WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.10 (2): 207-216. 2012
www.world-food.net

Concentrations of total phenols and antioxidant activity in apple do not differ between conventional and organic orchard management

José Antonio Yuri [1]*, Francisco J. Maldonado [1], Iván Razmilic [2], Amalia Neira [1], Álvaro Quilodran [1] and Iván Palomo [3]

[1] *Pomaceas Center, Agricultural Sciences Faculty,* [2] *Aromatic Plants Laboratory, Chemistry of Natural Resources Institute, Talca University,* [3] *Clinical Biochemistry and Immunohaematology Department, Health Sciences Faculty, Talca University, P.O. Box 747, Talca, Chile, P.O. Box 747 Talca, Chile. e-mail: ayuri@utalca.cl, aneira@utalca.cl, alquilodran@utalca.cl*

Received 10 January 2012, accepted 4 May 2012.

Abstract

The apple is one of the most widely consumed fresh fruits in the world. It constitutes a major contribution of phytochemical compounds to the diet, which are associated with a reduced risk to develop degenerative diseases. The main objective of this study was to evaluate the effects of conventional and organic management of apple cultivation, the stage of development and sunburn damage on polyphenol concentrations, antioxidant activity and pigments in three apple cultivars. Two experiments were carried out during the 2009/2010 season to study (1) the effect of the type of management and the development stage of the fruit during the season on the concentration and content of total and specific phenolics, antioxidant activity in the whole fruit, and pigments (chlorophylls, carotenoids and anthocyanins) in the peel of cvs. Gala (Galaxy and Brookfield), Granny Smith and Fuji (Raku Raku and Stripped) and (2) the effect of the type of management and the presence of sunburn at harvest on phenolics concentrations and antioxidant activity in both the whole fruit and peel, as well as pigments in peel, in two cultivars. Phenolics concentrations and antioxidant activity increased in the first weeks of fruit development and then decreased until harvest. The concentration of chlorophyll and carotenoids tended to decrease throughout the season, while anthocyanin concentration increased. In the case of tissue damaged by sunburn, phenolics concentrations and antioxidant activity were higher in damaged fruit, while changes in pigment concentrations varied according to the cultivar. The practices of conventional and organic management did neither influence significantly phenolics and pigments concentrations and antioxidant activity, except at certain stages of fruit development.

Key words: Apples, organic orchard, phenolics, antioxidant activity, pigments, sunburn damage.

Introduction

A diet rich in fruits and vegetables is associated with decreased risk of developing degenerative diseases (e.g. cardiovascular diseases and cancer) [1-5]. These beneficial effects for health are due to the presence of bioactive compounds, such as pigments (e.g. carotenoids, chlorophylls and anthocyanins) [6, 7, 9], vitamins (e.g. ascorbic acid) [5] and polyphenols (e.g. flavonoids) [8, 10, 11]. In this context, apples represent a contribution to public health owing to their high content of polyphenols and common consumption of apples as a fresh and processed product [12].

The levels of phenolic compounds and some other pigments can vary in apples according to the cultivar [15], season [19], agroclimatic region [17], management practices (conventional and organic) [18, 19], stage of fruit development [16], tissue type (e.g. peel or flesh) [17], conditions of biotic stress (e.g. attacks by pathogens) and abiotic stress (e.g. excess UV radiation, nutritional deficiencies) [13, 14]. In this context, this study evaluated the effect of conventional and organic management, the development stage and sunburn damage on the concentrations of phenolics and pigments and antioxidant activity in different apple cultivars.

Materials and Methods

***Plant material**:* During the 2009/10 season apple cultivars (cvs). Gala (Galaxy and Brookfield) and Fuji (Raku Raku and Striped), both bi-colored, and Granny Smith, a green cultivar, were collected from conventional (CO) and organic (OO) orchards located in Chimbarongo, O'Higgins Region, Chile (34°40'S, 71°1'W; 314 m above sea level). The proximity between the conventional and organic orchards was 10 km.

Experiment 1. Effect of the type of management and development stage on different apple cultivars

a) Determination of total and specific phenols and antioxidant activity in the whole fruit: Healthy fruit of cvs. Gala, Granny Smith and Fuji were collected at different stages of development (25, 32, 39, 52 and 88 days after full bloom - DAFB) and commercial harvest (128, 159,191 DAFB for each cultivar), from the conventional and organic orchards.

b) Determination of pigments in peel: Peel was collected from 25 DAFB until commercial harvest from cvs. Gala, Granny Smith and Fuji, from the same orchards.

Experiment 2. Effect of the type of management on fruit with and without sunburn among different cultivars at harvest

a) Determination of total phenolics and antioxidant activity in the whole fruit: Fruit of the cvs. Granny Smith and Fuji with (damaged) and without (healthy) sunburn were collected during a commercial harvest from the orchards mentioned in Experiment 1.

b) Determination of total and specific phenols, antioxidant activity and pigments in the peel: Healthy and damaged peels of the cvs. Granny Smith and Fuji were collected at the commercial harvest from the orchards described above.

Tissue extraction: Sixteen fruits for each treatment group, with four replications for each treatment, were selected randomly. The apples were cut into lengthwise slices and the seeds and core were removed. Each sample consisted of 1 g of the whole fruit (peel and flesh) per replication. The samples were immediately frozen with liquid nitrogen, pulverized in a mortar and pestle, and extracted by using the procedure described by Coseteng *et al*. [20] with modifications. Briefly, the tissue was extracted twice with a solution of 80% ethanol (ethanol:water 80:20, v/v) for 10 and 5 min at 100°C and then filtered. Samples were adjusted to 10 ml with 80% ethanol and kept at -20°C until use.

In the case of healthy and sunburn-damaged fruit, 2 g of whole fruit and 1 g of peel were used. The experimental design and procedure applied was the same as that described above.

Determination of pigment concentration

Chlorophyll and carotenoids: Chlorophyll (Chl) and carotenoids (Car) concentrations were determined by the method described by Lichtenthaler [21] with modifications. Two disks of peel (62 mg) were extracted with 1 ml of acetone (acetone: water 80:20, v/v) for 24 h at 4°C in darkness. Then, they were centrifuged at 200 g for three min and the disks were re-extracted with the same procedure described above. Both supernatants were mixed and absorbance read at 663.3, 646.8 and 420.0 nm with a spectrophotometer (Spectronic 1201, Milton Roy Co., Rochester, NY). The results were expressed in µg of Chl or Car cm^{-2} of fresh weight (FW) and calculations were carried out according to the following formulas:

Total chlorophyll (Chl T) = $7.15\ A_{663.2} + 18.71\ A_{646.8}$

Chlorophyll *a* (Chl *a*) = $12.25\ A_{663.2} - 2.79\ A_{646.8}$

Chlorophyll *b* (Chl *b*) = $21.50\ A_{646.8} - 5.10 A_{663.2}$

Carotenoids (Car) = $[1000\ A_{470} - 1.82\ (Ca) - 85.02\ (Cb)]\ 198^{-1}$

Anthocyanins: The concentration of anthocyanins (Ant) was determined according to the procedure of Fuleki *et al*. [22] with modification. Two disks of peel (62 mg) were treated with 0.5 ml of a mixture of 95% ethanol and 1.5 N HCl (15:85 in proportion to the volume) at 4°C in darkness for 1 day. The sample was then centrifuged at 200 g for three min and the supernatant was placed in a new microtube. The sediment was washed with a volume of 0.5 m HCl: ethanol for another day and then centrifuged for three min. The supernatants were mixed and absorbance was read at 533 nm. The results were expressed in µg Ant cm^{-2} FW.

