Scientia Horticulturae 129 (2011) 752–760

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of position on canopy and harvest time on fruit physico-chemical and antioxidant properties in different apple cultivars

Pavlina D. Drogoudi*, Georgios Pantelidis

Pomology Institute, National Agricultural Research Foundation, 38 R.R. Station, 59200 Naoussa, Greece

ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 17 May 2011
Accepted 31 May 2011

Keywords:
Antioxidant capacity
Apple quality
Fyriki
Fuji Kiku 8
Granny Smith
Imperial Double Red Delicious
Phenolics

ABSTRACT

Fruit from Granny Smith, Fyriki, Fuji Kiku 8, and Imperial Double Red Delicious apple (*Malus domestica* Borkh.) trees planted in single or double rows, were harvested from different positions in the canopy, during the last month before commercial harvest. Fruit physico-chemical and antioxidant capacity, using the radical DPPH, were measured, in skin and flesh tissue. Greater total phenol content and total antioxidant capacity per fresh weight and serving portion of skin and flesh tissue, were found in Imperial Double Red Delicious and Fyriki apples, compared to Granny Smith and Fuji Kiku 8. The variation among cultivars in antioxidant contents was greater in peeled, compared with unpeeled fruit, highlighting the importance of eating unpeeled fruit. During the last two weeks before commercial harvest the total phenol content and total antioxidant capacity per fresh weight increased in skin of Granny Smith (by 24% and 42%, respectively) and Fuji Kiku 8 (by 19% and 27%, respectively). Fruit from the more sun—exposed parts of canopy had usually better red coloration and the effect was more pronounced in Fuji Kiku 8 and Fyriki, followed by Imperial Double Red Delicious and a lower effect was found in Granny Smith. Greater total soluble solid content, but not flesh firmness, were also found in fruit from the more sun—exposed parts of canopy, in all cultivars. The skin of fruit from the upper positions in the canopy had greater total phenol content and total antioxidant capacity, in all cultivars, apart from Fyriki. Antioxidant contents in flesh tissue were also greater in the upper positions of canopy in Fuji Kiku 8 and Imperial Double Red Delicious, and to our knowledge this is the first report on plant canopy effects on apple flesh antioxidant compounds.

1. Introduction

The apple (*Malus domestica* Borkh.) fruit is the second most frequently consumed, after bananas and this is attributed not only to its organoleptic characteristics but also to its long storability and availability to the markets for many months. Some quality characteristics of apples directed for fresh consumption are size, color, aroma, freedom from blemishes, total soluble solid and total acid content. The apple quality is also determined by their phenolic content, as they provide taste characteristics such as flavour, bitterness and astringency and induce oxidative browning (Bengoechea et al., 1997; Fuleki et al., 1994). For apples directed to the juice industry, phenolic compounds also induce the formation of hazes and precipitates, resulting from a strong interaction of tannins with macromolecules such as proteins and polysaccharides (Siebert et al., 1996). More importantly apple phenolics have beneficial implications in human health. Apples are a good source of phenolic compounds (Eberhardt et al., 2000), and their "protective" property is attributed to its scavenging activity towards free radicals, which are thought to be responsible for many degenerative diseases (Liu et al., 2009; Sun and Liu, 2008; Wolfe et al., 2003).The strongest parameter affecting the content of phenolic compounds in apple is its tissue type. Apple skin has for a few to several times greater content of phenolic compounds than apple flesh, and has unique flavonoids, such as quercetin glycosides, not found in the flesh (Awad et al., 2000; Chinnici et al., 2004; Drogoudi et al., 2008; Łata and Tomala, 2007; Wolfe et al., 2003). Following, great differences have been found among apple cultivar in their phenolic content, when grown under the same ecological conditions (Drogoudi et al., 2008; Łata and Tomala, 2007; Lee et al., 2003; Tsao et al., 2003; Vieira et al., 2009; Wojdyło et al., 2008). Environmental parameters and cultivation practices are also known to affect the phenolic content in apple fruit (Jakopic et al., 2009; Iglesias et al., 2002; Stopar et al., 2002; van der Sluis et al., 2001), and their effects sometimes exceed the effect of genotype (Łata, 2007; Łata et al., 2005).

Fruit maturation and ripening have large effects on the red color development and anthocyanin accumulation in apple fruit (Arakawa et al., 1999; Faragher and Brohier, 1984). Anthocyanin biosynthesis may be induced by light, particularly UV, and vari-

* Corresponding author at: Pomology Institute, National Agricultural Research Foundation, 38 R.R. Station, 59200 Naoussa, Greece. Tel.: +30 23320 41548; fax: +30 23320 41178.
E-mail addresses: drogoudi@otenet.gr (P.D. Drogoudi), stratig@agro.auth.gr (G. Pantelidis).

0304-4238/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2011.05.036

ous stress treatments including cold (Arakawa et al., 1999; Awad et al., 2001a; Dong et al., 1998; Kim et al., 2003). The fruit position on canopy relates to the amount of light that it will receive and apples from the outer compared with the inner canopy have greater anthocyanin content (Awad et al., 2000; Jakopic et al., 2009). The fruit response to light has been reported to vary considerably among apple cultivars (Arakawa, 1988).

Although many studies have characterized the variation in apple phenolics due to cultivar, fruit position on canopy and harvesting date (Arakawa et al., 1999; Awad et al., 2000; Drogoudi et al., 2008; Faragher and Brohier, 1984; Jakopic et al., 2009; Łata and Tomala, 2007; Lee et al., 2003; Tsao et al., 2003; Vieira et al., 2009; Wojdyło et al., 2008), to our knowledge there is no report on effects on fruit quality characteristics and antioxidant contents in both skin and flesh tissue. The objectives of the present study were to investigate the variability in fruit physico-chemical and antioxidant properties in different apple cultivars, positions on canopy, harvesting dates and apple tissues.

2. Materials and methods

The experiment was conducted in the "Athanasakis brothers" commercial apple (*M. domestica* Borkh., cvs Granny Smith, Fyriki, Fuji Kiku 8 and Imperial Double Red Delicious (Imperial D.R.D.)) orchard located in Imathia, central Macedonia, which is a prefecture, where the majority of Greek apple cultivation takes place (49%, in 2006) (Hellenic Statistical Authority). The cultivars Granny Smith, Fuji Kiku 8 and Imperial Double Red Delicious are, or belong to a family that is, of the most widely cultivated in the world, and cultivar Fyriki, is a local genotype originating from Magnisia (central Greece).

All trees were raised as slender spindles, and planted in north-south direction and in twin-rows in Granny Smith and Fyriki (4 m distance between double rows, 1 m distance inside the twin-rows, and 2 m distance on the rows), and single rows in Fuji Kiku 8 and Imperial D.R.D. (4 m × 1 m). Granny Smith and Fyriki were 10 year-old, and Fuji Kiku 8 and Imperial D.R.D. were 8 year-old. Granny Smith was grafted on M26 rootstock, and the rest of cultivars were grafted on M9 rootstock. All trees received routine horticultural care.

