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Total phenol and quercetin content and antioxidant activity in apples in response to thermal, light stress and to organic management

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Summary

Flavonoids are the most abundant phenol compound group in apples, the concentration of which varies with the cultivars and climatic conditions. The objective of this study was to evaluate the effects of temperature, solar radiation, sunburn damage of the peel and the state of development of fruit on total phenol concentrations, quercetin glycosides and antioxidant activity. Three assays were conducted during the 2008/09 season to evaluate aforementioned variables on these parameters. The following season, the effect of the state of development on the fruit was evaluated. Sunburn increased phenol concentrations from 5.5 to 8.7 mg CAE* g FW^{-1}. In relation to the state of development of the fruit, phenol concentrations decreased from 14 to 1.3 mg CAE* g FW^{-1} between 32 DAFB to harvest, respectively. Fruit that was bagged until one month before harvest had significantly higher concentrations of quercetin rutinoside (28 mg*g^{-1}FW), galactoside (484 mg*g^{-1}FW) and glucoside (54 mg*g^{-1}FW) than fruit that remained bagged until harvest (6, 161 and 21 mg*g^{-1}FW, respectively). Temperature did influence phenol concentrations. This study determined that sunburn, the state of development and bagging the fruit are factors that determine phenol concentration in apples.

Introduction

The apple (*Malus domestica* Borkh.) is a temperate-cold climate zone cultivar that has nevertheless been adapted to a wide variety of environmental conditions (Feree, 2003). In Chile, with its Mediterranean climate, apple production is mainly concentrated between 34.5° and 38.4° latitude south (Gil, 2000), covering an area of 37,200 hectares (ODEPA – CIREN, 2007).

It is recognized that apples and in particular the peel have high concentrations of phenol compounds, which result in a significant level of antioxidant activity, which is associated with reducing the risks of developing chronic non-transmissible diseases (e.g. cancer and cardiovascular illnesses) (Steinmetz and Potter, 1996; Nakamura et al., 2008; Liu, 2004). The phenol content of the fruit is influenced by a number of factors, among them the cultivar (Henriquez et al., 2010; Van der Sluis, 2001), the state of development (Labbe et al., 2010; Kondo et al., 2002), tissue (peel or pulp) (Yuri et al., 2009), management (conventional and organic) (Asami et al., 2003; Stracke et al., 2009) mineral nutrition of the plant (Treutter, 2010), the agroclimatic region (Yuri et al., 2009), refrigerated storage (Martinez et al., 2002), light and temperature (Dixon and Paiva, 1995; Martinez et al., 2002).

Flavonoids are the most abundant phenol compound group in apples (Dueñas et al., 2011), among them notably are quercetin glycosides, which make a major contribution to total antioxidant activity (Dueñas et al., 2011; Lee et al., 2003). This study evaluated the effects of temperature, light, sunburn and the state of development of the fruit on phenol compound concentrations, quercetin glycosides and antioxidant activity in two cultivars under the agro climatic conditions of central Chile.

Materials and methods

Assay 1. Effect of temperature on the peel

The effects of thermal stress on fruit of cv. Fuji were evaluated in the 2008/09 at the Panguilemo Experimental Station of the Universidad de Talca (35° 23'L.S., 71° 40'L.W., 105 m.a.s.l). The trees used were grafted in 2003 onto EMLA 9 rootstock planted at 4.0 m x 2.0 m. Measurements were taken 150 days after full bloom (DAFB). The measurements were taken from three branches of each sampled tree: the first submitted to 35 °C, the second to 45 ºC and the third as a control with ambient temperature. In the case of the first two branches, the exposure time was 5 hours, between 10:00 am and 3:00 pm. Representative fruit were collected at 24 hours post-stress. To control the temperature of the treatments the branches were wrapped in plastic film for greenhouse. Thermocouples were installed in the plastic wrapping and connected to a computer and thermal ventilator by means of an Arduino microcontroller board. Eight apples were collected for each treatment and the control (four replications with 2 fruit each) from which peel was obtained for the measurements.