Determination of total phenolics concentration: Total phenolics were determined by the Folin-Ciocalteu method. Briefly, 0.1 ml of extract was mixed with 0.5 ml of the Folin-Ciocalteu phenol reagent (Merck, Darmstadt, Germany). The mixture was incubated for 5 min and then 0.5 ml of sodium carbonate (Na_2CO_3; 10%, w/v) was added and incubated for 15 min at room temperature. Absorbance was measured at 640 nm with the spectrophotometer. Total phenolic concentrations in the peel and whole fruit were expressed as mg of chlorogenic acid equivalents (CAE) g^{-1} FW.

Determination of antioxidant activity: The capture of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH; Fluka Chemie, Buchs, Switzerland) was measured by the method described by Von Gadow *et al.*[23], with modifications. Briefly, 0.1 ml extracts were mixed with 2 ml of 8×10^{-5} M DPPH solution, and incubated for eight min at room temperature and the absorbance measured at 515 nm with the spectrophotometer. Ethanol was used as zero in the spectrophotometer. Chlorogenic acid in different concentrations was used as a standard and the capture of the DPPH free radicals was expressed as mg of chlorogenic acid equivalents (CAE) g^{-1} FW.

Total phenolic content and antioxidant activity of whole fruit: Total phenols and antioxidant activity of the whole fruit were calculated with the following formulas:

Total phenolic content (TPCT) = total phenolic concentration (TPCC) fresh weight (FW^{-1}) from whole fruit.

Antioxidant activity of whole fruit (AOAF) = antioxidant activity of extract (AOAE) fresh weight from whole fruit.

The content of total phenolics and antioxidant activity in the whole fruit is expressed in mg CAE per fruit.

Determination of specific phenolics by HPLC: Specific phenolics (chlorogenic acid, catechin, epicatechin, procyanidin B2, quercetins glycosides and phloridzin) in the samples were determined using a HPLC-DAD Merck Hitachi (LaChrom, Tokyo, Japan), equipment consisting of a LaChrom L-7100 pump and a diode array detector, L – 7455 LaChrom, and a 100-5 C18 Kromasil column of 259 mm x 4.6 mm with a pre-column of the same characteristics, maintained at 20°C. Briefly, 0.02 ml previously filtered (0.45 µm filter) extracts were injected. To identify the compounds, different standards of specific phenolics were used with the UV-VIS spectra. The chromatogram was monitored at 256 nm. The solvents of the mobile phase were: A: 1% formic acid in H_2O quality HPLC; B: 40% acetonitrile in H_2O, and C: acetonitrile. The elution parameters were: time 0-10 min: A (70), B (30), C (0) flow 1ml min^{-1}; time 45 min: A (25), B (75), C (0) flow 0.5 ml min^{-1}; time 52 min: A (0), B (0), C (100) flow 1 ml min^{-1}; and time 55 min: A (70), B (30), C (0) flow 1 ml min^{-1}. The results were expressed in µg of samples in g of FW^{-1}.

Statistical analysis: Conventional and organic management were compared in Experiment 1 for each cultivar (Gala, Granny Smith and Fuji) independently. Experiment 2 separately compared healthy and sun damaged tissue and types of management. Both assays were carried out in a completely random design. The software SPSS v15.0 (SPSS Inc., Chicago, Illinois) was used for variance analysis and separation. Tukey's HSD test was used to compare treatments when the ANOVA was significant ($p \leq 0.05$). Correlations were assessed between total phenols and antioxidant activity for all the cultivars and both types of management.

Results

The results are presented according to the sequence in which they were indicated in the assays.

Experiment 1a: The evolution of the concentration and content of total phenolics for all the studied cultivars and both types of management showed similar tendencies throughout the season (Fig.1 A, C and E). The concentration of total phenolics at 25 DAFB started at about around 8 mg CAE g^{-1} FW, rose to 13-15 mg CAE g^{-1} FW at 32 DAFB, and then decreased progressively until harvest, with values of 1.5-3.5 mg CAE g^{-1} FW. Comparing the types of management, higher concentrations of total phenolics were found in organic fruit at 52 and 88 DAFB in cv. Gala ($p = 0.02$), and at 25, 32 and 52 DAFB in Granny Smith ($p = 0.04$). In the case of cv. Fuji, at 25 DAFB the concentration was higher in organic fruit ($p = 0.004$), while at 88 DAFB it was higher in conventional fruit ($p \leq 0.01$). Phenolic content increased progressively from 25 DAFB until harvest (Fig. 1 A, C and E). Comparing the type of management, higher phenol content was found in organic fruits at 39 DAFB in cvs. Granny Smith and Fuji, and at 88 DAFB in Gala ($p = 0.04$).

The tendency for antioxidant activity was similar in all the cultivars and for both types of management, increasing slightly during the first stages of development (25-39 DAFB) and then decreasing until harvest (Fig. 1 B, D and F). Comparing the management types, we found that at 25 DAFB, the antioxidant activity of the extracts of organic fruits was higher than that of the conventionally extracts in all cultivars ($p \leq 0.01$), while at the later

