianna') up to 14.4% ('Red Gold') (Table 2), well above the minimum threshold value (8%) that EU has established to market peaches and nectarines (R-CE no. 1861/2004). It should additionally taken into consideration that SSC values below 11% are usually unacceptable to consumers (Crisosto and Crisosto, 2005). Previous studies reported that SSC are associated with harvest date on peach (Cantín et al., 2010; Dirlewanger et al., 1999), apricot (Drogoudi et al., 2008; Ruiz and Egea, 2008) and plum cultivars (Drogoudi et al., unpublished data).

Cultivars varied in the TA content, with values ranging from 0.53% malic acid ('Royal Glory', a typical sub-acid peach cultivar) to 1.23% malic acid ('Red Gold') (Table 2). It is worth to note that new breeding programs in Spain are focusing in cultivars with TA lower than 0.5% through a marker assisted selection. It is well known that the flavour intensity of peaches and nectarines is mainly linked to the RI (SSC/TA ratio) and appears to be a key factor influencing the taste perception and consumer acceptance (Crisosto et al., 2006; Di Miceli et al., 2010). In the present study, cvs. 'Big Bang', 'Royal Glory', 'Fayette' and 'Sun Cloud' had the greatest values of SSC/TA ratio (17.0–19.3), whereas the lowest values were found in 'Opsimo Naoussas', 'Andrianna', 'Royal Gem', 'Tasty Free' and 'May Crest' (9.2–10.8). The variation in SSC, TA and RI values was relatively low among the cultivars tested (CV% = 14%, 19% and 21%, respectively).

Lately, the cultivar antioxidants content has become a quality parameter, due to their beneficial effects on health and an increasing demand of consumers for high antioxidant content in fruits. A wide variation in the antioxidant contents were found among the examined peach and nectarine cultivars. The majority of peach samples contained TPs in the range of 11.7 and 38.6, and greatest values (42.3–90.1 mg GAE 100^{-1} g FW) were found in descending order in cultivars 'Sun Cloud', 'Gladys', 'Sun Crest', 'Opsimo Naoussas', 'Fayette' and 'Rubidoux' (Fig. 1a). It should be noted that 'Sun Cloud' is one of the top-producing cultivars only in

Table 2
Soluble solid (SSC) (%) and total acid (TA) (% malic acid) contents, ripening index (RI), color parameters of the exocarp (L, a* and b*) and respiration (ml CO_2 kg^{-1} h^{-1}) rates, in 17 peach and 9 nectarine cultivars, at harvest. LSD = least significant difference; CV% = variation coefficient.