Assay 2. Sunburn effect

a) Different levels of damage

During the commercial harvest of 2008/09 season, cv. Granny Smith apples, were collected from a commercial orchard located in Los Niches, Maule Region, Chile (35°0'S, 71°08'W; 299 m.a.s.l.). Undamaged (healthy) and different grade of damage peel fruit (mild and moderate) were collected and analyzed.

b) In conventional (CO) and organic orchards (OO)

During the 2009/10 season apples cvs. Fuji (Raku Raku and Striped) and Granny Smith were collected from conventional and organic orchards located in Chimbarongo, O'Higgins Region, Chile (34° 40'S, 71° 1'W; 314 m.a.s.l.). The conventional and organic orchards were 10 km apart. The measurements were made at commercial harvest (191 and 159 DAFB, respectively) using peels of healthy and moderately sunburnt fruit, with four replications with four fruit in each for each evaluated cultivar.

Assay 3. Influence of the growth stages of the fruit

Healthy cvs. Fuji and Granny Smith apples at different stages of development were collected during the 2009/10 season (25, 32, 39, 52 and 88 DAFB), and commercial harvest (191 and 159 DAFB, respectively), from the conventional and organic orchards described in assay 2b. The measurements were made with the entire fruit (peel and flesh). Sixteen fruit were randomly selected for each stage for four replications with four fruit in each replication.

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Assay 4. Effect of bagging
In the commercial harvest of 2008/09 season, cv. Fuji apples, unbagged (Control), bagged until one month before harvest (commercial bagging) and bagged until harvest, were collected from a commercial orchard located in San Clemente (35°30'S, 71°28'W; 83 m.a.s.l). The determinations were made with peel from fruit, with four replications of 2 apples in each replication. The apples were covered with double-layer paper bags (Qingdao Keentop Paper Enterprise Ltd., China). The outer bags were blue on the outside and black on the inside while the inner bags were blue.

Tissue Extraction
Assay 1: A hole punch was used to obtain disks of 95 mm^2 in area and 5 mm deep until reaching one gram of peel.

Assays 2 and 4: 1 and 2 g of peel were obtained from the equatorial zone of the apple for assays 2 and 4, respectively.

Assay 3: The apples were cut into lengthwise slices until reaching one gram of tissue (peel and flesh). The seeds and core were previously removed.

The tissues were frozen with liquid nitrogen, pulverized and homogenized in a mortar and pestle. The method for extraction described by Coseteng and Lee (1987) was used with some modification. Briefly, the tissue was extracted twice with a solution of ethanol at 80 % (ethanol: water 80:20, v/v) for 10 and 5 minutes at 100 °C. Subsequently, it was filtered, graduated at 10 mL with ethanol at 80 % and stored at -20 °C until use.

Total phenol content
Total phenol content was determined by the Folin-Ciocalteu method described by Coseteng and Lee (1987). Briefly, 0.1 mL of the extract was mixed with 0.5 mL of the Folin-Ciocalteu phenol reagent (Merck, Darmstadt, Germany). The mixture was incubated for five minutes and then 0.5 mL of sodium carbonate (Na_2CO_3; 10 %, w/v) added and incubated for 15 minutes at room temperature (20 °C). Absorbance was measured at 640 nm. Total phenol concentrations were expressed as mg of chlorogenic acid equivalents * g FW^{-1}.

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. (1997), with modifications. Briefly, 0.01 mL of each extract was used, adjusted to a final volume of 0.5 mL with 80 % ethanol. They were then mixed with 2 mL of DPPH $8x10^{-5}$ M solution and incubated for 8 minutes at room temperature. Their absorbance was measured at 515 nm using ethanol as blank. 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 * g FW^{-1}.