Twelve fruit were collected from the upper part of canopy, and from the middle and lower part of the two sides of linear shaped canopies. Thus, in Granny Smith and Fyriki, samplings were made from the upper canopy, middle- and lower-lighted (outside the twin-rows), and middle- and lower-shaded (inside the twin-rows) parts of canopy. In Fuji Kiku 8 and Imperial D.R.D., samplings were made from the upper-, middle- and lower-east and west directions. Fruit were sampled from six to twelve trees, selected for vegetative and crop load uniformity. Samplings of Granny Smith apples were made at 35 d, 28 d, 14 d, 7 d, and 0 d before commercial harvest. Samplings of Fyriki and Fuji Kiku 8 apples were made at 18 d, 12 d, and 0 d before commercial harvest. In cultivar Imperial D.R.D. sampling was made only at commercial harvest.

Harvested fruit were immediately transferred to the lab. Six apples were rinsed with distilled water, dried on tissue paper and stored at −20 °C until measurements of total phenol (TPh) content and ascorbate equivalent antioxidant capacity (AEAC) were made. Measurements of fruit fresh weight, flesh firmness, color, soluble solid (SSC), and total acid (TA) contents were made from another six apples.

Flesh firmness was measured after removal of the skin, using a Chatillon penetrometer, fitted with an 11 mm tip (AME-TEK, Largo, USA). Soluble solid contents were analysed in the juice using a digital refractometer (Model PR-1; Atago, Tokyo, Japan) and TA was assessed for the same samples by titration with 0.1 N NaOH and expressed as malic acid contents ($g\,l^{-1}$). The color parameter a^* was measured using a Minolta Chromatometer (Model CR-200, Minolta, Ramsey, NJ, USA), and measurements were made in the more and least red colored cheek of each apple, and a mean value was calculated.

2.1. TPhs and AEAC assays

Skin samples were peeled using a peeler, all along the middle equatorial axis. Flesh samples were removed using a sharp knife, from the flesh below the most and least red colored part of skin. Each extraction was used directly for analyses, in triplicate.

All chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Assays were performed using an automated UV/visible spectrophotometer (Model U-2001 UV/Vis, Hitachi Instruments Inc., San Francisco, USA).

Frozen samples (approx. 1 g) of flesh from three replicate fruit were homogenised in 10 ml 80% (v/v) MeOH/H_2O in a micro-dismembrator (Micro-Dismembrator U, B. Braun Biotech International GmbH, Melsungen, Germany) for 3 min at 2000 rpm. The extract was centrifuged at 5000 × *g* for 10 min, and the supernatant was recovered.

Antioxidant capacities were measured using the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical (Blois, 1958), which has an intense violet color, but turns colorless as unpaired electrons are sequestered by antioxidants. Reaction mixtures containing 0 or 10 μl extract, 2.3 ml 106.5 μM DPPH in MeOH, and 690 μl H_2O were vortexed, then kept at room temperature in the darkness for 4 h. The absorbance of each reaction mixture was measured at 517 nm and the concentration of ascorbate equivalent antioxidant capacity (AEAC) (mM) was extrapolated from a standard curve prepared using 0–2.7 mM ascorbate.

Total soluble phenolics contents were determined according to the Folin-Ciocalteu's procedure (Singleton and Rossi, 1965) and the results were expressed as mg gallic acid equivalent $100\,g^{-1}$ fresh weight.

2.2. Statistical analyses

Statistical analyses were performed for a multi-factor ANOVA (fruit position on canopy and day of harvest), based upon the replicate fruit, using the Statsoft statistical package SPSS (version 9.0, Chicago, IL, USA). LSDs were calculated in cases which were significant at $P < 0.05$ variance.

3. Results and discussion

3.1. Cultivar

All cultivars were grown under the same geographical conditions and with the same applied agronomic practices, apart from the planting design, being twin-rows in Granny Smith and Fyriki, and single rows in Fuji Kiku 8 and Imperial D.R.D. A comparison on the fruit quality characteristics was made for apples harvested from the middle-lighted (outside twin-rows) or middle-east parts of canopy, at commercial harvest (Table 1).

In skin tissue, the TPh content (mg gallic acid equivalent 100^{-1} g fresh weight) was greatest in Imperial D.R.D. (630.0 mg) followed by Fyriki (403.0 mg), and even lower values were found in Granny Smith and Fuki Kiku 8 (303.8 and 285.7 mg, respectively). AEAC in skin tissue (mg ascorbate equivalent 100^{-1} g fresh weight) was similarly greatest in Imperial D.R.D. (895.9 mg) followed by the rest cultivars (mean value of 393.7 mg). In flesh tissue, the TPh content ranged between 31.2 and 129.2 mg 100^{-1} g fresh weight, and the cultivars followed the order: Imperial D.R.D. > Fyriki > Fuji

Table 1
Mean (±SE) fruit fresh weight, firmness, color parameter a^*, total soluble solids, total acid, total phenols (TPh), and ascorbate equivalent antioxidant capacity (AEAC) in peel and flesh tissue, and serving portions, in Granny Smith, Fyriki, Fuji Kiku 8 and Imperial Double Red Delicious apples. Fruit were sampled from the middle-lighted or middle-eastern parts of canopy, at commercial harvest.

	Granny Smith.	Fyriki	Fuji Kiku 8	Imperial D.R.D.
Fruit fresh weight (g)	199.9 ± 16.3	137.2 ± 8.3	175.4 ± 9.4	164.2 ± 5.3
Firmness (kg)	6.4 ± 0.2	9.3 ± 0.2	8.4 ± 0.2	7.8 ± 0.1
a^*	−15.5 ± 0.5	1.0 ± 4.3	3.5 ± 1.5	24.7 ± 1.2
Total soluble solids (%)	11.3 ± 0.1	12.9 ± 0.35	14.7 ± 0.2	13.0 ± 0.1
Total acids (g mallic acid l^{-1})	7.1 ± 0.2	2.3 ± 0.01	3.4 ± 0.2	2.5 ± 0.1
Peel—TPh[a]	303.8 ± 5.9	403.0 ± 22.7	285.7 ± 4.8	630.0 ± 11.1
Peel—AEAC[b]	412.2 ± 18.4	390.3 ± 30.9	378.5 ± 11.3	895.9 ± 22.6
Flesh—TPh[a]	31.2 ± 1.0	129.2 ± 0.9	72.5 ± 3.0	83.5 ± 2.0
Flesh—AEAC[b]	34.3 ± 7.3	197.4 ± 14.8	121.2 ± 8.8	174.8 ± 1.7
TPh per serving[a,c]	70.5 ± 1.6	163.8 ± 2.9	100.9 ± 2.7	161.1 ± 2.5
AEAC per serving[b]	88.3 ± 5.7	216.5 ± 14.5	153.7 ± 6.1	274.2 ± 3.5

[a] Expressed as mg gallic acid equivalents 100 g^{-1} fw.
[b] Expressed as mg ascorbic acid equivalent 100 g^{-1} fw.
[c] Serving = 100 g of fruit (80% flesh + 15% peel).