Peach	SSC	TA	RI	L	a*	b*	Respiration
May Crest	10.3	0.96	10.8	44.4	25.5	20.6	111.8
Spring Belle	9.7	0.81	12.0	49.4	24.0	24.8	45.5
Royal Gem	10.3	0.98	10.6	42.0	26.6	17.8	42.2
June Gold	11.6	0.98	11.9	53.0	23.0	28.6	59.1
Royal Glory	10.1	0.53	19.3	40.3	23.9	15.0	43.5
Rich Lady	12.1	1.11	10.9	37.6	24.8	11.6	56.8
Maria Bianca	12.2	0.83	14.7	73.3	-5.1	33.4	45.5
Red Haven	10.3	0.73	14.1	69.1	9.8	46.9	13.6
Sun Cloud	11.9	0.70	17.0	66.9	12.7	46.8	27.7
Kori	11.0	0.84	13.1	57.9	19.4	35.7	30.7
Sun Crest	12.7	0.82	15.4	62.3	9.4	40.8	39.2
Elegant Lady	13.0	1.01	12.9	52.9	21.2	32.5	39.0
Symphonie	11.2	0.71	15.8	49.9	28.9	28.5	37.3
Fayette	13.4	0.78	17.3	58.4	17.4	36.8	34.3
Rubidoux	12.3	0.85	14.4	68.9	5.6	46.3	33.3
Gladys	13.4	1.02	13.1	72.0	4.3	31.8	40.2
Ops.Naoussas	9.6	1.04	9.2	64.0	10.8	41.1	31.7
mean	11.5	0.86	13.7	56.6	16.6	31.7	43.0
min	9.6	0.53	9.2	37.6	-5.1	11.6	13.6
max	13.4	1.11	19.3	73.3	28.9	46.9	111.8
LSD	0.8	0.1	1.3	3.6	4.2	3.8	7.8
CV%	11.0	17.6	17.6	20.4	57.7	34.9	45.7
Nectarine							
Andrianna	9.5	0.91	10.4	57.8	24.5	32.5	52.7
Big Bang	12.2	0.63	19.3	41.6	30.4	16.8	41.4
Rose Diamond	11.4	0.79	14.5	55.3	25.5	30.3	44.0
Rita Star	9.8	0.83	11.8	48.7	28.7	25.3	2.8
Big Top	11.9	0.88	13.6	44.3	27.2	19.8	51.5
Caldesi 2000	10.8	0.95	11.4	45.9	31.7	18.0	46.2
Red Gold	14.4	1.23	11.8	59.3	13.7	38.5	39.0
Venus	13.5	1.00	13.5	52.5	26.8	30.8	40.9
Tasty Free	11.9	1.12	10.6	70.8	2.3	48.1	35.2
Mean	11.7	0.93	13.0	52.9	23.4	28.9	39.3
Min	9.5	0.63	10.4	41.6	2.3	16.8	2.8
Max	14.4	1.23	19.3	70.8	31.7	48.1	52.7
LSD	1.0	0.1	1.5	3.9	4.2	3.9	5.1
CV%	13.7	19.2	21.3	17.2	40.5	35.5	38.2

