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Total phenolics, quercetin glycosides and antioxidant activity in organic and conventional orchards in three apple cultivars during fruit growth

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Abstract

Aim of study: To evaluate whether organic and conventional management practices, cultivar and fruit growth stage affect total phenolic content, antioxidant activity and quercetin glycosides in apples of three cultivars.

Area of study: The trials were conducted in commercial orchards located in Chimbarongo, O'Higgins Region, Chile.

Material and methods: Two types of orchard management (organic and conventional) were studied in three apple cultivars: Gala 'Brookfield', Granny Smith and Fuji 'Raku Raku'. Total phenolic content, antioxidant activity and quercetin glycosides were evaluated according to management practices, fruit growth stage, cultivar, fruit weight and skin surface. Data were analyzed statistically using a truncated multiple regression model.

Main results: No differences were found between organic and conventional management regarding polyphenol concentration and antioxidant activity, except for specific quercetin glycosides. However, significant differences were observed between cultivars in both variables, as well as in fruit development throughout the season, which showed a significant dilution of polyphenols and antioxidant activity as the fruit grew.

Research highlights: Cultivar and fruit growth stage were decisive in total phenolic content, glycosidic quercetins and apples antioxidant activity. Conventional and organic management practices were significant for quercetin glycoside concentration, which is the main polyphenol in apples.

Additional key words: bioactive compounds; consumer; fresh apple; *Malus* × *domestica*; quercetin glycosides.

Abbreviation used: CAE (chlorogenic acid equivalents); CUL (cultivar); DAFB (days after full bloom); DPPH (2,2-diphenyl-1-picrylhydrazyl); FGS (fruit growth stage); FW (fresh weight); MAN (management practice); ORAC (oxygen radical absorbance capacity); SSA (skin surface area); TE (Trolox equivalent).

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Introduction

Phenolic compounds are key metabolites for plants, since they are involved in growth and reproduction (Babenko *et al.,* 2019), as chemical signals, allelopathic source among plants (Chou, 1999), as pest and disease defense (Dixon & Paiva, 1995), and as response to abiotic stress conditions (Cheynier *et al.,* 2013). Interest in such compounds has increased in recent decades, since evidence links a diet rich in fruits and vegetables, high in phenolic compounds and antioxidants, with a decrease in degenerative diseases and oxidative stress-associated diseases (Krüger *et al.,* 2011; Simioni *et al.,* 2018; Leyane *et al.,* 2022). Apples (*Malus* × *domestica* Borkh.*)* are of

great relevance in this context, due to their high content of these bioactive compounds, and their large and widespread consumption as fresh and processed products (Chun *et al.,* 2005; Hyson, 2011).

Apple trees hold the second place in world fruit production, with an area of 4,622 thousand hectares (FAOSTAT, 2020). In Chile, the growth of apple acreage reaches 31,000 ha, of which the area dedicated to organic agriculture has increased significantly, due to the high international demand for this type of product and the higher price obtained for it (ODEPA, 2021). Controversy continues, however, among consumers and researchers about the higher nutritional and health content of organic fruit, a topic often discussed in the industry.

In apples, phenolic content may vary according to cultivar (Henríquez *et al.,* 2010), climatic conditions, tissue type (skin *vs.* flesh) (Yuri *et al.,* 2009), phenological stage (Yuri *et al.,* 2014) orchard management and abiotic and biotic stress (Dixon & Paiva, 1995; Gill & Tuteja, 2010; Yuri *et al.,* 2012). However, it is relevant to consider that, under natural conditions, the plant is constantly interacting with multiple stimuli or stressful conditions.

The present study evaluated the orchard management practices (conventional and organic), growth stage, cultivar, skin size and surface area, with phenolic content, antioxidant activity and quercetin glycosides concentration in fresh apple, from commercial orchards of three cultivars.

Material and methods

Plant material and site description

Trials were conducted with fruit from commercial apple orchards, located in Chimbarongo, O'Higgins Region, Chile. The cultivars evaluated were Gala 'Brookfield', Granny Smith and Fuji 'Raku Raku', planted in 2000 on MM106 rootstock. The conventional Granny Smith and Fuji 'Raku Raku' fruit was obtained from the orchard "Agrocasan" (34° 40' S, 70° 53' W), Gala 'Brookfield' fruit from "Los Alamos" (34° 44' S, 71° 3' W), and organic fruit of three cultivars was obtained from the orchard "Los Pretiles" (34° 44' S, 70° 57' W), in 2011–2012 seasons.