Kiku 8 > Granny Smith. Similarly, AEAC was greatest in flesh of Fyriki and Imperial D.R.D. (mean value of 186.1 mg 100^{-1} g fw). Similar values of TPh contents were previously reported for the apple skin (105–270 mg 100^{-1} g fresh weight, Vieira et al., 2009; 309–589 mg 100^{-1} g fresh weight, Wolfe et al., 2003), and flesh (32–38 mg 100^{-1} g fresh weight, Chinnici et al., 2004). The apple cultivar Starkrimson which is genetically close to Imperial D.R.D. (clones from the family Delicious) together with Fyriki were also previously reported to have relatively greater TPh content and AEAC, compared to other five apple cultivars (Drogoudi et al., 2008).

The skin of apples is frequently discarded as a waste product during manufacturing or before eating. Nevertheless, the skins are the healthier part of fruit, since the TPh content in skin was up to 9.7 times, and the AEAC was up to 10.4 times, greater compared to the flesh. Similar results were previously described by other authors (Awad et al., 2000; Chinnici et al., 2004; Drogoudi et al., 2008; Łata and Tomala, 2007; Veberic et al., 2005; Wolfe et al., 2003). To evaluate the dietary impact of apple consumption on the intake of antioxidant compounds, their supplies by fruit serving were determined and these calculations were based on a fruit serving of 100 g (15 g skin + 80 g flesh + 5 g ovary with seeds). The TPh content ranged from 70.5 to 163.8 mg/serving, and the AEAC ranged from 88.3 to 274.2 mg/serving, with greater values in Imperial D.R.D. and Fyriki (Table 1). Considering that the skin represents up to 10% of the whole fruit it would be assumed that the phenolic compounds in the flesh are of greater importance to the consumer than the phenolic compounds in the skin (Veberic et al., 2005). Nevertheless, when considering the consumption of unpeeled apple, the variation in TPh and AEAC among cultivars was up to 2.3 and 2.9 times, respectively, and when considering the consumption of only the apple flesh the variation among cultivars was up to 4.2 and 4.7 times, respectively. Therefore, the apple cultivar may influence more the dietary impact when consuming peeled, compared with unpeeled fruit, highlighting the importance of eating unpeeled fruit.

Red coloration in skin was greater in Imperial D.R.D., followed by Fuji Kiku 8 and Fyriki, and was even lower in Granny Smith (24.7, 3.5, 1.0 and −15.5 a^* values, respectively). Color values are the mean of measurements made in the sun exposed, and the opposite, side of fruit. Therefore, although mean values of a^* were similar in Fyriki and Fuji Kiku 8, the red coloration in the sun exposed side of fruit was greater, in the bicolor cultivar Fyriki. The red color in skin is due to anthocyanin pigments (mainly cyaniding 3-galactoside), which are phenolics with antioxidant properties (Wang et al., 1997). Cultivars Imperial D.R.D. and Fyriki were also low sugar (<13.5% SSC) and low acid (<4 mg 100^{-1} g fw), compared to Granny Smith being low sugar and high acid (>6 mg 100^{-1} g fw), and Fuji Kiku 8 being sweet (>13.5% SSC) and low acid cultivar. Therefore, cultivars Imperial D.R.D. and Fyriki had relatively high antioxidants in skin and flesh, better red coloration in at least one cheek of fruit, and low SSC and TA contents, which coincides with results of Drogoudi et al. (2008) finding from a principal component analysis that a more nutritional skin may be darker and redder and a more nutritional flesh may have lower SSC.

3.2. Fruit position on tree

In all cultivars, apart from Fyriki, the TPh content and AEAC were usually greater in skin of apples harvested from parts of canopy being more exposed to the sun, such as the upper, middle-lighted and lower-lighted parts in Granny Smith, and the upper and middle-east parts in Imperial D.R.D. and Fuji Kiku 8, and additionally the middle-west part in Imperial D.R.D. (Figs. 1, 3, 5 and 7). The increase in skin TPh content and AEAC in the upper compared with the lower positions on canopy was up to 52–55% in Granny Smith, 43–59% in Fuji Kiku 8, and 20–22% in Imperial D.R.D.

The apples from all studied cultivars had usually greater a^* value in the upper, middle-lighted or middle-east, parts of canopy, compared with parts of canopy being less exposed to the sun (Figs. 2, 4, 6 and 8). The increase in a^* value in more exposed parts of canopy was greater in Fyriki and Fuji Kiku 8 (increase by 81%, 70%, respectively), followed by Imperial D.R.D. (increase by 39%) and a lower effect was found in the green cultivar Granny Smith (increase by 13%), suggesting that position on canopy was more important for getting better red coloration in cultivars Fyriki and Fuji Kiku 8. Arakawa (1988) also found that Fuji has difficulty coloring well, compared to Jonathan and Red Delicious.

Similarly, the SSC (Figs. 2, 4, 6 and 8) and SSC/TA ratio (data not shown) were usually greater in the upper, middle-lighted or middle-east parts of canopy. Minor changes were found in flesh firmness among the different positions on canopy in all cultivars (Figs. 2, 4, 6 and 8). Results form SSC and color, but not flesh firmness, suggest that apples from the upper and middle-lighted or middle-east parts of canopy were in a more advanced maturing stage. Other authors similarly found greater SSC in fruit from the upper, compared with the lower, canopy (Taylor and Ferre, 1984), or the outer compared with the inner canopy (Ju et al., 1999). Better red coloration in fruit from the outer, compared with the inner, canopy was also found in other studies, without changes in flesh firmness, ethylene or starch index (Awad et al., 2001a,b; Jakopic et al., 2009; Ju, 1998; Ju et al., 1999).