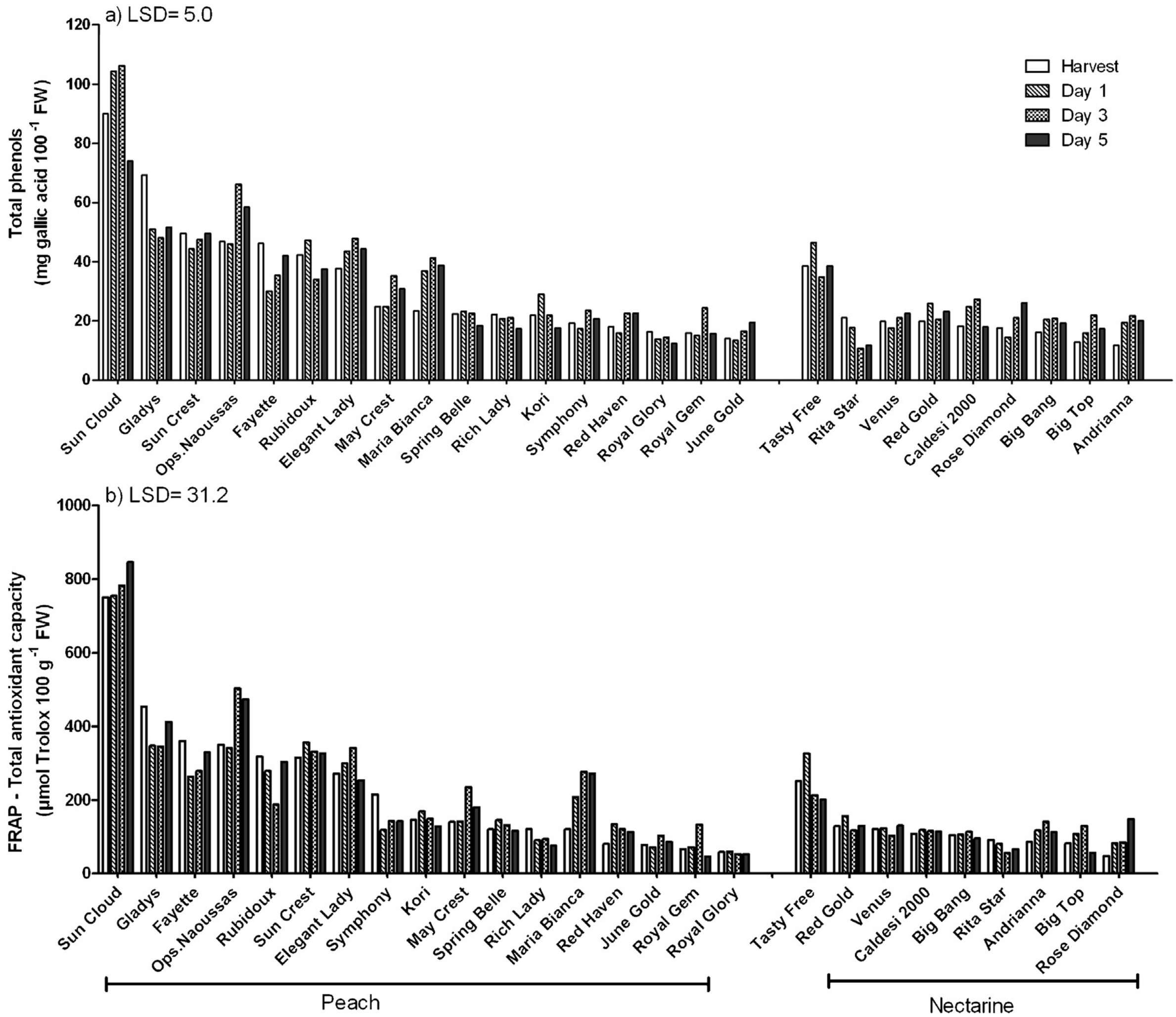

Fig. 1. (a) Total phenolics (mg GAE 100 g^{-1} FW) and (b) total antioxidant capacity using the FRAP method (TAC_{FRAP}) (μmol Trolox 100 g^{-1} FW), in 17 peach and 9 nectarine cultivars at harvest and during 1, 3 and 5 day shelf life storage. LSD = Least significant difference.

Greece, but is not being cultivated in other Mediterranean countries like Spain and Italy (Iglesias I. and Giovannini D., personal communications), while 'Gladys' is the most popular late white flesh cultivar, still interesting nowadays because of its high quality (aromatic, juicy with a balanced taste), yield potential and excellent fruit size.

Fruit from the 17 peach cultivars compared with the 9 nectarine cultivars contained 1.7, 2.5, 2.1, 1.4 and 1.5 fold higher TPs (Fig. 1a), TF (data not shown), TAC_{FRAP} (Fig. 1b), TAC_{DPPH} and TAC_{ABTS} (data not shown), respectively. Similarly, Di Vaio et al. (2008) and Gil et al. (2002) also found greater antioxidant contents in peach compared to nectarines cultivars.

It should also be mentioned that the three antioxidant assays generally showed comparable data in terms of cultivar ranking regarding their antioxidant potency; nevertheless differences among the *in vitro* assays were monitored. Indicatively, TAC_{FRAP} values in 'Sun Cloud' fruit were greater compared with all the rest cultivars (Fig. 1b), while TAC_{DPPH} and TAC_{ABTS} values were similar for the cultivars 'Sun Cloud', 'Elegant Lady', 'Tasty Free', 'Roubidoux' and 'Fayette' (data not shown).

The highest correlation coefficient between antioxidant assays and total phenolics was found for TAC_{FRAP} method ($r = 0.974$), followed by the TAC_{ABTS} ($r = 0.761$) and TAC_{DPPH} ($r = 0.748$) methods. Similar results were also documented in other peach and plum cultivars (Gil et al., 2002; Scalzo et al., 2005). The different chemical principles of antioxidant assays is rather responsible for the diversity in correlation coefficients: DPPH and ABTS activities are based on the scavenging of a relative stable radical, whereas the ability of the antioxidants to reduce Fe^{3+} to Fe^{2+} is the chemistry behind FRAP assay.