Determination of quercetin glycosides by HPLC
The determination of quercetin glycosides in the samples was performed using HPLC-DAD Merck Hitachi (LaChrom, Tokyo, Japan), equipment, which consisted of a LaChrom L-7100 pump and a diode array detector, L-7455 LaChrom, and a 100-5 C18 Kromasil column of 259x4.6 mm with a pre-column of the same characteristics, maintained at 20 °C. Briefly, 0.02 mL, previously filtered (0.45 µm filter), were injected. To identify the compounds, different standards of quercetin glycosides were used with the UV-VIS spectra. The chromatogram was monitored at 300 nm. The solvents of the mobile phase were: A: formic acid 1 % in H_2O quality HPLC; B: acetonitrile 40 % in H_2O, and C: acetonitrile. The elution used was: 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 mg equivalents of quercetin glycosides* 1 g of FW^{-1} (Yuri et al., 2010).

Statistical analysis
The assay was carried out with a completely random design. An ANOVA was applied for the statistical analysis and separation of means, using the SPSS v15.0 computer program (SPSS Inc., Chicago, Illinois). Tukey's HSD test was used to compare treatments when significant differences were found ($p \leq 0.05$).

Results

Assay 1
In cv. Fuji exposure to high temperatures had no effect on total phenolic compounds concentrations and antioxidant activity, with values ranging from 4.5 to 5.3 mg CAE* g^{-1} FW and from 3.9 to 4.7 mg CAE* g^{-1} FW, respectively (Tab. 1).

Quercetin glycoside concentrations in the peel of Fuji apples exposed to 35 and 45 °C were not affected by the high temperature (Tab. 2).

Tab. 1: Effect of temperature in phenolic concentration and antioxidant activity in peel of Fuji apple.

	Total Phenolic (mg CAE* g^{-1} FW)	Antioxidant Activity (mg CAE* g^{-1} FW)
Initial Condition	4.13 a	3.54 a
35 ºC x 5 h	4.58 a	3.96 a
35 ºC x 24 h	4.71 a	4.65 a
45 ºC x 5 h	4.00 a	3.19 a
45 ºC x 24 h	5.31 a	4.40 a
Significance	n.s	n.s

Statistically significant differences between treatments at $p \leq 0.05$ (Tukey's test) are expressed with *. Significance: * $p \leq 0.05$; ** $p \leq 0.01$: n.s, no significance.

Assay 2 a
Tissue of cv. Granny Smith apples damaged by sunburn had higher concentrations of phenolics compounds (8.7 mg CAE* g FW^{-1}) than tissue of healthy apples (5.5 mg CAE* g FW^{-1}) ($p = 0.001$), as well as higher levels of antioxidant activity (7.4 mg CAE* g FW^{-1}) compared to healthy peel (5.2 mg CAE* g FW^{-1}) in healthy tissue, although the differences were not significant.

Concentrations of quercetin rutinoside, galactoside and glucoside in solar damaged tissue (Tab. 3) were 6 to 20 times as high as in healthy peel, while quercetins xyloside, arabinoside and rhamnoside were 3 times as high as in healthy tissue.

Assay 2 b
Moderate sunburn significantly increased total phenol concentration ($p < 0.01$) in the damaged tissue of both conventionally and organically grown fruit, with values ranging from 7.3 to 16.3 mg CAE* g FW^{-1}. A similar tendency was observed with antioxidant activity, which increased until reaching ranging between 8.4 and 13.8 mg CAE* g FW^{-1} for both management systems and cultivars (Tab. 4).

Tab. 2: Concentration of quercetin glycosides in peel cv. Fuji exposed to 35 °C and 45 °C (150 DAFB).

	Quercetin type (mg* g^{-1} FW)					
	Rutinoside	Galactoside	Glucoside	Xyloside	Arabinoside	Rhamnoside
Initial Condition	0.009 a	0.275 a	0.041 a	0.097 a	0.145 a	0.119 a
35 ºC x 5 h	0.012 a	0.398 a	0.056 a	0.139 a	0.210 a	0.158 a
35 ºC x 24 h	0.016 a	0.382 a	0.073 a	0.128 a	0.200 a	0.145 a
45 ºC x 5 h	0.012 a	0.309 a	0.052 a	0.112 a	0.167 a	0.141 a
45 ºC x 24 h	0.022 a	0.493 a	0.078 a	0.157	0.238 a	0.170 a
Significance	n.s	n.s	n.s	n.s	n.s	n.s

Statistically significant differences between treatments at p≤ 0.05 (Tukey's test) are expressed with *. Significance: * p≤ 0.05; ** p≤ 0.01: n.s, no significance.