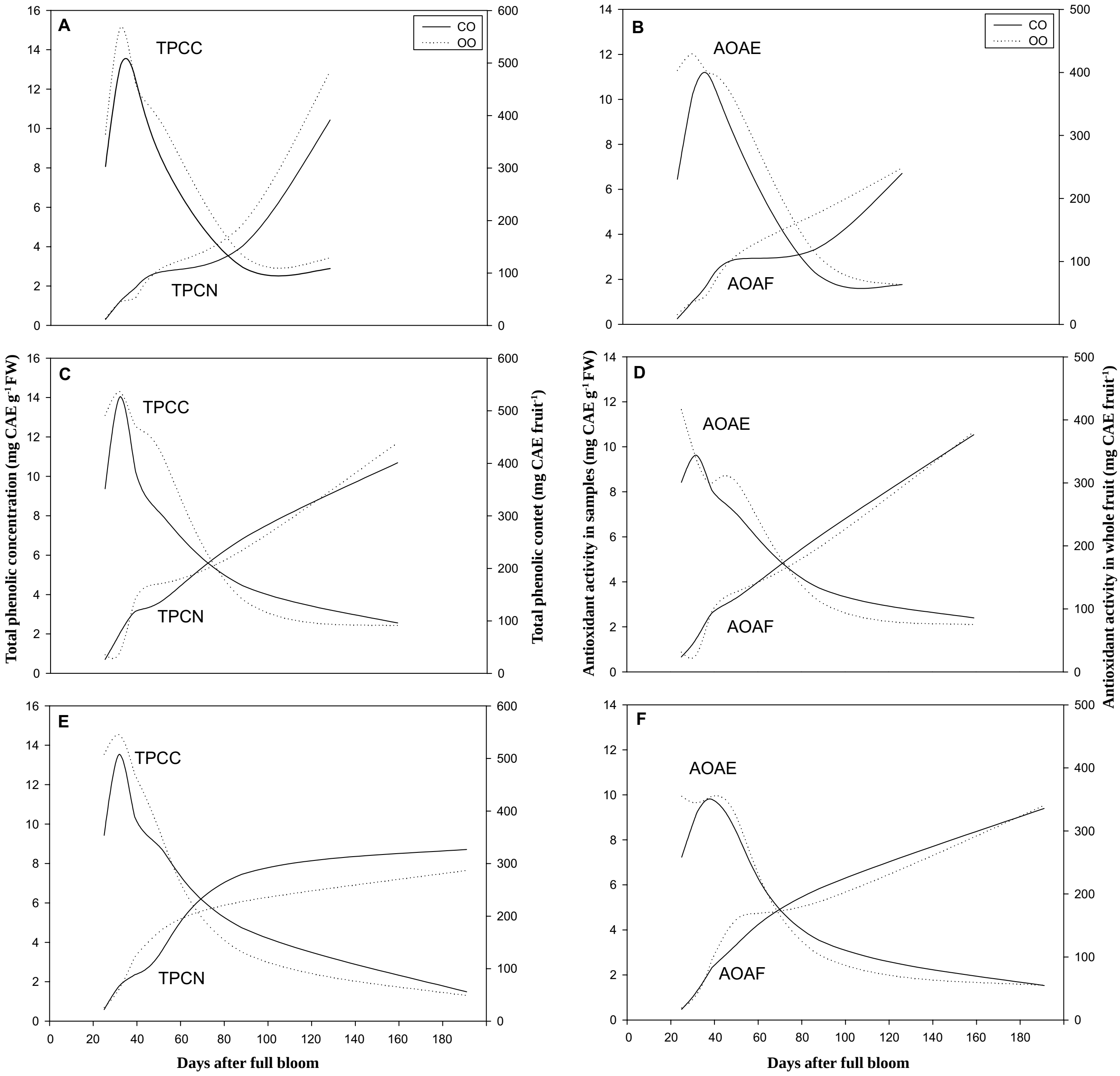

Figure 1. Evolution of total phenolic concentration, total phenolic content, antioxidant activity in extracts and antioxidant activity in whole fruit from apples cvs. Gala (A and B), Granny Smith (C and D) and Fuji (E and F), from conventional and organic orchards, in different stages of development, during the 2009/2010 season. Curves end at harvest. TPCC: total phenolic concentration; TPCN: total phenolic content; AOAS: antioxidant activity of extract; AOAE: antioxidant activity of whole fruit.

dates differences were only observed at 39 and 88 DAFB the cv. Gala, in which the levels were higher in the organically grown fruit (p = 0.03). The antioxidant activity of the whole fruit increased throughout the season (Fig. 1 B, D and F). Comparing the two orchards, the fruit produced organically presented higher levels of antioxidant activity than the conventionally produced fruit at 25 DAFB in cv. Gala, and at 39 and 52 DAFB in cv. Fuji (p = 0.03). Considering the different stages of the development of the fruit, cultivars and types of management, a good correlation was found between total phenolics concentration and the antioxidant activity of the extract: in fruit with conventional management the correlation (*r*) was 0.95 (Gala), 0.97 (Granny Smith) and 0.95 (Fuji), and in the case of organic management, the correlations were 0.96, 0.92 and 0.95 for the same cultivars, respectively.

Throughout the season the specific phenolics that showed the highest concentrations were chlorogenic acid, phloridzin and procyanidin B2, varying according to the cultivar and the stage of develop of the fruit (Table 1). It is interesting to note that concentrations of chlorogenic acid, procyanidin B2 and quercetins glycosides presented similar tendencies to those observed for total phenolics; their concentrations increased until approximately 32-39 DAFB and then decreased until harvest. Phloridzin in contrast, decreased progressively from 25 DAFB until harvest. With respect to the type of management, differences were only

Table 1. Specific phenolic concentrations (Ca, chlorogenic acid; Cat, catechin; Epi-cat, epicatechin; Pro-B2, procyanidin B2; Que-gly, quercetins glycosides; Phl, phloridzin) in whole fruits from conventional and organic orchards in differents stages of development during the 2009/2010 season.

Cultivars	DAFB	Management	Specific phenolic (μg*g⁻¹ FW)					
			Ca	Cat	Epi-Cat	Pro-B2	Que-gly	Phl
Gala	25	CO	1393	90	481	845	192	2319
		OO	1732	140	165	1078	263	2129
		Significance	n.s	n.s	*	n.s	n.s	n.s
	32	CO	2545	85	376	1460	322	1217
		OO	3017	78	289	1386	436	1521
		Significance	n.s	n.s	*	n.s	*	n.s
	52	CO	1529	55	1200	1456	268	284
		OO	2019	141	1556	1493	353	426
		Significance	n.s	**	n.s	n.s	n.s	*
	88	CO	376	36	39	583	98	49
		OO	400	21	60	450	153	53
		Significance	n.s	n.s	n.s	*	**	n.s
	Harvest	CO	209	43	153	77	201	12
		OO	321	38	266	144	244	23
		Significance	**	n.s	n.s	**	n.s	*
Granny Smith	25	CO	1315	45	339	703	197	1336
		OO	1796	48	183	473	286	2685
		Significance	n.s	n.s	n.s	n.s	n.s	**
	32	CO	1404	106	845	809	483	923
		OO	1029	97	713	717	417	936
		Significance	*	n.s	n.s	n.s	n.s	n.s
	52	CO	1091	57	474	1217	287	264
		OO	1104	53	423	1421	347	433
		Significance	n.s	n.s	n.s	n.s	n.s	n.s
	88	CO	202	30	115	325	133	40
		OO	197	26	136	260	148	36
		Significance	n.s	n.s	n.s	n.s	n.s	n.s
	Harvest	CO	62	36	64	188	107	12
		OO	47	33	49	168	98	9
		Significance	n.s	n.s	n.s	n.s	n.s	n.s
Fuji	25	CO	1988	91	276	973	183	2306
		OO	2864	109	338	1242	198	4419
		Significance	*	n.s	n.s	n.s	n.s	**
	32	CO	2939	74	410	1551	334	1633
		OO	3014	68	514	836	374	1674
		Significance	n.s	n.s	*	**	n.s	n.s
	52	CO	1996	69	452	1989	228	425
		OO	2061	65	360	1675	301	424
		Significance	n.s	n.s	n.s	n.s	**	n.s
	88	CO	513	35	155	1732	132	76
		OO	517	45	148	524	180	57
		Significance	n.s	n.s	n.s	**	n.s	n.s
	Harvest	CO	233	13	29	232	61	11
		OO	205	20	23	242	54	12
		Significance	n.s	*	n.s	n.s	n.s	n.s

Statistically significant differences between whole fruit from CO and OO at $p \leq 0.05$ (Tukey's test) are expressed with *. * $p \leq 0.05$; ** $p \leq 0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.; DAFB: days after full bloom.

observed in the concentrations of specific phenols in some stages of development of the fruit (Table 1).

Experiment 1 b: Evaluated the concentration of various pigments of the different apple cultivars, according to the management system and stage of development of the fruit.

The concentration of chlorophylls (total, *a* and *b*) in the peels of all studied cultivars and with both management systems decreased during the development of the fruit, with the decrease being most pronounced in cvs. Gala and Fuji, from maximum values of about 12 µg cm^{-2} FW to minimum values close to 2 µg cm^{-2} FW (Fig. 2). Comparing the two management systems, it was observed that the peel from organically grown fruit presented the highest concentrations of chlorophyll in all three cultivars in the majority of the development stages, although not always at statistically significant levels (Fig. 2). The Chl *a*/*b* ratio remained practically constant, with values that ranged between 2 and 3 until 88 DAFB, and then decreased until harvest.

As with the chlorophylls, carotenoids decreased as the fruit developed (Fig. 3). Comparing carotenoid concentration according to the type of management it was observed that the peel of organically grown fruit of all cultivars had higher concentrations, except in some growth phases (Fig. 3). The Car/Chl ratio ranged between 0.2 and 0.4, the lowest level being with cv. Granny Smith. Anthocyanin concentrations increased in cvs. Gala and Fuji from 25 to 32 DAFB, decreased until 52-88 DAFB and then increased until harvest (Fig. 4). In cv. Granny Smith, an increase in anthocyanin concentrations was observed only in the first stages of development (25-32 DAFB). In general, the concentration profiles described above were higher in organic fruit (Fig. 4).