Correlations between harvest date and phenol content or antioxidant capacities ($r = 0.566–0.721$) suggest that late-harvested peach and nectarine cultivars tend to have a higher antioxidant potential (Table 3). Similar observations were also made among nine processing peach cultivars (Drogoudi and Tsipouridis, 2007) and in a recent study of 45 peach and nectarine cultivars (Drogoudi et al., unpublished data).

Principal component analysis was applied to describe all the information contained in the data set to detect the most important variables for data structure determination on the date of harvest for peach and nectarine cultivars (Fig. 2). The main features of PCA are the coordinates of the data in the new base (score plot) and the contribution to each component of the sensors (load plot). This can help to select a set of cultivars with better quality performance and to determine the most appropriate cultivars for each fruit type. In peach cultivars the first two components explained 54.9% of variation. The first component (38.8% of variation) positively correlated with the color parameters L* and b*, TPs, TF, TAC_{DPPH}, TAC_{ABTS} and TAC_{FRAP}, and negatively with the color parameter a*. The second component (16.1% of variation) was positively

Table 3
Pearson correlation (r) coefficients between ripening related traits and antioxidant parameters in 26 peach and nectarine cultivars at harvest. Abbreviations are explained in Table 1; HD, harvest day; FF, flesh firmness; TPs, total phenolics; TF, total flavanods; TAC_{FRAP}, TAC_{DPPH} and TAC_{ABTS}, total antioxidant capacity using the FRAP, DPPH and ABTS methods, respectively; ns. non significant; Absolute linear correlations ≥|0.80| are marked in bold. Significant differences: $^{*}P < 0.05$; $^{**}P < 0.01$.

	HD	I_{AD}	FF	SSC	TA	RI	L*	a*	b*	Hue	Chroma	TPs	TF	TAC_{DPPH}	TAC_{ABTS}	TAC_{FRAP}
HD																
I_{AD}	ns															
FF	.410*	.413*														
SSC	.443*	ns	.473*													
TA	ns	ns	.472*	ns												
RI	ns	ns	ns	ns	**-.803****											
L*	.620**	ns	ns	ns	ns	ns										
a*	-.606**	ns	ns	ns	ns	ns	**-.891****									
b*	.617**	ns	ns	ns	ns	ns	**.894****	-.728**								
Hue	.629**	ns	ns	ns	ns	ns	**.981****	**-.945****	**,878****							
Chroma	.487*	-.397*	ns	ns	ns	ns	.696**	-.426*	**.917****	.643**						
TPs	.566**	ns	ns	ns	ns	ns	.570**	-.542**	.548**	.568**	.397*					
TF	.443*	ns	ns	ns	ns	ns	.491*	-.430*	.500**	.478*	.394*	**.962****				
TAC_{DPPH}	.621**	ns	ns	ns	ns	ns	.510**	-.567**	.522**	.561**	ns	.748**	.643**			
TAC_{ABTS}	.721**	ns	ns	ns	ns	ns	.533**	-.497**	.601**	.567**	.475*	.761**	.649**	**.911****		
TAC_{FRAP}	.574**	ns	ns	ns	ns	ns	.532**	-.469*	.559**	.525**	.456*	**.974****	**.973****	.732**	.765**	

correlated with TA content and negatively with RI and the third component (10.4% of variation) was positively correlated with I_{AD} and RI. 'Sun Cloud' was separated, having high antioxidant contents and high RI, cvs. 'Gladys', 'Opsimo Naoussas' and 'Elegand Lady' having high antioxidant and TA contents, while cultivar 'Royal Glory' having low antioxidant and TA contents, and high red coloration.

As for nectarine cultivars, the first two components accounted for 67.3% of the total variance (Fig. 2b). Antioxidant contents, TA, L* and b* color parameters exhibited positive values for PC1, while a* color parameter exhibited negative value. The second component accounted for 18% of variance with I_{AD}, FF and SSC, exhibiting positive values. 'Tasty Free' fruit were separated, having high antioxidant contents and TA, and low I_{AD} and a* color parameter, while 'Red Gold' was separated having high I_{AD} and SSC values.