Tab. 3: Concentration of quercetin glycosides in healthy peel and moderate sunburn damage.

	Quercetin type (mg* g^{-1} FW)					
	Rutinoside	Galactoside	Glucoside	Xyloside	Arabinoside	Rhamnoside
Healthy	0.03 b	0.30 b	0.13 b	0.14 b	0.17 b	0.13 b
Mild Sunburn	0.46 a	1.57 a	1.21 a	0.31 a	0.52 a	0.33 a
Moderate Sunburn	0.60 a	1.94 a	1.54 a	0.31 a	0.55 a	0.34 a
Significance	**	**	**	**	**	**

Statistically significant differences between treatments at p≤ 0.05 (Tukey's test) are expressed with *. Significance: * p≤ 0.05; ** p≤ 0.01: n.s, no significance.

Tab. 4: Total phenol concentration and antioxidant capacity in healthy peel and moderate sunburn damage.

Cultivars	Management		Total Phenolic (mg CAE* g^{-1} FW)	Antioxidant Activity (mg CAE* g^{-1} FW)
Granny Smith	Conventional	Health	5.5 b	5.1 b
		Damage	16.3 a	13.8 a
		Significance	**	**
	Organic	Health	5.7 b	4.1 b
		Damage	12.9 a	10.3 a
		Significance	**	**
Fuji	Conventional	Health	4.8 b	5.6 b
		Damage	7.8 a	8.4 a
		Significance	**	**
	Organic	Health	4.1 b	5.3 b
		Damage	7.3 a	9.3 a
		Significance	**	**

Statistically significant differences between treatments at p≤ 0.05 (Tukey`s test) are expressed with *. Significance: * p≤ 0.05; ** p≤ 0.01: n.s, no significance.

As a result of sunburn there were increases in the concentrations of all types of quercetins in both cultivars and both management approaches (Tab. 5). In cv. Granny Smith, quercetin rutinoside and galactoside were the quercetins that increased most, followed by glucoside, xyloside, arabinoside and rhamnoside. Fuji showed a similar tendency, although with increases of lower magnitude. There were no differences between the two types of management.

Assay 3

In studying the evolution of total phenols and antioxidant activity during the growth stages of the fruit of cvs. Granny Smith and Fuji, we observed that phenol concentrations increased from 9 mg CAE* g FW^{-1} to approximately 14 mg CAE* g FW^{-1} from 25 to 32 DAFB and then began to decrease until harvest, with values ranging from 1.3 to 2.6 mg CAE* g FW^{-1}. Phenol content, in contrast, increased throughout the development period, from 22-35 mg CAE* $Fruit^{-1}$ to 287-401 mg CAE* $Fruit^{-1}$ (Tab. 6).

As with total phenol concentrations, antioxidant activity increased from 25 to 32 DAFB and then began to decrease until harvest (Tab. 6), with values ranged between 1.5 and 2.4 mg CAE* g FW^{-1}. The antioxidant activity of the whole fruit increased throughout the growth period, from 17-32 mg CAE* $Fruit^{-1}$ to 337-380 mg CAE* $Fruit^{-1}$ at harvest.

Quercetin glycoside concentrations in both Granny Smith and Fuji apples during fruit development increased until 32 DAFB and then

Tab. 5: Concentration of quercetin glycosides in cv. Granny Smith with different sunburn damage level.