Experiment 2 a: Determined the concentrations of total phenolics and antioxidant activity in the whole fruit at harvest in fruit with and without sun damage, in conventionally and organically grown fruit of cvs. Granny Smith and Fuji.

Phenolics concentrations were higher in damaged than in healthy fruit in both cultivars and management systems (Fig. 5 A). Differences in phenolics concentrations between management systems were only found in damaged tissue, where conventionally grown Granny Smith apples had higher concentrations than those obtained by organic production ($p = 0.03$). With respect to antioxidant activity, fruit with sun damage presented higher levels of activity than healthy fruit in both cultivars (Fig. 5 B). Differences in antioxidant activity according to the management system were only observed in damaged fruit, in which conventionally grown Granny Smith had a higher level of antioxidant activity than organic fruit ($p \leq 0.01$).

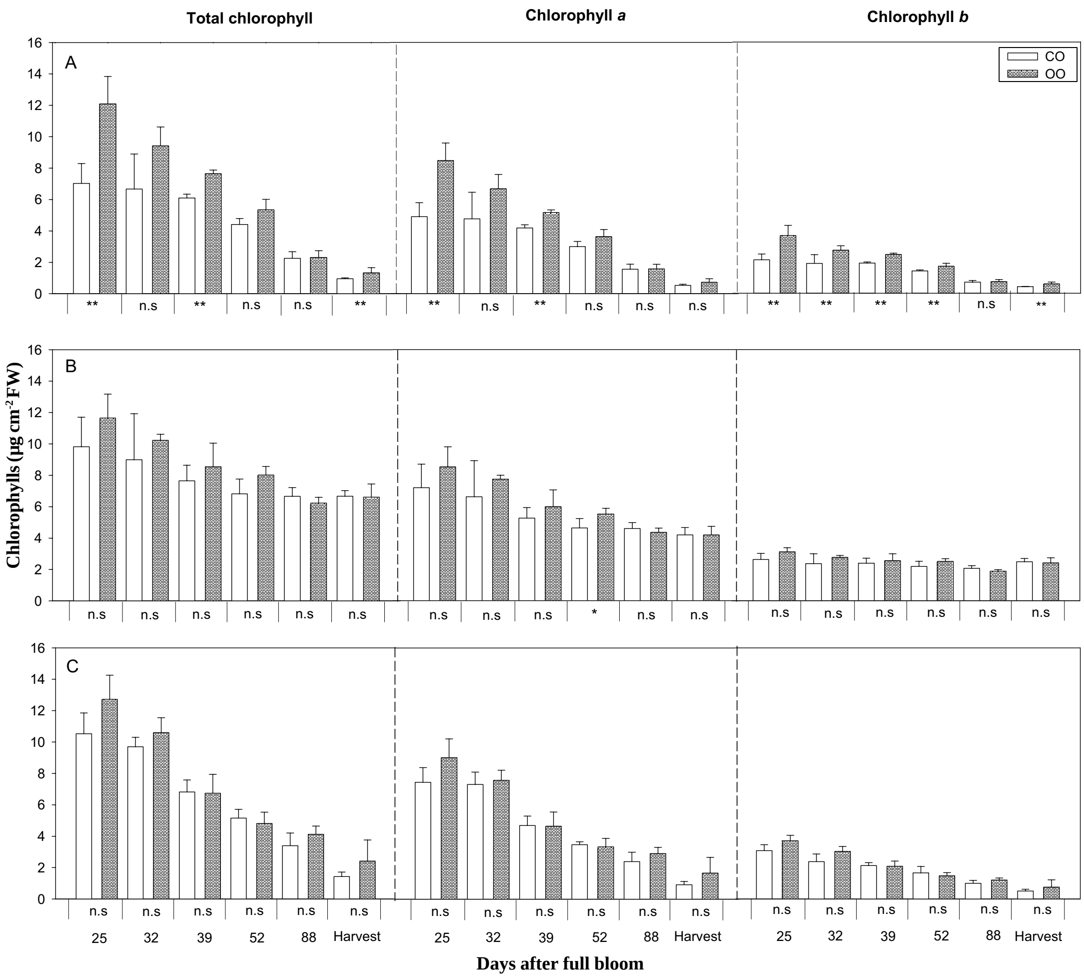

Figure 2. Chlorophylls (total, *a* and *b*) concentrations in apple peel from Gala (A), Granny Smith (B) and Fuji (C) from conventional and organic orchards in different stages of development during the 2009/2010 season. Statistically significant differences between peel from conventional and organic orchards at $p \leq 0.05$ (Tukey's test) are expressed with *. Significance: * $p \leq 0.05$; ** $p \leq 0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.

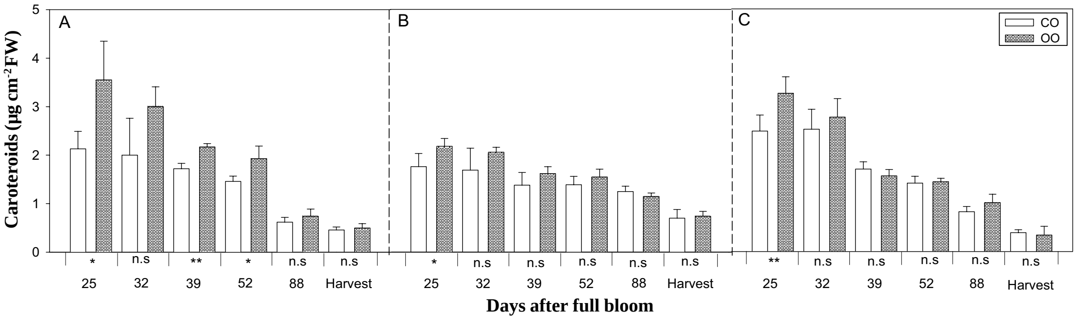

Figure 3. Carotenoids concentrations in apple peel from Gala (A), Granny Smith (B) and Fuji (C) from conventional and organic orchards in different stages of development during the 2009/2010 season. Statistically significant differences between peel from conventional and organic orchards at $p≤0.05$ (Tukey's test) are expressed with *. Significance: * $p≤0.05$; ** $p≤0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.

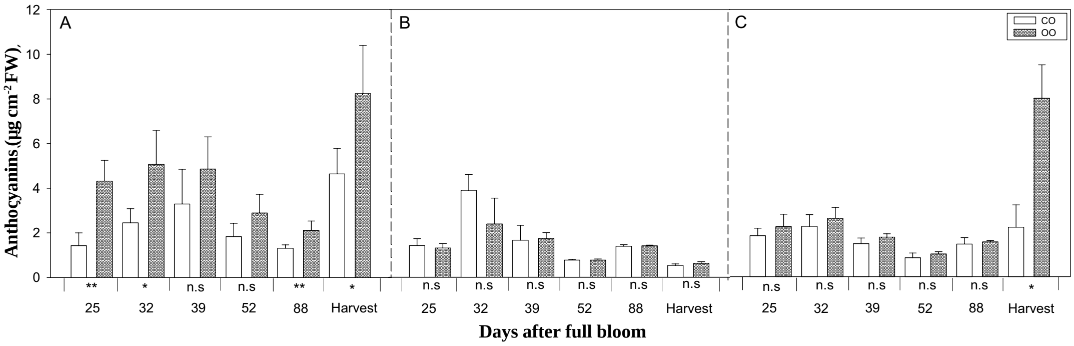

Figure 4. Anthocyanins concentrations in apple peel from Gala (A), Granny Smith (B) and Fuji (C) from conventional and organic orchards in different stages of development during the 2009/2010 season. Statistically significant differences between peel from conventional and organic orchards at $p≤0.05$ (Tukey's test) are expressed with *. Significance: * $p≤0.05$; ** $p≤0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.