The orchards are located in the same edaphoclimatic zone, at a distance of less than 10 km. The soils in the area are characterized by having a slight slope (less than 3%), being deep (more than 100 cm) and with a clay loam texture. The organic matter content of the orchards was 2.1%, 2.3% and 2.7% for "Agrocasan", "Los Alamos" and "Los Pretiles", respectively. The climate of the area is Mediterranean, with dry and warm summers and rainy and mild winters. The average annual rainfall is close to 388 mm. The average annual air temperature is 14.5 °C. Orchard management was according to commercial requirements, with a drip irrigation system.

The fruit of each cultivar was collected at different growth stages: 26, 34, 40, 68, 96 days after full bloom (DAFB) and at commercial harvest (124, 156, and 175 DAFB for Gala 'Brookfield', Granny Smith and Fuji 'Raku Raku', respectively).

Tissue extraction

A total of sixteen fruit for each treatment group, in four replicates of four apples each, were randomly selected (two apples per tree). Tissue samples consisted of 2 g of whole fruit (skin plus pulp) for each treatment. Samples were then frozen with liquid nitrogen, pulverized and homogenized in a mortar, and then extracted according to the method described by Coseteng & Lee (1987) with some modifications. The tissue was extracted twice (for 10 and 5 min) at 100 °C, with an 80% ethanol solution (ethanol:water 80:20, v/v), and then filtered. Samples were volumetrically diluted to 10 mL with 80% ethanol and kept at -20 °C until use.

Total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method described by Coseteng & 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 5 min and then 0.5 mL of Na_2CO_3 (10%, w/v) added and incubated for 15 min at room temperature (20 °C). Absorbance was measured at 640 nm with a spectrophotometer (Pharo 300, Merck KGaA, Darmstadt, Germany). Total phenolic content was expressed as mg of chlorogenic acid equivalents (CAE) per g fresh weight (FW). Moreover, mg of phenolics/ fruit was calculated.

Antioxidant activity

DPPH method: The capture of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH; Fluka Chemie, Buchs, Switzerland) was measured colormetry by the method described by von Gadow *et al.* (1997). 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 8×10^{-5} M DPPH solution and incubated for 8 min at room temperature. Their absorbance was measured at 515 nm with a spectrophotometer (Pharo 300, Merck KGaA, Darmstadt, Germany). 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) per g FW. Moreover, mg CAE/ fruit was calculated.

Oxygen radical absorbance capacity (ORAC) method:

Table 1. Description of variables used in the multiple regression model.

Variables	Description
Total phenolic content	Expressed in mg CAE/g FW
Antioxidant activity	Expressed in mg CAE/g FW or mmol TE/100 g FW
Quercetin glycosides	Expressed in µg/g FW
Management practices (MAN)	0: conventional management 1: organic management
Fruit growth stage (FGS)	1: 26 DAFB 2: 34 DAFB 3: 40 DAFB 4: 68 DAFB 5: 96 DAFB 6: Harvest
Cultivar (CUL)	Gala, Granny Smith and Fuji
Fruit weight (FW)	Expressed in g
Skin surface area (SSA)	Expressed in cm^2

CAE: chlorogenic acid equivalents. FW: fresh weight. TE: Trolox equivalent. DAFB: days after full bloom

Analysis was performed with a Synergy HT Multi-Detection kit (BioTek Instruments, Inc., Winooski, VT, USA), and a 96-well black-bottom microplate (Nunc, Denmark) was used. Fluorescence was measured from the plate top, wavelength of excitation 485 nm, and emission 528 nm. Equipment was controlled with Gen 5 software. The methodology used was described by Huang *et al.* (2002). Phosphate buffer 75 mM (pH 7.4) was used to prepare the reagents (Trolox®, AAPH and sodium fluorescein). Samples were diluted with 75 mM phosphate buffer (pH 7.4) until a phenolic content between 0.01 and 0.03 mg CAE/mL or g was obtained. Subsequently, 25 µL of each sample and Trolox® standards (Aldrich, USA) were placed on the plate. Then 150 µL of 0.04 µM sodium fluorescein (Sigma-Aldrich, USA) was added. The mixture was incubated for 30 min at 37 °C. Next, 25 µL of 300 µM AAPH (Aldrich, USA) was added and the equipment shook for 8 s. Kinetics was then started for 60 min, with fluorescence readings every minute. Antioxidant capacity was calculated by software, through interpolation of the Trolox® standard curve (0, 6.25, 12.5, 25, 50 and 100 µM) with the net area under the curve (Net-AUC). Values were expressed as mmol Trolox equivalent (TE) per 100 g FW. Moreover, mmol TE/ fruit was calculated.