3.2. Monitoring ripening related and antioxidant content changes during shelf-life

Ripening related parameters and quality indicators (weight loss, FF, color, SSC and TA), during five days of shelf-life showed that most cultivars deteriorated quickly and their response generally reflected fruit physiological development (Valero et al., 2007). Weight loss

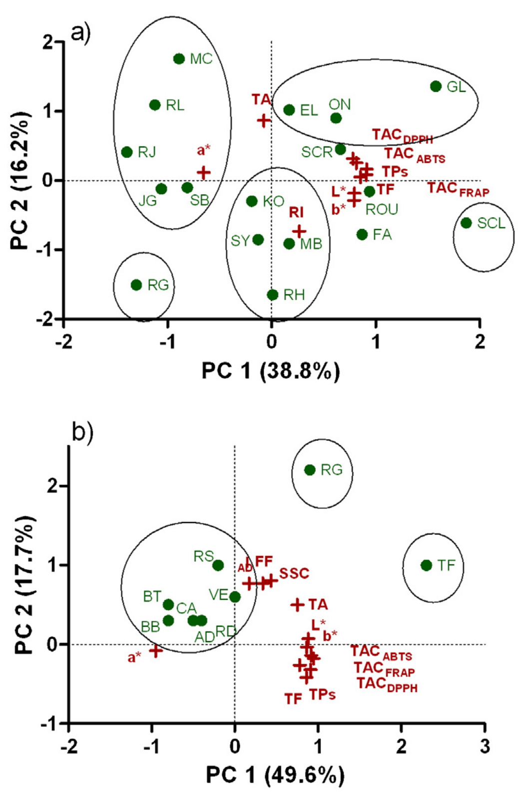

Fig. 2. Segregation of (A) 17 peach, and (B) 9 nectarine cultivars, according to their quality characteristics on day 0, determined by Principal Component Analysis (PCA). Crosses represent the loadings of quality traits data along with the principal component scores. Dotes represent peach and nectarine cultivars. *Abbreviations for peach cultivars: Fayette, FA; Gladys, GL; June Gold, JG; Kori, KO; Maria Bianca, MB; May Crest, MC; Opsimo Naoussas, ON; Red Haven, RH; Rich Lady, RL; Royal Gem, RGE; Royal Glory, RGL; Rubidoux, RU; Spring Belle, SB; Sun Cloud, SC; Sun Crest, SC; Symphony, SY; Elegant Lady, EL., Abbreviations for nectarine cultivars: Andrianna, AD; Big Bang, BB; Big Top, BT; Caldesi 2000, CA; Red Gold, RG; Rita Star, RS; Rose Diamond, RD; Tasty Free, TF; Venus, VE.*

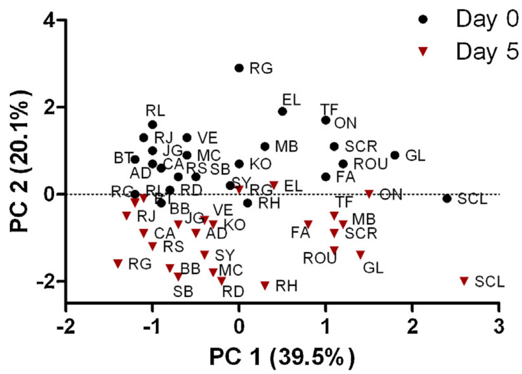

Fig. 3. Bi-plot principal component analysis (PCA) of ripeness indicators and antioxidant parameters of peaches and nectarines harvested at commercial ripening stage and then maintained for 5 days shelf life period. Fig. shows results only for day 0 and day 5.

ranged from 0.6% to 1.7% after 1 day of shelf life, reaching between 3.1% ('Red Gold') and 7.0% ('Rita Star') on day 5 in all cultivars, apart from the early harvested 'May Crest' were extensive weight loss occurred (Table 1).