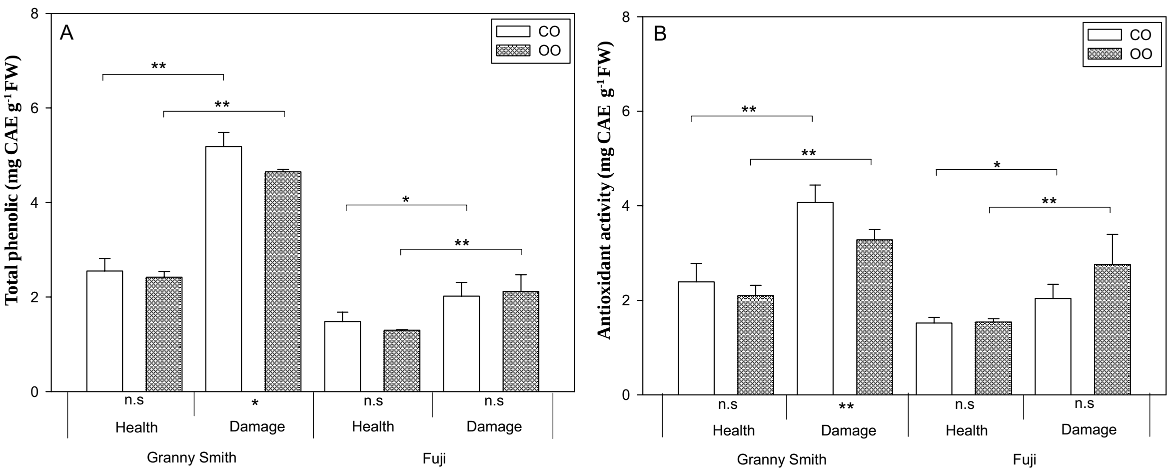

Figure 5. Total phenolic concentrations (A) and antioxidant activity (B), in whole fruit from different apples cultivars (Granny Smith and Fuji) from conventional and organic orchards in fruit with (damaged) and without (healthy) sunburn during the 2009/2010 season. Statistically significant differences between whole fruit from conventional and organic orchards at $p≤0.05$ (Tukey's test) are expressed with *. Significance: * $p≤0.05$; ** $p≤ 0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.

Experiment 2b: This assay determined the concentration of total and specific phenolics, antioxidant activity and pigments in peels at harvest, in healthy and damaged fruit of Granny Smith and Fuji, with conventional and organic management.

In both cultivars and management systems under study, the concentration of total phenolics in damaged peel was 2 to 3 times higher than in healthy peel (Fig. 6 A). No differences in phenolics concentrations between management practices could be found. With respect to antioxidant activity, as with phenolics concentrations, damaged peel showed higher levels than healthy peel (Fig. 6 B), while no differences were found between management systems.

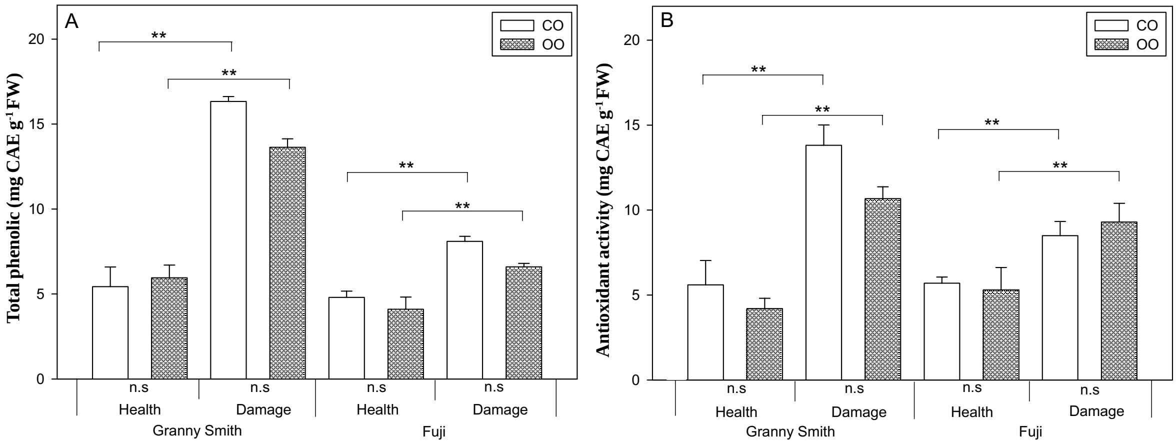

Figure 6. Total phenolics concentrations (A) and antioxidant activity (B), in apple peel from cvs. Granny Smith and Fuji, from conventional and organic orchard, with (damaged) and without (healthy) sunburn during the 2009/2010 season. Statistically significant differences between peel from conventional and organic orchards at $p \leq 0.05$ (Tukey's test) are expressed with *. Significance: * $p \leq 0.05$; ** $p \leq 0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.

At harvest concentration of most specific phenols was higher in sunburn than in healthy peel in all cultivars (Table 2). Differences between management systems were found only in damaged peel, in which the concentrations of procyanidin B2 was higher in Granny Smith and Fuji that came from conventional orchards (p = 0.03), while the concentration of chlorogenic acid was higher in organically grown Fuji ($p \leq 0.01$).

Comparing chlorophyll concentrations (total, *a* and *b*) in healthy and damaged peel, differences were only observed in Granny Smith, where healthy tissue presented a higher concentration (p = 0.001). No differences were found between the management practices (Fig 7). Damaged tissue showed a decrease of 65% in Chl *a* and 59% in Chl *b* in cv. Granny Smith, while in Fuji the Chl *a* and *b* levels decreased by 49 and 31%, respectively. However, the Chl *a*/*b* ratio ranged between 1.5 and 2.5 but, did not vary significantly between healthy and damaged fruit.

Differences in carotenoid concentrations were observed in both cultivars. In the case of cv. Granny Smith, the levels of carotenoids decreased in damaged fruit in relation to healthy tissue. However, statistically significant differences were found only in the peel of organically produced fruit, where the concentration in healthy tissue (0.74±0.1 µg cm^{-2} FW) was higher than that of damaged tissue (0.51±0.06 µg cm^{-2} FW) (p = 0.008). The carotenoid concentration in damaged peel of cv. Fuji was twice as high as in healthy peel, in both conventional (0.64±0.1 µg cm^{-2} FW) and organic (0.78±0.1 µg cm^{-2} FW) fruit (p = 0.008). On the other hand, no differences in carotenoid concentration were found according to the type of management.

In the evaluation of anthocyanins, differences were only observed in the cv. Fuji, in which the concentration in healthy tissue was 2.7 times higher than in damaged tissue, and 3.6 times higher in organically produced fruit than in conventional ($p \leq 0.01$; Fig. 8).

Table 2. Specific phenolics concentrations (Ca, chlorogenic acid; Cat, catechin; Epi-cat, epicatechin; Pro-B2, procyanidin B2; Que-gly, quercetins glycosides; Phl, phloridzin;) in apple peel with and without sunburn, from cvs. Granny Smith and Fuji, from conventional and organic orchards during the 2009/2010 season.