Quercetin glycosides

The determination of quercetin glycosides in 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 259 × 4.6 mm with a pre-column of the same characteristics, maintained at 20 °C. Briefly, 20 µL, previously filtered (0.45 µm filter), were injected. To identify the compounds, different standards of quercetin glycosides were used with the UV-VIS spectra: Quercetin 3-D-galactoside, Quercetin 3-glucoside, Quercetin 3-xyloside, Quercetin 3-arabinoside, Quercetin 3-rhamnoside (Extrasynthese, Genay, France). The chromatogram was monitored at 300 nm. The solvents of the mobile phase were: A: formic acid 1% in H_2O HPLC-grade; B: acetonitrile 40% in H_2O, and C: acetonitrile. The elution time used was: 0-10 min: A (70), B (30), C (0) flow 1 mL/min; 45 min: A (25), B (75), C (0) flow 0.5 mL/min; 52 min: A (0), B (0), C (100) flow 1 mL/min; and 55 min: A (70), B (30), C (0) flow 1 mL/min. Values were expressed as µg equivalents of quercetin glycosides per g FW.

Determination of skin surface area

In order to determine fruit skin surface, the nonlinear model presented by Clayton *et al*. (1995) for apple trees was used. Surface area (A) was determined by the Eq. (1):

$$A = d \times x^e \quad (1)$$

where: x = mass (kg) or volume (m^3) and d, e = parameters for each cultivar.

Statistical analysis

Data were analyzed statistically using a truncated multiple regression model (SAS software, SAS Inst., Inc., Cary, NC, USA), which considered management practices (MAN), fruit growth stage (FGS), cultivar (CUL), fruit weight (FW) and skin surface area (SSA) as independent

Table 2. Results of multiple regression model of management type, growth stage, cultivar, skin surface and fruit weight on total phenolic content and antioxidant activity measured by DPPH and ORAC method.

Parameters	Total phenolic			DPPH			ORAC		
	Coeff	Value Z	$p > Z$	Coeff	Value Z	$p > Z$	Coeff	Value Z	$p > Z$
Management	0.170	0.860	0.390	0.120	0.660	0.507	56	0.360	0.721
Growth stage	-0.800	-3.003	0.000	-0.830	-3.210	0.001	-1,784	-7.870	0.000
Gala × Fuji	-0.860	-3.360	0.001	-0.740	-3.170	0.002	61	0.330	0.744
Granny × Fuji	0.410	1.700	0.089	-0.870	-3.950	0.000	-476	-2.500	0.012
Gala × Granny	-1.260	-4.980	0.000	0.150	0.700	0.482	537	2.800	0.005
Skin surface	-0.110	-3.890	0.000	-0.014	-0.590	0.556	81	3.680	0.000
Fruit weight	0.060	0.340	0.640	0.004	0.210	0.831	53	-3.200	0.001
Constant	8.940	24.570	0.000	6.880	0.320	0.030	6,020	22.530	0.000

Parameter coefficients (Coeff) of total phenolic content and antioxidant activity measured by DPPH method are expressed in mg CAE/g FW. Parameter coefficients of antioxidant activity measured by ORAC method are expressed in mmol TE/100 g FW. CAE: chlorogenic acid equivalents. FW: fresh weight. TE: Trolox equivalent.

variables (Table 1). The variables evaluated were total phenolic content, antioxidant activity and quercetin glycosides. The statistical model used to calculate each variable was determined by the Eq. (2):

$$Variable\ evaluated = \alpha + \beta MAN + \beta FGS + \beta CUL + \beta FW + \beta SSA + \varepsilon \quad (2)$$

Results

Total phenolic content

According to the regression models for the total phenolic content, management practices and fruit weight factors had a positive correlation, although lower, in the cultivars (0.17 and 0.06 mg CAE/g FW, respectively) (Table 2).