Fruit in the top receive higher light intensity with relative more UV and red light, and relative less fared light than in other positions (Jakopic et al., 2009; Looney, 1968). The better light conditions

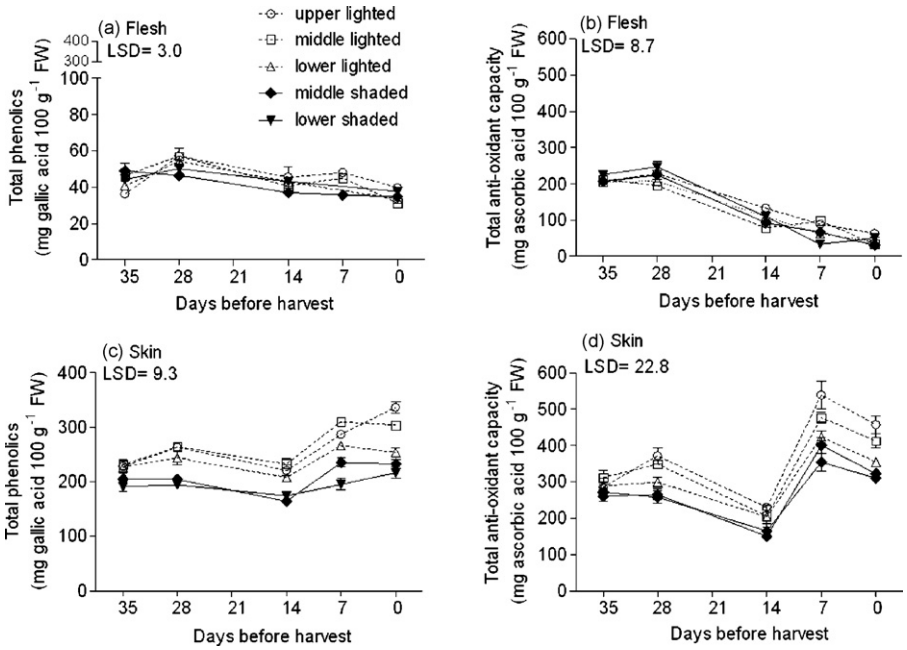

Fig. 1. Mean (±SE) total phenolic content (mg gallic acid 100 g^{-1} fresh weight) (a and c) and total antioxidant capacity (mg ascorbic acid 100 g^{-1} fresh weight) (b and d) in flesh (a and b) and skin (c and d) tissues of Granny Smith apples. Fruit were collected from the upper part of canopy, and from the middle and lower part of the two sides of linear shaped canopy; one being outside the twin-rows and more exposed to the sun (lighted), than the other (shaded). Samplings were made during 0–32 d before commercial harvest.

are known to induce anthocyanin synthesis suggested by increased levels of quercetin glycosides and cyanidin glycosides in the skin of sun exposed apples (Arakawa et al., 1999; Awad et al., 2001a,b; Jakopic et al., 2009; Ju et al., 1996). Similarly, in the present study the better light conditions in the upper, middle-lighted or middle-east parts of canopy has probably induced better red coloration in all cultivars, and greater TPh and AEAC in skin of Granny Smith, Fuji Kiku 8 and Imperial D.R.D.

Nevertheless, there were no important changes in the skin antioxidant contents of Fyriki among different positions in the canopy (Fig. 5), although red coloration was advanced in the sun exposed parts (Fig. 6). Fyriki is a bicolor cultivar and effects of

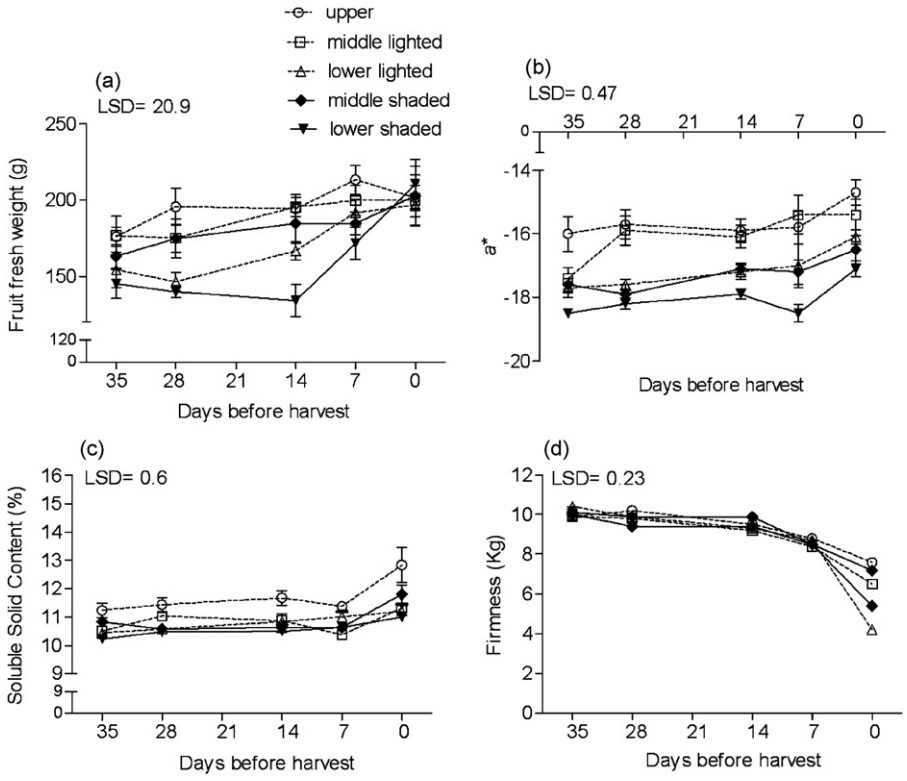

Fig. 2. Mean (±SE) (a) fruit fresh weight (g), (b) color parameter a^*, (c) soluble solid content, and (d) flesh firmness (kg) of Granny Smith apples, in different positions on canopy and sampling dates.

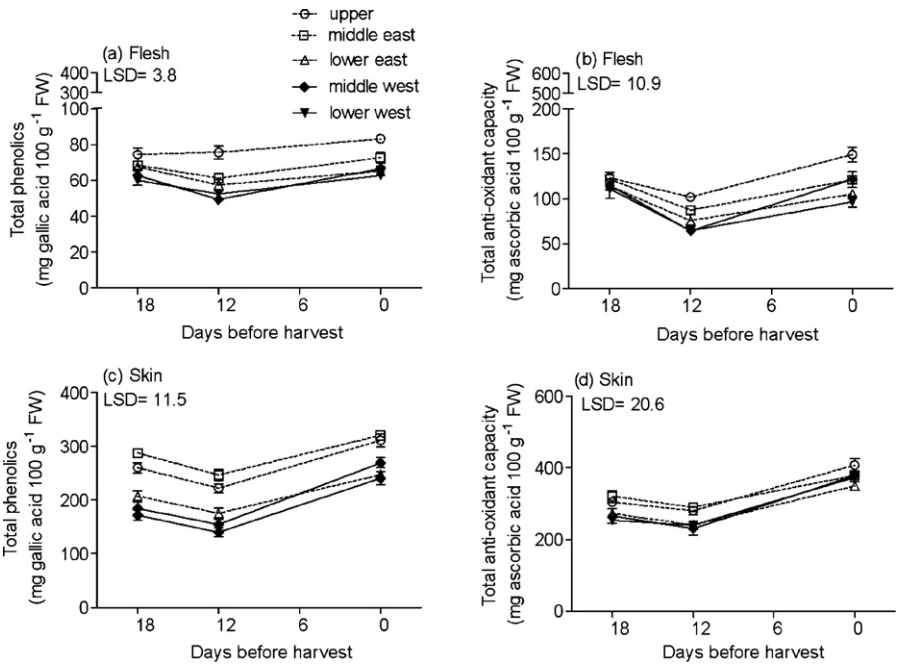

Fig. 3. Mean (±SE) total phenolic content (mg gallic acid 100 g^{-1} fresh weight) (a and c) and total antioxidant capacity (mg ascorbic acid 100 g^{-1} fresh weight) (b and d) in flesh (a and b) and skin (c and d) tissues of Fuji Kiku 8 apples. Fruit were collected from the upper part of canopy, and from the middle and lower part of the two sides of linear shaped canopy, having east (lighted) and west (shaded) directions. Samplings were made during 0–18 d before commercial harvest.