Cultivars	Management		Specific phenolic (µg*g^{-1} FW)					
			Ca	Cat	Epi-Cat	Pro-B2	Que-gly	Phl
Granny Smith	Conventional	Health	79	56	107	334	216	16
		Damage	171	421	84	523	6004	49
		Significance	*	**	n.s	**	**	**
	Organic	Health	79	53	83	481	229	16
		Damage	118	356	45	271	5539	44
		Significance	n.s	n.s	**	n.s	**	**
Fuji	Conventional	Health	146	28	12	27	898	393
		Damage	303	63	8	251	3463	459
		Significance	**	**	n.s	**	**	n.s
	Organic	Health	168	16	32	31	786	638
		Damage	427	80	16	279	3186	303
		Significance	**	*	n.s	**	**	*

Statistically significant differences between peel with (damaged) and without (healthy) sunburn from CO and OO at $p \leq 0.05$ (Tukey's test) are expressed with *. Significance: * $p \leq 0.05$; ** $p \leq 0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.

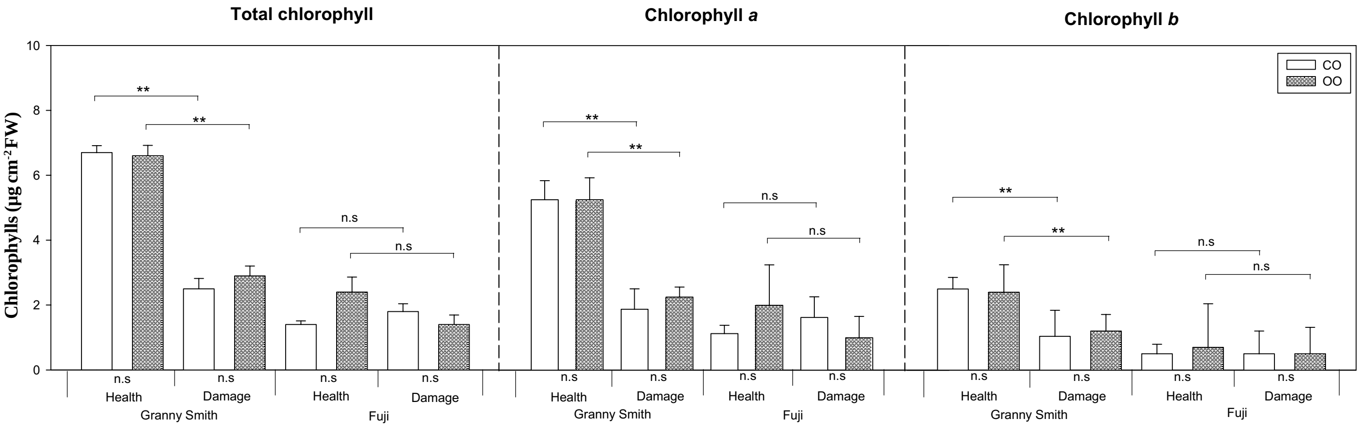

Figure 7. Chlorophylls (total, *a* and *b*) concentrations in apple peel from cvs. Granny Smith and Fuji from conventional and organic orchards with (damaged) and without (healthy) sunburn during the 2009/2010 season. Statistically significant differences between peel from conventional and organic orchards at $p \leq 0.05$ (Tukey's test) are expressed with *. Significance: * $p \leq 0.05$; ** $p \leq 0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.

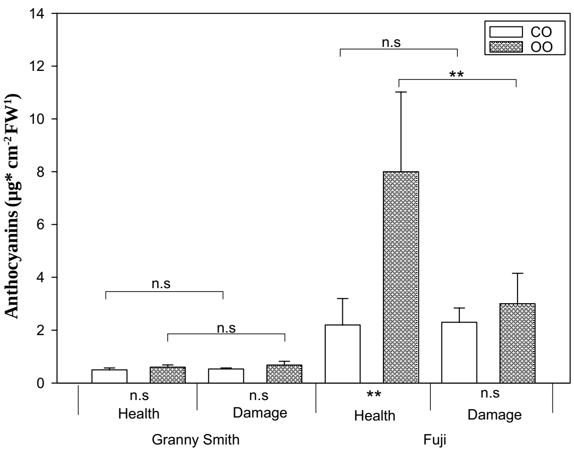

Figure 8. Anthocyanins concentration in apple peel from cvs. Granny Smith and Fuji from conventional and organic orchards with (damaged) and without (healthy) sunburn during the 2009/2010 season. Statistically significant differences between peel from conventional and organic orchards at $p \leq 0.05$ (Tukey's test) are expressed with *. Significance: * $p \leq 0.05$; ** $p \leq 0.01$; n.s, no significance. CO: conventional orchards; OO: organic orchards.

Discussion

The present study compared the effect of conventional and organic management, the stage of development of the fruit and damage by sunburn on the concentration of phenolic compounds, pigments and antioxidant activity in different apple cultivars.

The majority of phenolic compounds reached their highest concentration in the first stages of the development of the fruit and then gradually decreased until harvest, according to results described by several authors [20, 24-27, 31, 32]. However, although phenolic concentration decreased, total content always increased. This agrees with Kondo *et al.* [25], who determined that the phenolic content in apples is maintained or increases during development. The elevated initial concentration of polyphenols could be attributed to the high levels of enzymatic activity (e.g. PAL) and the biosynthesis of these compounds during cellular division [34, 35], while the subsequent decrease can be attributed to a decrease in the activity of these enzymes and dilution as the fruit grows [24, 26-27]. Awad *et al.* [26] suggested that the rate of accumulation of these metabolites decreases through the different growing phases of the fruit but never stops, which could explain increased phenolic content.

The behaviour of the antioxidant activity of the extract was similar to that of the phenolic compounds, increasing until 32 DAFB, and then decreasing until harvest, while the antioxidant activity of the whole fruit increased throughout the growth period. The similarity in the evolution of phenolic compounds and antioxidant activity can be attributed to the good correlation between both, which is according with previous studies [28, 36].

In relation to pigments, the concentration of chlorophylls (total, *a* and *b*) and carotenoids decreased throughout the development of the fruit, coinciding with results reported previously [30, 37, 38]. The Chl *a*/*b* ratio remained practically constant until 88 DAFB, and then decreased until harvest as a consequence of higher reduction of Chl *a*. With respect to the Car/Chl ratio, Reay *et al.* [30] observed that it is maintained until close to harvest and then increased, which concurs with the results of the present study in cv. Gala. Anthocyanins had two accumulation peaks during the season in the red cultivars, while in Granny Smith there was only one, which agrees with several authors [24, 33]. Lister and Lancaster [34] observed that the enzyme (UFGT; UDP-3-*O*-glucosyltransferase), which catalyzes the conversion of anthocyanidin to anthocyanin, was much more active in the red peel, which could explain the reduced accumulation of anthocyanins in green cultivars. Takos *et al.* [24] determined that the transcription of the gene that regulates the expression of the UFGT enzyme was higher in red peel.

Sunburn in apples is caused by exposure to high temperatures and elevated levels of solar radiation [39]. The expression of damage is located on the exposed side of the fruit and is characterized by discoloration in the affected area [39, 40]. When the tissue is exposed to these conditions the production of reactive oxygen species (ROS) and free radicals increases, which can provoke the inactivation of ARN and proteins, damage of DNA, destruction of membranes and eventually cell death [41]. In response to these stressful conditions, enzymatic and non-enzymatic mechanisms are activated to neutralize free radicals and prevent tissue damage [39, 42, 43]. Among the non-enzymatic mechanisms in apples are notably phenolic compounds, due to their high concentration and elevated level of antioxidant activity [28].