Cultivars	Management		Rutinoside	Galactoside	Glucoside	Xyloside	Arabinoside	Rhamnoside
Granny Smith	Conventional	Health	0.004 b	0.065 b	0.015 b	0.027 b	0.062 b	0.043 b
		Damage	0.543 a	2.190 a	1.756 a	0.351 a	0.686 a	0.477 a
		Significance	**	**	**	**	**	**
	Organic	Health	0.003 b	0.051 b	0.013 b	0.026 b	0.049 b	0.038 b
		Damage	0.527 a	2.162 a	1.574 a	0.305 a	0.593 a	0.379 a
		Significance	**	**	**	**	**	**
Fuji	Conventional	Health	0.027 b	0.354 b	0.057 b	0.114 b	0.213 b	0.131 b
		Damage	0.251 a	1.619 a	0.470 a	0.321 a	0.542 a	0.259 a
		Significance	**	**	**	**	**	**
	Organic	Health	0.031 b	0.337 b	0.054 b	0.104 b	0.170 b	0.090 b
		Damage	0.279 a	1.500 a	0.496 a	0.244 a	0.449 a	0.218 a
		Significance	**	**	**	**	**	**

Statistically significant differences between treatmeans at p≤ 0.05 (Tukey's test) are expressed with *. Significance: * p≤ 0.05; ** p≤ 0.01: n.s, no significance.

Tab. 6: Evolution of total phenolic concentration, total phenolic content, antioxidant activity in extracts and antioxidant activity in whole fruit from apples cvs. Granny Smith and Fuji , from conventional and organic orchards, in different stages of development, during the 2009/2010 season.

	Management	DAFB	Total Phenolic concentration (mg CAE* g^{-1} FW)	Total Phenolic content (mg CAE* $Fruit^{-1}$)	Antioxidant Activity in extract (mg CAE* g^{-1} FW)	Antioxidant Activity in whole fruit (mg CAE* $Fruit^{-1}$)
Granny Smith	Conventional	25	9.4	27	8.4	24
		32	14	78	9.6	54
		39	10	118	8.1	93
		52	7.9	141	6.8	125
		88	4.5	256	3.7	217
		159	2.6	401	2.4	380
	Organic	25	13	35	12	32
		32	14	81	9.4	53
		39	13	143	8.4	96
		52	11	172	8.2	131
		88	3.7	235	3.1	197
		159	2.4	439	2.1	381
Fuji	Conventional	25	9.4	22	7.2	17
		32	14	67	9.2	46
		39	11	88	9.9	83
		52	8.5	138	8.6	126
		88	4.7	282	2.9	212
		191	1.3	326	1.5	337
	Organic	25	12	26	9.6	19
		32	15	62	9.9	41
		39	13	122	9.9	96
		52	9.2	175	8.6	164
		88	3.5	225	2.9	186
		191	1.5	287	1.5	340

began to decrease until harvest (Fig. 1 and 2). At the same time, quercetin glycoside content increased during the season, from 0.5 mg* g FW^{-1}, until 0.5 to 6 mg* g FW^{-1}, depending on the type.

Assay 4

The phenols quantity and the antioxidant activity level were slightly lower (3.9 mg CAE* g FW^{-1} and 3.4 mg CAE* g FW^{-1} respectively) in fruit that were bagged until harvest compared to fruit that were bagged until one month before harvest, the latter with values of 5.4 and 4.2 mg CAE* g FW^{-1}, respectively (Tab. 7).

The concentrations of quercetins rutinoside (28 mg*g^{-1} FW), galactoside (484 mg*g^{-1} FW) and glucoside (54 mg*g^{-1} FW) were significantly higher with bagging until one month before harvest compared to levels with apples that remained bagged until harvest (6,