On the other hand, total phenolic content was negatively correlated with fruit growth stage; as fruit developed, concentration decreased on average by 0.8 mg CAE/g FW (Table 2), reaching harvest with concentrations fluctuating between 1.5 and 2 mg CAE/g FW (Fig. 1). However, even though concentration decreased, phenolic content per fruit increased steadily throughout the season (Fig. 2 and 3). Regarding fruit skin, as apples grew and surface area increased, total phenolic content decreased, reducing on average by 0.11 mg CAE/g FW for each cm^2 increase in surface area among each of the stages (Table 2).

Regarding the cultivar, this was a determining factor, with cv. Gala being the lowest, averaging 0.86 and 1.26 mg CAE/g FW less than cvs. Fuji and Granny Smith, respectively. Upon comparing the latter, it was observed that Granny Smith reached 0.41 mg CAE/g FW more than Fuji, although this difference was not significant (Table 2).

Antioxidant activity

Based on the regression models, antioxidant activity in fruits of cvs. Gala, Granny Smith and Fuji was not influenced by management practices (Table 2).

Antioxidant activity decreased as fruit grew, with values ranging from 8 mg CAE/g FW or 7 mmol TE 100/g FW at 20 DAFB to 1 mg CAE/g FW or 1 mmol TE/100 g FW at harvest for DPPH and ORAC methods, respectively (Fig. 1). As antioxidant activity decreased per gram tissue, however, when content per fruit was considered, it increased as apples grew, reaching values of 300 mg CAE/fruit or 300 mmol TE/fruit, according to DPPH and ORAC, respectively (Fig. 2).

Fruit skin surface area showed different results based on the methodology used for antioxidant activity determination. The regression model based on data from DPPH did not show a significant relationship between skin surface area and antioxidant activity (Table 2). The regression model based on data from ORAC showed a positive association between skin surface area and antioxidant activity, increasing by 81 mmol TE for each cm^2 increase in fruit surface area (Table 2).

Results for fruit weight did not coincide between the two methodologies. The regression model based on the data from DPPH method showed that no response of fruit weight on antioxidant activity was observed (Table 2). In contrast, the regression model that showed activity in ORAC units indicated an association between fruit weight and antioxidant activity, with the latter decreasing by 53 mmol TE for each gram increase in fruit (Table 2).

Cultivar factor was determinant in fruit antioxidant activity. Fuji showed the highest activity with values ranging between 7.7 and 1.7 mg CAE/g FW, followed by Gala and Granny Smith with mean values of 5.9 and 1.6 mg CAE/g

Table 3. Results of multiple regression model of management type, growth stage, cultivar, skin surface and fruit weight on quercetin glycoside concentration.

Parameters	Quercetin galactoside			Quercetin glucoside			Quercetin xyloside		
	Coeff	Value Z	$p > Z$	Coeff	Value Z	$p > Z$	Coeff	Value Z	$p > Z$
Management	0.960	2.930	0.003	0.240	3.6400	0.000	0.500	3.01	0.003
Growth stage	2.690	4.960	0.000	0.410	3.5100	0.000	2.190	7.50	0.000
Gala × Fuji	-1.570	-3.960	0.000	-0.210	-2.6100	0.009	-1.440	-6.80	0.000
Granny × Fuji	-1.570	-3.990	0.000	-0.135	-1.6700	0.094	-0.540	-2.81	0.005
Gala × Granny	0.005	0.010	0.988	-0.079	-0.9800	0.329	-0.900	-4.33	0.000
Skin surface	-0.170	-3.920	0.000	-0.021	-2.2700	0.023	-0.142	-5.95	0.000
Fruit weigh	0.110	3.730	0.000	0.015	2.3800	0.017	0.088	5.38	0.000
Constant	0.530	0.740	0.004	-0.128	-0.7500	0.040	0.057	0.14	0.005
	Quercetin arabinoside			**Quercetin rhamnoside**			**Total quercetin**		
Management	0.380	2.430	0.015	0.630	2.900	0.004	2.71	2,930	0.004
Growth stage	2.290	8.310	0.000	2.970	7.520	0.000	10,140.41	8,310	0.015
Gala × Fuji	-0.970	-4.800	0.000	-2.920	-9.33	0.000	-5,931.18	-9.33	0.000
Granny × Fuji	-0.410	-2.200	0.028	-1.110	-4.44	0.000	-2,681.08	-3,990	0.000
Gala × Granny	-0.560	-2.840	0.005	-1.810	-6.04	0.000	-1,811.53	-6.04	0.000
Skin surface	-0.170	-7.470	0.000	-0.216	-6.56	0.000	-0.719	-5.95	0.000
Fruit weigh	0.109	6.800	0.000	0.137	6.11	0.000	0.459	6,800	0.000
Constant	0.837	13.440	0.000	0.826	1.67	0.009	2,122	1.67	0.009

Parameter coefficients (Coeff) of quercetin glycoside concentration are expressed in µg/g of fresh weight.