The results of the present study indicate that the concentration of total phenolics and antioxidant activity in both the whole fruit and the peel is higher in damaged than in healthy tissue, according to other authors [42, 44, 45]. In general terms, the levels of specific compounds (e.g. chlorogenic acid and quercetins glycosides) were higher in damaged fruit, which agrees with other studies [45, 47, 48].

With respect to changes in pigments in tissue damaged by sunburn, the results of this study suggest that the chlorophyll levels (total, *a* and *b*) decrease in sunburn peel, which is in accordance to other authors [43, 44, 47, 49]. Chlorophyll loss is the consequence of degradation caused by photooxidative stress [44], although the levels of chlorophyll *b* decreased by a lower percentage than chlorophyll *a*, probably because the latter presents a higher degree of sensitivity to high temperatures [50]. However, no statistically significant differences were found between chlorophyll *a* and *b*, which contrasts with the reports of other authors that this ratio increases in damaged tissue [46, 49]. The increased concentration of carotenoids in the damaged peel of cv. Fuji is coincident with reported results [43, 44, 48, 49], while in cv. Granny Smith the concentration was higher in healthy tissue, which is similar to Felicetti and Schrader [46], that the levels of β-carotene, lutein and zeaxanthin were higher in undamaged peel, while the values of violaxanthin and antheraxanthin were similar in the two types of peel. The Car/Chl ratio decreased in damaged peel because the level of Chl decreased sharply, while Car increased or decreased slightly, depending on the cultivar. The high concentration of carotenoids in damaged tissue could be attributable to greater photostability and its importance in dissipating energy or its capacity as an antioxidant compound [43, 51]. With respect to anthocyanins, previous reports indicate that its concentration decreases nearer to the damaged area in red cultivars, while in green cultivars the concentration hardly varies between healthy and damaged tissue [45].

Earlier studies compared the concentration of phenolic compounds and antioxidant activity in fruits from conventional and organic orchards. However, the reports are not consistent on the effects of management practices on phytochemical compound concentrations [19, 29,52-54]. The results of this study agree with Valavinidis *et al.* [29], who did not find differences in phenolics concentrations and antioxidant activity according to the type of management, except in very specific cases. Similar results were found by Veberic and Stampar [52], who did not observe differences in the polyphenol levels among different apple cultivars, produced conventional and organically. This contrasts with other authors [19, 53], that the fruit produced in orchards with conventional or integrated management present lower phenolics concentrations and levels of antioxidant activity than in apples in organic orchards, because of the lower exposition to biotic and abiotic stress [53]. In relation to pigment composition, the results from this study suggest that the concentration of Chl (total, *a* and *b*) and Car are not conditioned by the management practice. This concurs with Cardoso *et al.* [55] for carotenoids in strawberries, while for chlorophyll no previous studies are available to contrast the results. The higher concentration of anthocyanins observed in cvs. Gala and Fuji, could be due to genetic differences among the different strains and not necessarily the product of organic management [56].

Conclusions

The cultivar, the development stage of the fruit and the stress provoked by high temperatures and high levels of solar radiation would be more determinant than the management of photochemical compound concentrations and content in apples. However, further studies are required to determine the effect of practices on the levels of these compounds.

Acknowledgements

This paper is part of the project AF 10I1022 supported by “Fondo de Fomento al Desarrollo Científico y Tecnológico” (FONDEF), Chile.

References

1. Steinmetz, K. A. and Potter, J. D. 1996. Vegetables, fruit, and cancer prevention: A review. Journal of the American Dietetic Association **96**(10):1027-1039.
2. Rimm, E. B. 2002. Fruit and vegetables–building a solid foundation. Am. J. Clin. Nutr. **76**(1):1-2.
3. Stanner, S. A., Hughes, J., Kelly, C. N. M. and Buttriss, J. 2003. A review of the epidemiological evidence for the antioxidant hypothesis. Public Health Nutrition **7**(3):407-422.
4. Liu, R. H. 2004. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. The Journal of Nutrition **134**(12):3479-3485.
5. Nakamura, K., Nagata, C., Oba, S., Takatsuka, N. and Shimizu, H. 2008. Fruit and vegetables intake and mortality from cardiovascular disease are inversely associated in Japanese women but not in men. The Journal of Nutrition **138**:1129-1134.
6. Smith, T. A. 1998. Carotenoids and cancer: Prevention and potential therapy. British Journal of Biomedical Science **55**:268-275.
7. Lanfer-Marquez, U. M., Barros, R. M. C. and Sinnecker, P. 2005. Antioxidant activity of chlorophylls and their derivatives. Food Research International **38**: 885-891.
8. Rizvi, S. Y. and Mishra, N. 2009. Anti-oxidant effect of quercetin on type 2 diabetic erythrocytes. Journal of Food Biochemistry **33**:404-415.
9. Shipp, J. and Abdel-Aal, E. 2010. Food applications and physiological effects of anthocyanins as functional food ingredients. The Open Food Science Journal **4**:7-22.
10. Oršolić, N., Gajski, G., Garaj-Vrhovac, V., Đikić, D., Prskalo, Z. Š. and Sirovina, D. 2011. DNA-protective effects of quercetin or naringenin in alloxan-induced diabetic mice. European Journal of Pharmacology **656**:110-118.
11. Camargo, C. A., da-Silva, M. E. F., da-Silva, R. A., Justo, G. Z., Gomes-Marcondes, M. C. C. and Aoyama, H. 2011. Inhibition of tumor growth by quercetin with increase of survival and prevention of cachexia in Walker 256 tumor-bearing rats. Biochemical and Biophysical Research Communications **406**:638-642.
12. Chun, O. K., Kim, D., Smith, N., Schroeder, D., Han, J. T. and Lee, C. Y. 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. Journal of the Science of Food and Agriculture **85**:1715-1724.
13. Dixon, R. A. and Paiva, N. L. 1995. Stress-induced phenylpropanoid metabolism. The Plant Cell **7**:1085-1097.
14. Gill, S. S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry **48**(12):909-930.
15. Henríquez, C., Almonacid, S., Chiffelle, I., Valenzuela, T., Araya, M., Cabezas, L, Simpson, R. and Speisky, H. 2010. Determination of antioxidant capacity, total phenolic content and mineral composition of different fruit tissue of five apple cultivars grown in Chile. Chilean Journal of Agricultural Research **70**(4):523-536.
16. Labbé, M., Pérez, F. and Sáenz, C. 2010. Influence of fruit maturity and growing region on phenolic content, antioxidant capacity and color of pomegranate juices. International Conference on Food Innovation, FOODINNOVA 10, 25-29 October 2010, Valencia, Spain.
17. Yuri, J. A., Quilodran, A., Motomura, Y. and Palomo, I. 2009. Antioxidant activity and total phenolics concentration in apple peel and flesh is determined by cultivar and agroclimatic growing regions in Chile. Journal