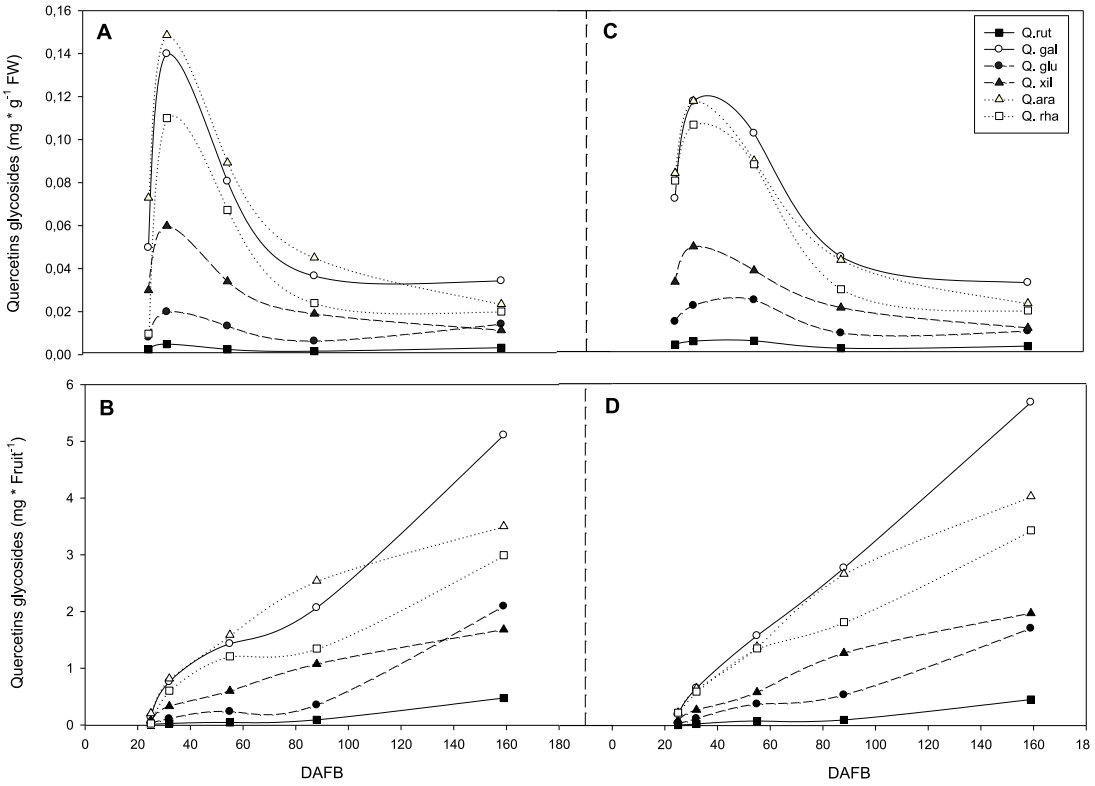

Fig. 1: Evolution of quercetins glycosides concentration and content in whole fruit from apple cv. Granny Smith, from conventional (A and B) and organic (C and D) orchards, in different stages of development, during 2009/2010 season. Curves end at harvest. Q.rut, rutinoside; Q.gal, galactoside; Q.glu, glucoside; Q.xil, xyloside; Q.ara, arabinoside; Q.rha, rhamnoside.