positions on canopy may have been diminished in the present study, because skin tissue samples were taken from all around the equatorial area of fruit, and not from the sun exposed part of fruit. It cannot be excluded that the lack of response of antioxidant contents of Fyriki in the different positions in canopy may be a cultivar characteristic. The fruit response to light has been reported to vary considerably among apple cultivars with some cultivars requiring higher intensity of light than do other cultivars in order to produce the same quantity of anthocyanins (Arakawa, 1988; Reay and Lancaster, 2001).

In flesh tissue, the antioxidant contents were greater in the upper and middle-east and some times in the middle-west parts

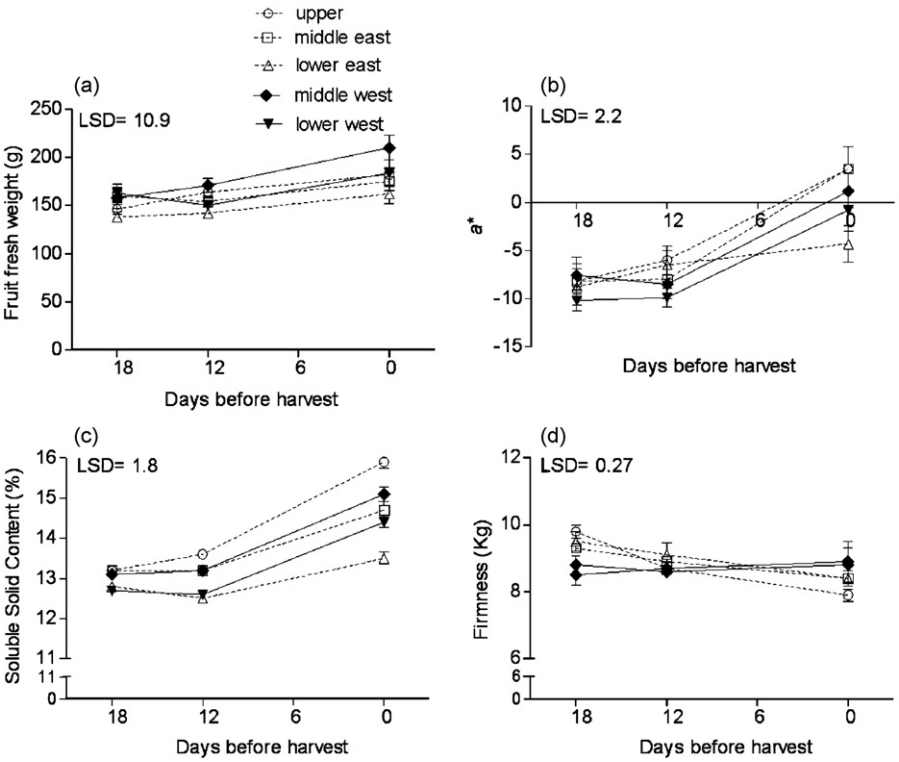

Fig. 4. Mean (±SE) (a) fruit fresh weight (g), (b) color parameter a^*, (c) soluble solid content, and (d) flesh firmness (kg) of Fuji Kiku 8 apples, in different positions on canopy and sampling dates.

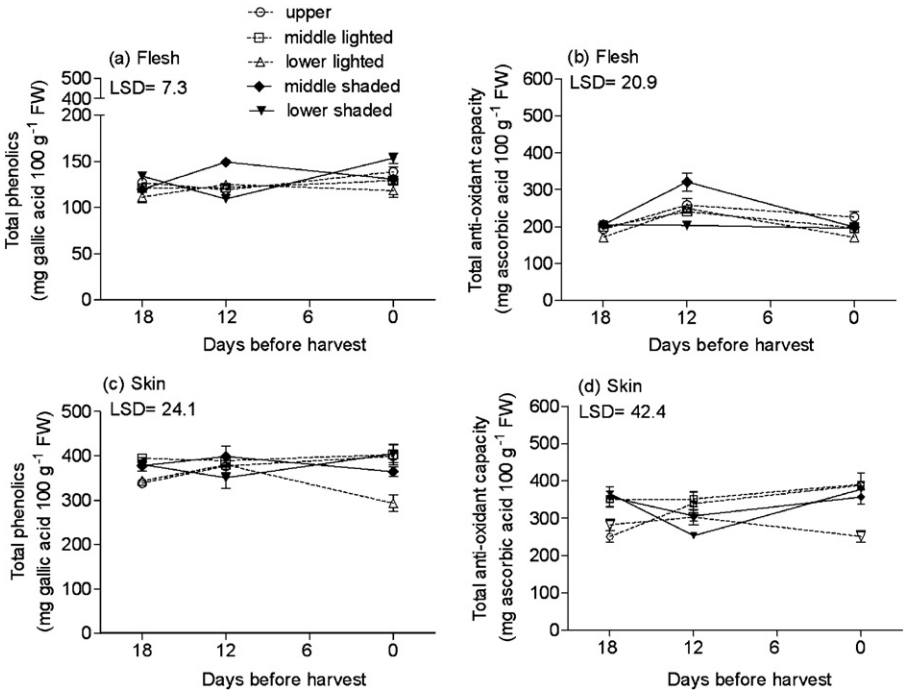

Fig. 5. Mean (±SE) total phenolic content (mg gallic acid 100 g^{-1} fresh weight) (a and c) and total antioxidant capacity (mg ascorbic acid 100 g^{-1} fresh weight) (b and d) in flesh (a and b) and skin (c and d) tissues, of Fyriki apples. Fruit were collected from the upper part of canopy, and from the middle and lower part of the two sides of linear shaped canopy; one being outside the twin-rows and more exposed to the sun (lighted), than the other (shaded). Samplings were made during 0–18 d before commercial harvest.

of canopy in Fuji Kiku 8 (increase up to 44% in TPh, and 58% in AEAC) (Fig. 3), and, in the upper, compared with the rest positions on canopy, in Imperial D.R.D. (increase up to 32% in TPh content, and 9% in AEAC) (Fig. 7). There were no important changes in the TPh and AEAC in the flesh of Granny Smith and Fyriki, among different canopy positions (Figs. 1 and 5). Therefore, fruit from the upper positions in canopy had greater TPh content and AEAC, not only in skin, but also in flesh tissue, in