of Food, Agriculture & Environment **7**(3-4):513-517.
[18]Asami, D. K., Hong, Y. J., Barrett, D. M. and Mitchell, A. E. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. Journal of Agricultural and Food Chemistry **51**:1237-1241.
[19]Stracke, B. A., Rufer, C. E., Weibel, F. P., Bub, A. and Watzl, B. 2009. Three year comparison of the polyphenol contents and antioxidant capacities in organically and conventionally produced apples (*Malus domestica* cultivar 'Golden Delicious'). Journal of Agricultural and Food Chemistry **57**:4598-4605.
[20]Coseteng, M. and Lee, C. 1987. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. J. Food Sci. **52**(4):986-989.
[21]Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic membranes. Methods Enzymol. **148**:350–382.
[22]Fuleki, T. and Francis, F. J. 1968. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. Journal of Food Science **33**:72-77.
[23]Von Gadow, A., Joubert, E. and Hansmann, C. 1997. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*) alfa-tocopherol, BHT, and BHA. J. Agric. Food Chem. **45**:632-638
[24]Takos, A. M., Ubi, B. E., Robinson, S. P. and Walker, A. R. 2006. Condensed tannin biosynthesis genes are regulated separately from other flavonoid biosynthesis genes in apple fruit skin. Plant Science **170**:487-499.
[25]Kondo, S., Tsuda, K., Muto, N. and Ueda, J. E. 2002. Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars. Scientia Horticulturae **96**:177-185.
[26]Awad, M. A., Jaeger, A., Van der Plas, L. H. W. and Van der Krol, A. 2001. Flavonoid and chlorogenic acid changes in skin of Elstar' and Jonagold' apples during development and ripening. Scientia Horticulturae **90**:69-83.
[27]Renard, C. M. G. C., Dupont, N. and Guillermin, P. 2007. Concentrations and characteristics of procyanidins and other phenolics in apples during fruit growth. Phytochemistry **68**:1128-1138.
[28]Lee, K. W., Kim, Y. J., Kim, D., Lee, H. J. and Lee, C. Y. 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. J. Agric. Food. Chem. **51**:6516-6520.
[29]Valavanidis, A., Vlachogianni, T., Psomas, A., Zovoili, A. and Siatis, V. 2009. Polyphenolic profile and antioxidant activity of five apple cultivars grown under organic and conventional agricultural practices. International Journal of Food Science and Technology **44**:1167-1175.
[30]Reay, P. F., Fletcher, R. H. and Thomas, V. J. 1998. Chlorophylls, carotenoids and anthocyanin concentration in the skin of 'Gala' apples during maturation and the influence of foliar application of nitrogen and magnesium. J. Sci. Food. Agric. **76**:63-71.
[31]Mayr, U., Treutter, D., Santos-Buelga, C., Bauer, H. and Feucht, W. 1995. Developmental changes in the phenol concentrations of 'Golden Delicious' apple fruits and leaves. Phytochemistry **38**(5):1151-1155.
[32]Murata, M., Tsurutani, M., Masami, T., Homma, S. and Kaneko, K. 1995. Relationship between apple ripening and browning: Changes in polyphenol content and polyphenol oxidase. Journal of Agricultural and Food Chemistry **43**(5):1115-1121.
[33]Lister, C. and Lancaster, J. 1994. Developmental changes in the concentration and composition of flavonoides in skin of a red and green apple cultivar. J. Sci. Food. Agric. **64**:155-161.
[34]Lister, C. and Lancaster, J. 1996. Developmental changes in enzymes of flavonoid biosynthesis in the skins of red and green apple cultivars. J. Sci. Food. Agric. **71**:313-320.
[35]Treutter, D. 2001. Biosynthesis of phenolic compounds and its regulation in apple. Plant Growth Regulation **34**:71-89.
[36]Drogoudi, P. D., Michailidis, Z. and Pantelidis, G. 2008. Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. Scientia Horticulturae **115**:149-153.
[37]Knee, M. 1972. Anthocyanin, carotenoid, and chlorophyll changes in the peel of Cox's Orange Pippin apples during ripening on and off the tree. Journal of Experimental Botany **23**(74):184-196.
[38]Mussini, E., Correa, N. and Crespo, G. 1985. Evolución de pigmentos en frutos de manzanas Granny Smith. Phyton **45**:79-84.
[39]Wünsche, J. N., Bowen, J., Ferguson, I., Woolf, A. and McGhie, T. 2004. Sunburn on apples – Causes and control mechanisms. Acta Hort. **636**:631-636.
[40]Andrews, P. K. and Johnson, J. R. 1996. Physiology of sunburn development in apples. Good Fruit Grower **47**(12):33-36.
[41]Lambers, H., Chapin, F. S., and Pons, T. L. 2008. Plant Physiological Ecology. 2nd edn. Springer, 623 p.
[42]Yuri, J. A., Torres, C., Bastias, R. and Neira, A. 2000. Golpe de sol en manzanas. II. Factores inductores y respuestas bioquímicas. AgroCiencia **16**(1):23-32.
[43]Chen, L. S., Li., P. and Cheng, L. 2008. Effects of high temperature coupled with high light on the balance between photooxidation and photoprotection in the sun-exposed peel of apple. Planta **228**:745-756.
[44]Yuri, J. A., Neira, A., Quilodran, A., Razmilic, I., Motomura, Y., Torres, C. and Palomo, I. 2010. Sunburn on apples is associated with increases in phenolic compounds and antioxidant activity as a function of the cultivar and areas of the fruit. Journal of Food, Agriculture & Environment **8**(2):920-925.
[45]Felicetti, D. A. and Schrader, L. E. 2009. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. II. Phenolics. Plant Science **176**:84-89.
[46]Felicetti, D. A. and Schrader, L. E. 2009. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and carotenoids. Plant Science **176**:78-83.
[47]Schrader, L. E., Zhang, J. and Duplaga, W. K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. Plant Health Prog. Doi:10.1094/PHP-2001-1004-01-RS.
[48]Wünsche, J. N., Greer, D. H., Palmer, J. W., Lang, A. and McGhie, T. 2001. Sunburn - The cost of a high light environment. Acta Hort. **557**:349-356.
[49]Felicetti, D. A. and Schrader, L. E. 2008. Changes in pigment concentrations associated with the degree of sunburn browning of 'Fuji' apple. J. Amer.Soc. Hort. Sci. **133**(1):27-34.
[50]Weemaes, C. A., Ooms, V., Van Loey, A. M. and Hendrickx, M. E. 1999. Kinetics of chlorophyll degradation and color loss in heated broccoli juice. J. Agric. Food. Chem. **47**(6):2404-2409.
[51]Merzlyak, M. N. and Solovchenko, A. E. 2002. Photostability of pigment in ripening apple fruit: A possible photoprotective role of carotenoids during plant senescence. Plant Sci. **163**:881-888.
[52]Veberic, R. and Stampar, F. 2005. Quality of apple fruits (*Malus domestica*) from organic versus integrated production. Information and Technology for Sustainable Fruit and Vegetable Production. FRUTIC. 05, 12-16.September 2005, Montpellier, France.
[53]Petrovsek, M. M., Slatnar, A., Stampar, F. and Veberic, R. 2010. The influence of organic/integrated production on the content of phenolic compounds in apple leaves and fruits in four different varieties over a 2-year period. J. Sci. Food. Agric. **90**:2366-2378.
[54]Wang, S. Y., Chen, C. T., Sciarappa, W., Wang, C. Y. and Camp, M. J. 2008. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. J. Agric. Food Chem. **56**:5788-5794.
[55]Cardoso, P. C., Tomazini, A. P. B., Stringheta, P. C., Ribeiro, S. M. R. and Pinheiro-SantAna, H. M. 2011. Vitamin C and carotenoids in organic and conventional fruits grown in Brazil. Food Chemistry **126**:411-416.
[56]Iglesias, I. and Echeverria, G. 2009. Does strain affect fruit color development, anthocyanin content and fruit quality in 'Gala' apples? A comparative study over three seasons. Journal of the American Pomological Society **63**(4):168-180.