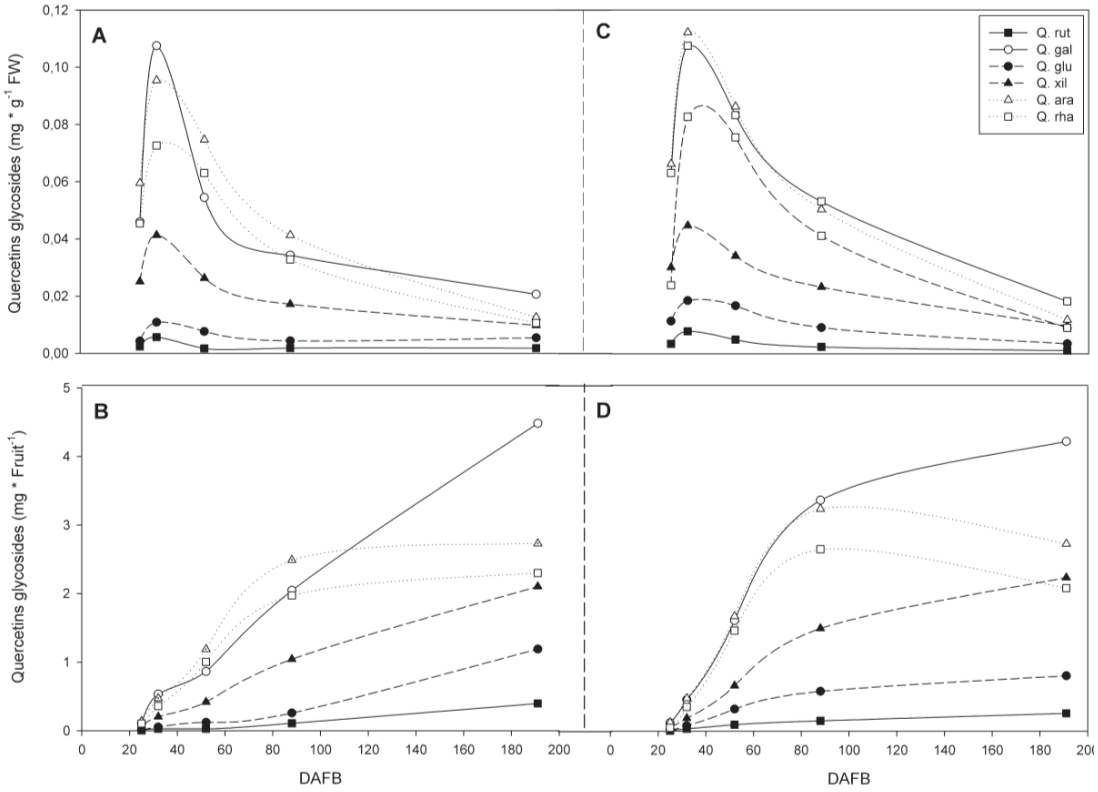

Fig. 2: Evolution of quercetins glycosides concentration and content in whole fruit from apple cv Fuji, from conventional (A and B) and organic (C and D) orchards, in different stages of development, during 2009/2010 season. Curves end at harvest. Q.rut, rutinoside; Q.gal, galactoside; Q. glu, glucoside; Q. xil, xyloside; Q. ara, arabinoside; Q.rha, rhamnoside.

161 and 21 mg*g^{-1} FW, respectively). The values observed in the unbagged fruit (controls) were similar to those of bagged fruit until one month before harvest (Tab. 8).

Discussion

The present study evaluated the effects of temperature, radiation, sunburn and the state of development of the fruit on total concentrations of phenols and quercetin glycosides, as well the levels of antioxidant activity in Granny Smith and Fuji apples.

No significant increases in total phenolic compounds and quercetin glycoside concentrations or levels of antioxidant activity were observed with higher temperatures.

Sunburn is a physiological disorder caused by the fruit being exposed to excessive levels of temperature and solar radiation (WÜNSCHE et al., 2004; RACSKO and SCHRADER, 2012), which induces photo- and thermal protective mechanisms in the fruit tissue, expressed as higher levels of antioxidant activity and the activity of certain enzymes (LEJA et al., 2003; LAMBERS et al., 2008). This coincides with the results of this study, in which total phenol, quercetins and antioxidant activity were higher in damaged tissue. FELICETTI and SCHRADER (2009) had similar results and observed that the concentrations of chlorogenic acid and quercetin glycosides were lower further away from the area of damaged tissue. YURI et al. (2010) observed that total phenol, flavonol concentrations and levels of antioxidant activity were higher in apples with sunburn in both seasons.

Total phenolic compounds concentration, quercetins and antioxidant activity decreased during the growth period of the both conventionally and organically grown, while the content and activity of these compounds at the level of the entire fruit presented a tendency to increase. These results concur with observations described previously by other authors (MAYR et al., 1995; HAMAUZU et al., 1999; WANG and LIN, 2000). TAKOS et al. (2006) observed that the concentration of flavonols is high during the first stages of the development of the fruit (32 DAFB) and then decreases during development and maturation. RENARD et al., (2007) determined that the concentrations of hydroxycinnamic acids and flavanols reached their maximum value during the period of cellular division and then decreased abruptly until the end of the season. This is because the activity of phenylalanine ammonia lyase (PAL) and other enzymes related to the biosynthesis of phenols reach their peak in the stage of cell division (JU et al., 1995; LISTER and LANCASTER, 1996; TREUTTER, 2001) until 30 DAFB and then decrease sharply until the stage of cellular expansion. According to RENARD et al. (2007), once the strong initial synthesis of phenol compounds is detained, the decrease in phenolic compounds concentrations is due to the growth of the fruit. Thus, although the concentration of phenols decreases, phenol content in the fruit increases, which is in agreement with what was observed by AWAD et al. (2001).