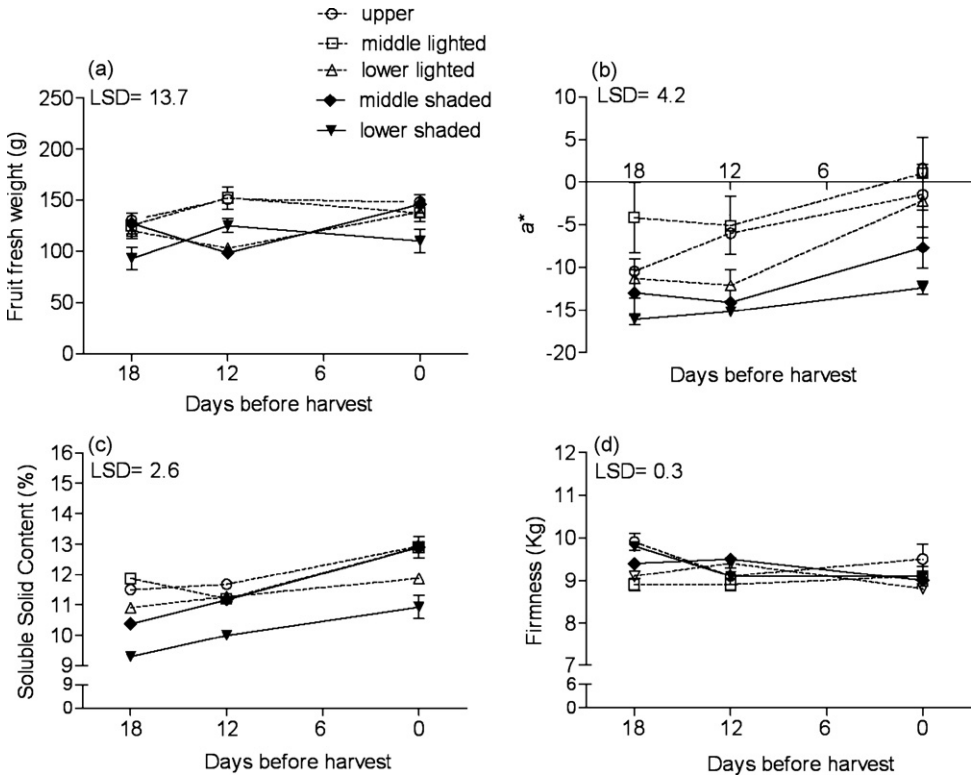

Fig. 6. Mean (±SE) (a) fruit fresh weight (g), (b) color parameter a^*, (c) soluble solid content, and (d) flesh firmness (kg) of Fyriki apples in different positions on canopy and sampling dates.

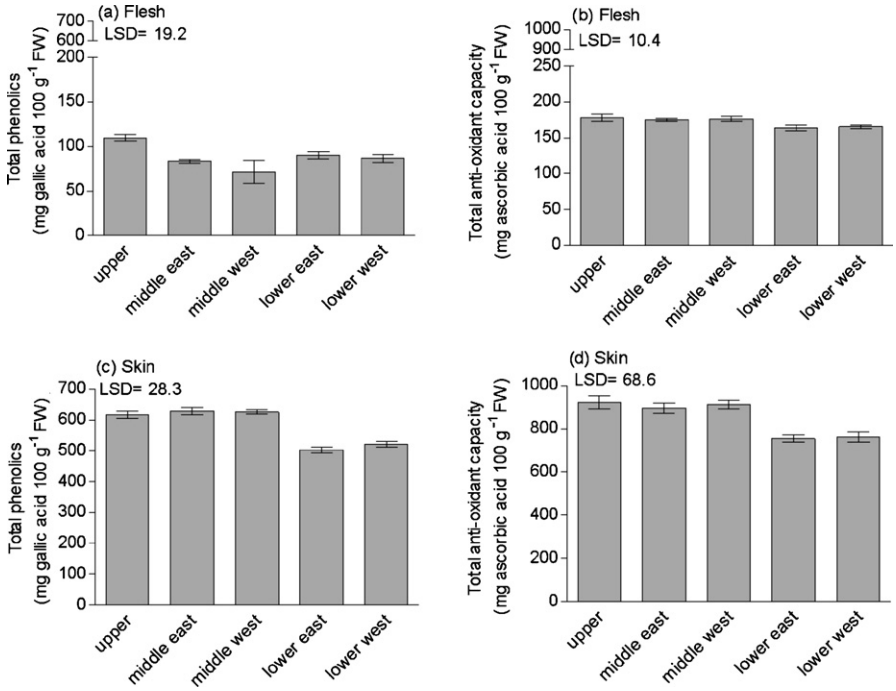

Fig. 7. Mean (±SE) total phenolic content (mg gallic acid 100 g^{-1} fresh weight) (a and c) and total antioxidant capacity (mg ascorbic acid 100 g^{-1} fresh weight) (b and d) in flesh (a and b) and skin (c and d) tissues of Imperial D.R.D. apples. Fruit were collected from the upper, middle-east, middle-west, lower-east and lower-west parts of canopy, at commercial harvest.

Fuji Kiku 8 and Imperial D.R.D., but not in Granny Smith and Fyriki. It is unlikely that the increase in TPh content and AEAC is attributed to greater quercetin 3-glycosides and anthocyanin levels since these metabolites are almost exclusive found in the apple skin (Awad et al., 2000; Mayr et al., 1995), unless in red-fleshed apple genotypes. The apple flesh contain relatively greater levels of chlorogenic acid and epicatechin and procyanidin B1 compared to skin (Awad et al., 2000; Mayr et al., 1995). To our knowledge this is the first report on the variation in antioxidant contents among different positions on canopy in flesh tissue, and future studies need to consider the mechanism behind this light induction pathway.