Flesh firmness is an important quality parameter since it is related to susceptibility to mechanical damage during postharvest (Crisosto et al., 2001) and is directly related to the postharvest potential of stone fruits (Zerbini et al., 2006). Flesh firmness went descending with the progress of shelf life period concomitant with an ethylene increase that occurs when fruit had already softened (Lu et al., 2008). Such changes occurred on a cultivar dependent basis. Flesh firmness reduced substantially after 3 days of shelf life, except for the early-harvested cultivars 'May Crest' and 'Spring Belle', where advance softening occurred after one day maintenance at room temperature. At the end of shelf life period, FF reduction was ranging from 66.3% up to 90.3%, receiving values below 22 Newtons. A significant lower percentage of FF reduction was monitored in 'Opsimo Naoussas' (40.2%) and 'Royal Gem' (47.7%) fruits and most promptly in the slow softening melting nectarine cultivars 'Big Top' (23.4%), receiving values in the range 27.9–46.0 Newtons.

An appreciable increase of SSC content was monitored after 5 days shelf life only in the late harvested cultivars, namely 'Opsimo Naoussas' (22%), 'Gladys' (15%) and 'Tasty Free' (16%); fruit variability, tissue dehydration and/or contribution of soluble pectins may partially explain this increase. Titratable acidity decreased by 10–43% when ripening related changes were extensive (5 days shelf life). The percentage increase in RI after 5 days of shelf life was greatest in 'Gladys' (102%) and 'Tasty Free' (86%).

A wide variation in the response of antioxidant contents among the examined peach and nectarine cultivars, during ripening was observed. During 5 days shelf life the % change in TPs content ranged from −44% ('Rita Star') to +70% ('Adrianna') (Fig. 1a). The % change in TAC_{FRAP} ranged from −37% to +210% (Fig. 1b), the % change in TAC_{DPPH} ranged between −65% and +108%, and the % change in TAC_{ABTS} ranged between −52% and +127% (data not shown). Accordingly, no consistent pattern of phenolic changes during storage has been reported on peach (Dalla Valle et al., 2007; Di Vaio et al., 2008; Aubert et al., 2014) or other stone and pome fruit (Gonçalves et al., 2004; Adyanthaya et al., 2010; Faniadis et al., 2010; Napolitano et al., 2004; Awad and De Jager, 2003; Kevers et al., 2011; Goulas et al., 2014). The wide variation in the response of phenolics during shelf life may be the result of differences in their phenolic composition since the content of some compounds

Table 4
Pearson correlation coefficient (r) between the I_{AD} index and various ripening related traits and antioxidant parameters, during 5 days shelf-life storage. Ns, non significant; *$P<0.05$; **$P<0.01$; ***$P<0.001$. Abbreviations are explained in Tables 1 and 2.