Tab. 7: Effect of bagging in total phenol concentration and antioxidant activity in cv. Fuji peel.

	Total Phenolic (mg CAE* g^{-1} FW)	Antioxidant Activity (mg CAE* g^{-1} FW)
Unbagged	4.54 ab	3.29 a
Comercial Bagging	5.40 a	4.16 a
Bagging until harvest	3.87 b	3.44 a
Significance	*	n.s

Statistically significant differences between treatments at p≤ 0.05 (Tukey's test) are expressed with *. Significance: * p≤ 0.05; ** p≤ 0.01: n.s, no significance.

Bagging is a used agricultural practice in some countries to protect cv. Fuji fruit from pests and pathogens, as well as preventing sunburn. However, the most important motive for this practice is its positive effect on the color of the fruit. Removing the bag promotes the synthesis of anthocyanins in tissue that lacks chlorophyll (CHEN et al., 2012). However, re-exposure to radiation increases the level not only of anthocyanins, but also of other phenol compounds. JU et al. (1995) observed that when the fruit is unbagged the activity of the PAL enzyme and simple phenol concentrations of anthocyanins and flavanoids increases to levels similar to those reached by the fruit during the first development stages. This evidences that the precursor PAL enzyme and some phenol compounds are dependent on light (LANCASTER, 1992; JU et al., 1995; JU, 1998; AWAD et al., 2000; OH et al., 2009). Gene expression and enzyme activity related to the metabolism of phenol compounds are equally regulated by light (JU et al., 1995; TAKOS et al., 2006; QIAN et al., 2013).

Although the fruit that were bagged until harvest did not develop red pigmentation (anthocyanins), they did have similar phenolic compounds content and levels of antioxidant activity to those of unbagged and commercially bagged fruit, which to some extent contradicts what has been indicated in terms of the need for direct solar radiation for the synthesis of phenolic compounds, opening the possibility that the these are transported to the fruit from adjacent leaves. PERKINS et al. (2009) and KŪKA et al. (2010) determined the presence of catechins, kaempferol, and quercetins in the sap of *Betula pendula* and *Acer platinoides*. This is supported by JU (1998), who observed that bagged apples developed simple phenols, procyanidins and quercetin glycosides. In contrast, CHEN et al. (2012) observed that bagging fruit reduced the concentrations of anthocyanins, hydroxycinnamic acids and flavanols in the peels of the three apple cultivars. Consequently, the theme should be studied in greater amplitude and depth.

Tab. 8: Effect of bagging in quercetin glycosides of cv. Fuji. Efecto del embolsado en la concentración de quercetinas glicosiladas en piel de cv. Fuji.

	Quercetin type (mg* g-1 FW)					
	Rutinoside	Galactoside	Glucoside	Xyloside	Arabinoside	Rhamnoside
Unbagged	25 a	320 ab	65 a	103 a	187 a	102 a
Normal Bagging	28 a	484 a	54 ab	92 a	187 a	114 a
Bagging until harvest	6 b	161 b	21 b	61 a	122 a	82 a
Significance	**	**	*	n.s	n.s	n.s

Statistically significant differences between treatments at p≤ 0.05 (Tukey's test) are expressed with *. Significance: * p≤ 0.05; ** p≤ 0.01: n.s, no significance.

Conclusion

Sunburn, light and the state of development of the fruit appear to be more important factors in determining the levels of phenol compounds and antioxidant activity in apples by than transient high temperature stress. However, more studies are required to reaffirm the effect of these factors on the concentrations of phytochemical compounds.

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