FW, although the latter showed no differences between them (Table 2). Antioxidant activity expressed in ORAC units was equal between Fuji and Gala, with values ranging between 6.6 and 1.6 mmol TE/100 g FW, while Granny Smith had the lowest activity with values between 5.4 and 1.5 mmol TE/100 g FW (Table 2).

Quercetin glycosides

A significant association between management practices and quercetin glycoside concentration was found in the regression models (Table 3). This was evident in organic fruit, where mean tissue concentration was between 0.23 to 0.63 µg higher than in conventional tissue, depending on cultivar and quercetin type (Table 3).

During the season, the dynamics of quercetin glycoside concentration showed a relatively stable content, although with an increasing trend in its concentration (Fig. 4). This coincided with the results obtained at statistical level, where fruit growth stage showed a positive association with quercetin glycoside concentration, resulting in an increase in these compounds as the fruit began to grow. Concentration of quercetin galactoside, xyloside, arabinoside, and rhamnoside increased on average by 2 µg/g tissue per measurement date, while quercetin glucoside only increased by 0.4 µg/g per stage (Table 3).

Skin surface area showed a negative association with quercetin glycoside concentration, meaning that as surface area increased, these metabolites concentration decreased. The phenols that showed the greatest association on surface area increase was quercetin rhamnoside, which decreased by 0.21 µg per cm^2 increase in area, followed by quercetins galactoside, arabinoside and xyloside, which decreased by 0.17 µg per cm^2 increase. The compound with the lowest dependence on skin surface area was quercetin glucoside, which only decreased by 0.02 µg per cm^2 increase in fruit area.

Cultivar was also a determining factor in fruit quercetin concentration. Both quercetins galactoside and glucoside showed higher concentrations in cv. Fuji, while cvs. Granny Smith and Gala showed no differences between them. Content of quercetins xyloside, arabinoside and rhamnoside was higher in cv. Fuji, followed by Granny Smith, with the lowest concentration in cv. Gala.

A positive association was observed between fruit weight and quercetin glycoside concentration, increasing concentration between 2 µg and 14 µg per 100 g increase in fruit weight, according to compound type.