	% WL	FF	SSC	TA	RI	L*	a*	b*	Hue	Chroma	Eth.	Resp.	TP	TF	TAC_{FRAP}	TAC_{DPPH}	TAC_{ABTS}
Peach																	
May Brest	−1.000*	.988*	ns	ns	ns	ns	ns	ns	ns	ns	-.971*	ns	ns	ns	ns	ns	ns
Spring Belle	−.999*	.934*	ns	ns	ns	ns	−.979*	ns	,980*	ns	ns	ns	ns	ns	ns	ns	.985*
Royal Gem	−.973*	.893*	ns	.987**	ns	.884*	ns	ns	ns	ns	−.979**	ns	ns	ns	ns	ns	ns
June Gold	−.999*	.968*	ns	ns	−.984*	ns	ns	ns	ns	ns	−.974*	ns	−.957*	ns	ns	ns	.975*
Royal Glory	−.978*	.986**	ns	.934*	−.965*	ns	ns	ns	ns	ns	−.973**	ns	ns	ns	ns	ns	ns
Rich Lady	ns	.992**	ns	.966*	−.998**	.921*	ns	.934*	,955*	,892*	ns	ns	ns	ns	ns	ns	ns
Maria Bianca	ns	.975*	ns	ns	ns	−.992**	−.989*	.996**	,986*	,998**	ns	ns	ns	ns	ns	ns	−.956*
Red Haven	ns	.959*	ns	ns	−.954*	.966*	ns	ns	ns	ns	−.958*	ns	ns	ns	ns	ns	ns
Sun Cloud	ns	.952*	ns	.965*	ns	ns	−.988*	ns	,993**	ns	−.959*	ns	ns	ns	ns	ns	ns
Kori	−.957*	.983**	ns	.945*	−.968*	ns	ns	ns	ns	ns	−.950*	−.925*	ns	ns	ns	ns	ns
Sun Crest	ns	.978**	ns	ns	ns	ns	ns	ns	ns	ns	−.945*	ns	ns	ns	ns	ns	ns
Elegant Lady	−.957*	.972**	ns	.931*	ns	ns	ns	ns	ns	ns	ns	−.947*	ns	ns	ns	ns	ns
Symphonie	ns	.994**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fayette	−.999**	.961**	ns	.962**	−.973*	.992**	−.929*	ns	,956*	ns	−.988**	ns	ns	ns	ns	ns	ns
Roubidoux	ns	.988*	ns	.964*	−.986*	ns	ns	ns	ns	ns	−.979*	ns	ns	ns	ns	ns	ns
Gladys	−.999*	.967*	−.997**	.980*	−.984*	.981*	ns	ns	,950*	ns	ns	ns	ns	ns	ns	ns	ns
Opsimo Naoussas	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−.974*	ns	ns
Nectarine																	
Adrianna	ns	.969*	ns	ns	−.987*	ns	ns	ns	ns	−.970*	−.993**	ns	ns	ns	ns	ns	ns
Big Bang	ns	.994**	ns	.999**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rose Diamond	ns	.906*	ns	ns	−.963*	ns	ns	ns	ns	ns	ns	ns	ns	ns	−.962*	ns	ns
Rita Star	ns	.963*	ns	.966*	−.986*	ns	ns	ns	ns	ns	ns	ns	ns	.952*	ns	.998**	.958*
Big Top	−.983*	.961**	ns	.939*	ns	ns	−.976**	ns	ns	ns	−.972*	ns	ns	ns	ns	ns	ns
Caldesi 2000	ns	.988*	−.998**	.993**	−.996**	ns	−.964*	ns	.964*	ns	ns	ns	ns	ns	ns	ns	ns
Red Gold	ns	.983**	ns	.984**	−.981*	ns	ns	ns	ns	−.943*	ns	ns	ns	ns	ns	ns	ns
Venus	ns	.999**	ns	ns	ns	ns	ns	ns	.976*	ns	ns	ns	ns	ns	ns	ns	ns
Tasty Free	ns	ns	−.997**	.978*	−.953*	ns	ns	ns	ns	ns	−.973*	ns	ns	ns	ns	ns	ns

has been found to increase and of others to decrease (Awad and De Jager, 2003). Postharvest enzymatic activity could hydrolyse some bound polyphenols, thus enhancing the total phenols amount (Dalla Valle et al., 2007). Imeh and Khokhar (2002) reported that in peaches the 80% of phenolics are in conjugated form; it is surmised that some conjugated polyphenols were degraded to extractable single phenolics during cold storage. Furthermore, phenolic compounds are known to be responsible for the induction of enzymatic browning in the presence of oxygen and the enzyme polyphenol oxidase (Radi et al., 1997).

In the present study, no significant correlation was found between ethylene production rate after 5 days of shelf life and percentage change in TPs (data not shown). It should be also noted that ethylene production is being culminated in peach fruit during their ripening after cold storage compared to ripening after harvest (Manganaris et al., 2006).

A PCA was applied on ripening related parameters and antioxidant contents during all measurements dates, and a bi-plot graph with results from harvest (day 0) and day 5 (Fig. 3). The first two principal component axes explained 59.6% of the total variance (PC1 = 39.5%, and PC2 = 20.1%). PC1 was defined positively by L, b* and all antioxidant parameters (factor loadings between 0.8–0.9) measured, and negatively with a* (factor loading −0.7). PC2 was positively associated with I_{AD}, flesh FF, TA and RI (factor loadings between 0.7–0.9).