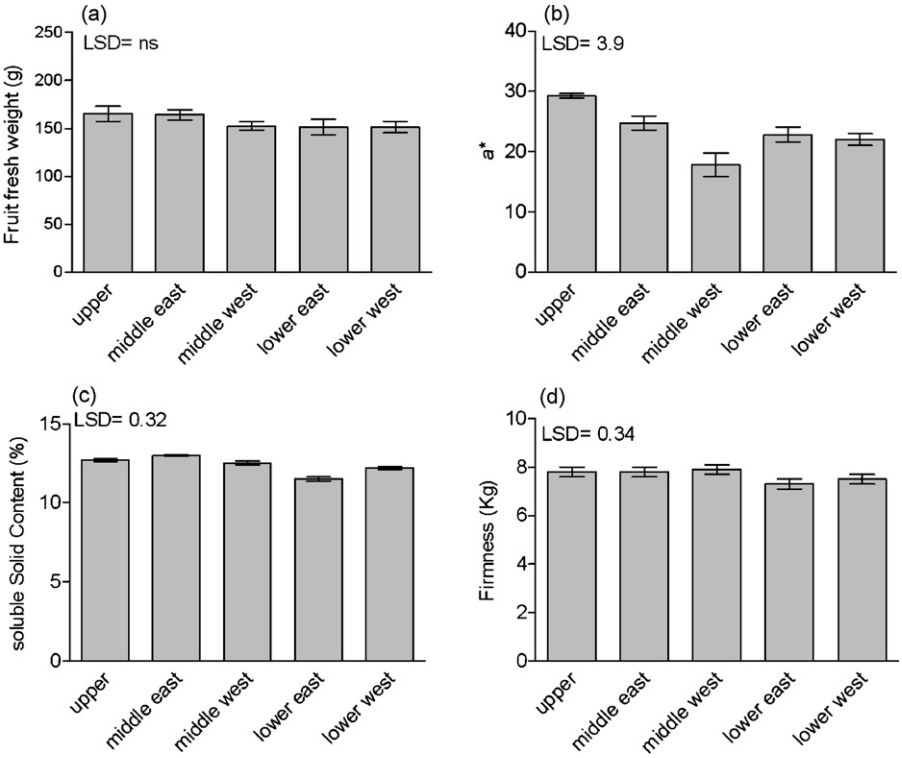

Fig. 8. Mean (±SE) (a) fruit fresh weight (g), (b) color parameter a^*, (c) soluble solid content (%), and (d) flesh firmness (kg) of Imperial D.R.D. apples. Fruit were collected from the upper, middle-east, middle-west, lower-east and lower-west parts of canopy, at commercial harvest.

3.3. Harvest time

The effects of harvest time on fruit physico-chemical and antioxidant properties were studied only in Granny Smith, Fyriki and Fuji Kiku 8, for 35, 18 and 18 d, before commercial harvest, respectively. The harvest dates were chosen to determine the changes in fruit maturity, obtaining early to optimum-picked fruit, since during the last month before apple commercial harvest the onset of ethylene evolution would be expected to occur (Cin et al., 2007).

Fruit fresh weight, SSC, SSC/TA ratio and red coloration in skin tissue increased in Granny Smith and Fuji Kiku 8, during 18–35 d before harvest, which could be the result of natural carbohydrate transfer from leaves to fruit during maturation (Figs. 2 and 4). Flesh firmness decreased during the last two weeks before harvest only in Granny Smith, which has probably resulted by ethylene production during ripening, inducing flesh softening. During the last two weeks before harvest, the TPh content and AEAC increased in the skin of Granny Smith (by 24% and 42%, respectively) and Fuji Kiku 8 (by 19% and 27%, respectively) (Figs. 1 and 3). A similar increase in the flavonoids content was also found in a red colored (Splendour) and to a lesser extent in a green colored (Granny Smith) apple cultivar, during the last weeks before harvest (Lister et al., 1994) and may be attributed to a light regulated anthocyanin biosynthesis in skin during ripening (Faragher and Brohier, 1984).

Kondo et al. (2002), Renard et al. (2007) and Takos et al. (2006) found that accumulation of polyphenolics run antiparallel to the fruit growth, suggesting that the evolution of concentrations during fruit growth and maturation was due to dilution of an initially accumulated store. Similarly, in the present study the TPh content and antioxidant capacity reduced in the flesh of Granny Smith during the last 28 d before harvest (by 27% in TPh and 80% in AEAC), which may be attributed to a dilution effect linked to fruit growth (Fig. 1). The fruit position on canopy did not appear to alter the response of antioxidants during maturation.

There were not important changes in the fruit fresh weight, SSC, flesh firmness and antioxidant contents in the flesh or skin of Fyriki (Figs. 5 and 6). The a^* color parameter increased during the last 3 weeks before harvest, but the increase was to a lesser extent compared with Fuji Kiku 8 (mean increase of 6 and 10 units, respectively). The above minor changes in physico-chemical and antioxidant properties in Fyriki during the last three weeks before harvest may be a cultivar characteristic.

4. Conclusions

The cultivar, fruit position on canopy and harvesting time have a significant role in determining the antioxidant contents in apple fruit. Cultivars Imperial D.R.D. and Fyriki contain relative greater TPh content and AEAC in fruit skin and flesh tissues. The variations among cultivars in the antioxidant contents per serving portion were reduced when calculating the antioxidant contents in unpeeled, compared with peeled, fruit.

Good light conditions are particularly important for producing apples with health-promoting compounds in skin and flesh tissue, although there were cultivar dependent changes.

The natural maturation changes in physico-chemical characteristics, during the last weeks before harvest, were combined with changes in the TPh and AEAC in skin and flesh tissue in Granny Smith and Fuji Kiku 8, but not in Fyriki, and these changes were not influenced by the position of fruit on canopy.

Acknowledgements

This research was supported by a grant of European Union, thought Project "LOWJUICE", Collective Research Project in FP6.