3.3. I_{AD} *values and prediction of ripening related parameters*

The I_{AD} index has been proven as a rapid and simple measuring technique to determine maturity, or to sort fruit into categories of maturity (Herrero-Langreo et al., 2011; Bonora et al., 2013; Shinya et al., 2013). The 26 peach and nectarine cultivars studied exhibited considerable variation in the I_{AD} index [variation coefficient (CV) = 32%] at the time of commercial harvest, with mean values ranging from 0.3 ('Red Haven') to 1.2 ('Royal Glory') (Table 1). Changes in the I_{AD} index during shelf life were highly correlated with changes in FF in 24 cultivars, with TA in 15 cultivars, and ethylene production rate in 13 cultivars (Table 4). In all cases that a significant correlation was found the r value was $\geq|0.88|$, suggesting absolute linear correlations. Flesh firmness was not significantly correlated with the I_{AD} index only in the peach 'Opsimo Naoussas' and the nectarine 'Tasty Free', both late-harvested cultivars with slow-softening rate (Supplementary Table 1). The changes in SSC did not correlate with I_{AD} in most cultivars, although for apples a close prediction of SSC content and I_{AD} index has been reported (McGlone et al., 2002; Nyasordzi et al., 2013).

Minor changes in h° during shelf ripening were found (data not shown) and as result the I_{AD} parameter was not correlated with h° in most cultivars tested (Table 4). Shinya et al. (2013), similarly, found no correlation between I_{AD} and h° during postharvest ripening. Nevertheless, these parameters, together with FF, were closely related during on tree maturation, when pronounced color changes occur (Shinya et al., 2013). Moreover, the same h° could be associated with different levels of FF and I_{AD} during on tree ripening since fruit color is influenced by the light environment in which the fruit develops, and therefore needs to be treated with caution (Lewallen and Marini, 2003).

NIR technology has shown significant applicability for predicting chemical and textural parameters, including FF, SSC, TA, chlorophyll, pectins and carotenoids (Sirisomboon et al., 2007; Solovchenko et al., 2005; Zude-Sasse et al., 2002); to our knowledge there is no report for antioxidant contents. In the present study, antioxidant contents (TPs and TF) and antioxidant capacity (TAC_{DPPH}, TAC_{ABTS} and TAC_{FRAP}) exhibited a wide variation in their response, showing an increase, decrease or no change during shelf life, depending on the cultivar, and as expected there was no correlation with changes in the I_{AD} index (Table 4; Fig. 1a,b).

4. Conclusions

A considerable variation in the I_{AD} values of peach and nectarine cultivars was found; such I_{AD} values can be used as reference indexes per cultivar in future studies. Noteworthy, changes in the I_{AD} index were closely correlated with changes on firmness retention. Segregation of peach and nectarine cultivars, according to their quality and ripening characteristics, was performed. Most cultivars deteriorated quickly, evident as rapid reduction of firmness retention under shelf life conditions, apart from 'Opsimo Naoussas', 'Big Top' and 'Royal Gem'. Great phenotypic differences in fruit quality parameters, phenolic contents and ripening behavior, among the 26 examined cultivars were monitored. Furthermore, phenolic and flavonoid contents and antioxidant capacities were differentially changed among cultivars with the progress of shelf life ripening. Cultivars with high antioxidant contents were indicated; most promptly 'Sun Cloud', top-producing cultivar in Greece, although it is not being cultivated in other main European peach producing countries. Interestingly, the reference nectarine cultivar 'Big Top', highly appreciated by the consumers due to particular pomological traits (slow softening rate, size, skin color, sweet taste), was ranked among the cultivars with the lower antioxidant potency. Further, late compared to early-season harvested cultivars, and peach compared to nectarine cultivars, tended to be characterized by higher polyphenolic contents. Such data needs to be further confirmed in successive growing seasons or with the examination of additional cultivars or lots of the same cultivar from different orchards.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2015.12.002.

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