References

Arakawa, O., 1988. Characteristics of color development in some apple cultivars: changes in anthocyanin synthesis during maturation as affected by bagging and light quality. J. Jpn. Soc. Hortic. Sci. 57, 373–380 (Japanese with English summary).
Arakawa, O., Shinoda, M., Hiraga, M., Wang, H., 1999. Comparison of anthocyanin synthesis of true-to-type 'Tsugaru' apple and its red sport strains. J. Hortic. Sci. Biotechnol. 74, 738–742.
Awad, M.A., de Jager, A., van der Plas, L.H.W., van der Krol, A.R., 2001b. Flavonoid and chlorogenic acid changes in skin of 'Elstar' and 'Jonagold' apples during development and ripening. Sci. Hortic. 90, 69–83.
Awad, M.A., de Jager, A., van Westing, L.M., 2000. Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. Sci. Hortic. 83, 249–263.
Awad, M.A., Wagenmakers, P.S., de Jager, A., 2001a. Effects of light on flavonoid and chlorogenic acid levels in the skin of 'Jonagold' apples. Sci. Hortic. 88, 289–298.
Bengoechea, M.L., Sancho, A.I., Bartolome, B., 1997. Phenolic composition of industrially manufactured purees and concentrates from peach and apple fruits. J. Agric. Food Chem. 45, 4071–4075.
Blois, M.S., 1958. Antioxidant determination by the use of stable free radicals. Nature 181, 1199–1200.
Chinnici, F., Bendini, A., Gaiani, A., Riponi, C., 2004. Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. J. Agric. Food Chem. 52, 4684–4689.
Cin, V.D., Danesin, M., Botton, A., Boschetti, A., Dorigoni, A., Ramina, A., 2007. Fruit load and elevation affect ethylene biosynthesis and action in apple fruit (*Malus domestica* L. Borkh.) during development, maturation and ripening. Plant Cell Environ. 30, 1480–1485.
Dong, Y.H., Mitra, D., Kootstra, A., Lister, C., Lancaster, J., 1998. Postharvest stimulation of skin colour in Royal Gala apple. J. Am. Soc. Hortic. Sci. 120, 95–100.
Drogoudi, P.D., Michailidis, Z., Pantelidis, G., 2008. Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. Sci. Hortic. 115, 149–153.
Eberhardt, M.V., Lee, C.Y., Liu, R.H., 2000. Antioxidant activity of fresh apples. Nature 405, 903–904.
Faragher, J.D., Brohier, R.L., 1984. Anthocyanin accumulation in apple skin during ripening: regulation by ethylene and phenylalanine ammonia-lyase. Sci. Hortic. 22, 89–96.
Fuleki, T., Pelayo, E., Palabay, R.B., 1994. Sugar composition of varietal juices produced from fresh and stored apples. J. Agric. Food Chem. 42, 1266–1275.
Hellenic Statistical Authority. http://www.statistics.gr/portal/page/portal/ESYE.
Iglesias, I., Salvia, J., Torguet, L., Cabús, C., 2002. Orchard cooling with overtree microsprinkler irrigation to improve fruit colour and quality of 'Topred Delicious' apples. Sci. Hortic. 93, 39–51.
Jakopic, J., Stampar, F., Veberic, R., 2009. The influence of exposure to light on the phenolic content of 'Fuji' apple. Sci. Hortic. 123, 234–239.
Ju, Z., 1998. Fruit bagging, a useful method for studying anthocyanin synthesis and gene expression in apples. Sci. Hortic. 77, 155–164.
Ju, Z., Duan, Y., Ju, Z., 1999. Effects of covering the orchard floor with reflecting films on pigment accumulation and fruit coloration in 'Fuji' apples. Sci. Hortic. 82, 47–56.
Ju, Z., Yuan, Y., Liu, C., Zhan, S., Wang, M., 1996. Relationships among simple phenol, flavonoid and anthocyanin in apple fruit peel at harvest and scald susceptibility. Postharvest Biol. Technol. 8, 83–93.
Kim, S.H., Lee, J.R., Hong, S.T., Yoo, Y.K., An, G., Kim, S.R., 2003. Molecular cloning and analysis of anthocyanin biosynthesis genes preferentially expressed in apple skin. Plant Sci. 165, 403–413.
Kondo, S., Tsuda, K., Muto, N., Ueda, J., 2002. Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars. Sci. Hortic. 96, 177–185.
Łata, B., 2007. Relationship between apple peel and the whole fruit antioxidant content: year and cultivar variation. J. Agric. Food Chem. 55, 663–671.
Łata, B., Przeradzka, M., Kowska, M.B., 2005. Great differences in antioxidant properties exist between 56 apple cultivars and vegetation seasons. J. Agric. Food Chem. 53, 8970–8978.
Łata, B., Tomala, K., 2007. Apple peel as a contributor to whole fruit quantity of potentially healthful bioactive compounds. Cultivar and year implication. J. Agric. Food Chem. 55, 10795–10802.
Lee, K.W., Kim, Y.J., Kim, D.O., Lee, H.J., Lee, C.Y., 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. J. Agric. Food Chem. 51, 6516–6520.
Lister, C.E., Lacaster, J.E., Sutton, K.H., 1994. Developmental changes in the concentration and composition of flavonoids in skin of a red and a green apple cultivar. J. Sci. Food Agric. 64, 155–161.
Liu, J.-R., Dong, H.-W., Chen, B.-Q., Zhao, P., Liu, R.H., 2009. Fresh apples suppress mammary carcinogenesis and proliferative activity and induce apoptosis in mammary tumors of the Sprague-Dawley rat. J. Agric. Food Chem. 57, 297–304.
Looney, N.E., 1968. Light regimes within standard size apple trees as determined spectrophotometrically. Proc. Am. Soc. Hortic. Sci. 93, 1–6.
Mayr, U., Treutter, D., Santos-Buelga, C., Bauer, H., Feucht, W., 1995. Developmental changes in the phenol concentrations of 'Golden delicious' apple fruits and leaves. Phytochemistry 38, 1151–1155.
Reay, P.F., Lancaster, J.E., 2001. Accumulation of anthocyanins and quercetin glycosides in 'Gala' and 'Royal Gala' apple fruit skin with UV-B–Visible irradiation: modifying effects of fruit maturity, fruit side, and temperature. Sci. Hortic. 90, 57–68.

Renard, C.M.G.C., Dupont, N., Guillermin, P., 2007. Concentrations and characteristics of procyanidins and other phenolics in apples during fruit growth. Phytochemistry 68, 1128–1138.
Siebert, K.J., Troukhanova, N.V., Lynn, P.Y., 1996. Nature of polyphenol–protein interactions. J. Agric. Food Chem. 44, 80–85.
Singleton, V.L., Rossi, J.A., 1965. Calorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagent. Am. J. Enol. Viticult. 16, 144–158.
Stopar, M., Bolcina, U., Vanzo, A., Vrhovsek, U., 2002. Lower crop load for Cv. Jonagold apples (*Malus* × *domestica* Borkh.) increases polyphenol content and fruit quality. J. Agric. Food Chem. 50, 1643–1646.
Sun, J., Liu, R.H., 2008. Apple phytochemical extracts inhibit proliferation of estrogen-dependent and estrogen-independent human breast cancer cells through cell cycle modulation. J. Agric. Food Chem. 56, 11661–11667.
Takos, A.M., Ubi, B.E., Robinson, S.P., Walker, A.R., 2006. Condensed tannin biosynthesis genes are regulated separately from other flavonoid biosynthesis genes in apple fruit skin. Plant Sci. 170, 487–499.
Taylor, B.H., Ferre, D.C., 1984. The influence of summer pruning and cropping on growth and fruiting of apple. J. Am. Soc. Hortic. Sci. 109, 19–24.
Tsao, R., Yang, R., Christopher, J., Zhu, Y., Zhu, H.H., 2003. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). J. Agric. Food Chem. 51, 6347–6353.
van der Sluis, A.A., Dekker, M., de Jager, A., Jongen, W.M., 2001. Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. J. Agric. Food Chem. 49, 3606–3613.
Veberic, R., Trobec, M., Herbinger, K., Hofer, M., Grill, D., Stampar, F., 2005. Phenolic compounds in some apple (*Malus domestica* Borkh.) cultivars of organic and integrated production. J. Sci. Food Agric. 85, 1687–1694.
Vieira, F.G., Borges, G.D., Copetti, C., Amboni, R.D., Denardi, F., Fett, R., 2009. Physico-chemical and antioxidant properties of six apple cultivars (*Malus domestica* Borkh.) grown in southern Brazil. Sci. Hortic. 122, 421–425.
Wang, H., Cao, G., Prior, R., 1997. Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem. 45, 304–309.
Wojdyło, A., Oszmiański, J., Laskowski, P., 2008. Polyphenolic compounds and antioxidant activity of new and old apple varieties. J. Agric. Food Chem. 56, 6520–6530.
Wolfe, K., Wu, X., Liu, R.H., 2003. Antioxidant activity of apple peels. J. Agric. Food Chem. 51, 609–614.