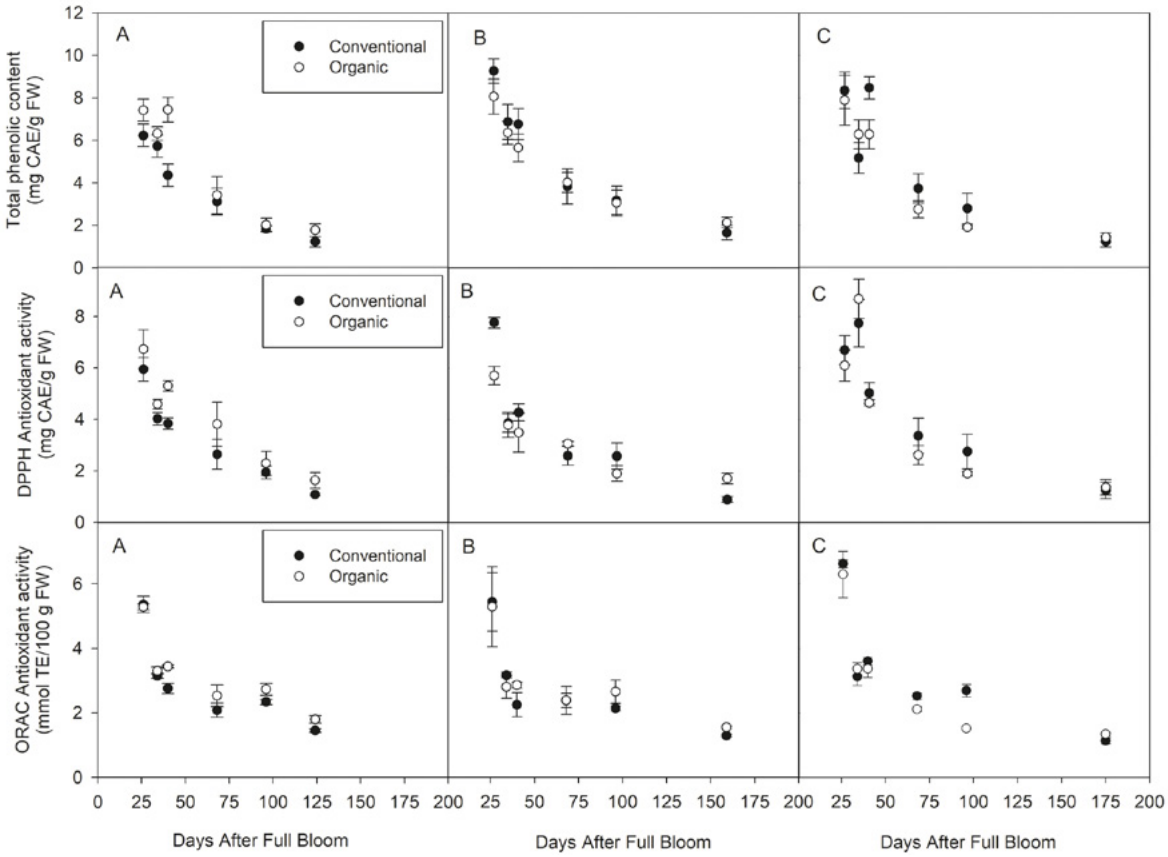

Figure 1. Evolution of total phenolic content, DPPH and ORAC antioxidant activity during fruit development in Gala (A), Granny Smith (B) and Fuji (C) apples in conventional and organic management. Mean ± SD. DPPH: 2,2-diphenyl-1-picrylhydrazyl. ORAC: oxygen radical absorbance capacity.

Discussion

In early developmental stages of apple fruit, total phenolic content reached its highest level and then decreased until harvest (Fig. 1). Conversely, phenolic content per fruit increased steadily by late season (Fig. 2). High initial concentration of polyphenols may be attributed to high levels of enzyme activity (*e.g.* phenylalanine ammonia-lyase (PAL), chalcone isomerase (CHI) and glycosyltransferase (UFGT)) and biosynthesis of these compounds at cell division (Lister *et al.,* 1996; Treutter, 2001; Yuri *et al.,* 2012). The subsequent decline may be linked to an enzyme activity decrease and dilution due to fruit growth (Awad *et al.,* 2001; Takos *et al.,* 2006; Renard *et al.,* 2007).

Phenolic accumulation rate decreases throughout the fruit growth phases, although it never ceases, possibly explaining the increase in total phenolic content per fruit (Fig. 3). However, even though total phenol concentration showed a strong decreasing tendency, that of specific phenols such as glycosidic quercetins showed a relatively stable concentration that tended to increase late in the season (Fig. 4). Similar results were found by Takos *et al*. (2006).

According to Awad *et al.* (2001) changes in the concentration and amount of individual flavonoids and chlorogenic acid during development and ripening were investigated by reversed-phase high performance liquid chromatography (RP-HPLC), quercetin glycoside content tends to increase during fruit growth and development, but with levels and fluctuations mainly attributed to cultivar. In contrast, Yuri *et al.* (2014) observed that quercetin glycoside concentration in whole fruit from cvs. Granny Smith and Fuji increases up to 32 DAFB, and then decreases until harvest, while the content per fruit increased progressively. However, in another study, Yuri *et al.* (2012) found that quercetin glycoside levels in three apple cultivars increased during early developmental stages, then decreased and finally increased again. Likewise, Bizjak *et al.* (2013) reported an increase in quercetin content in cv. Braeburn apples throughout fruit ripening, tending to decrease in the last week before harvest. These results coincide with those observed in the present study, and with those noticed by Takos *et al.* (2006), who determined that the transcription of the MdFLS gene (flavonol synthetase enzyme), which modulates flavonol synthesis, reached its maximum values at 48 and 147 DAFB in cv. Pink Lady™, so that there would be synthesis peaks after cell division.

The trend for antioxidant activity in all cultivars was similar to that observed for total phenolic content, decreas-

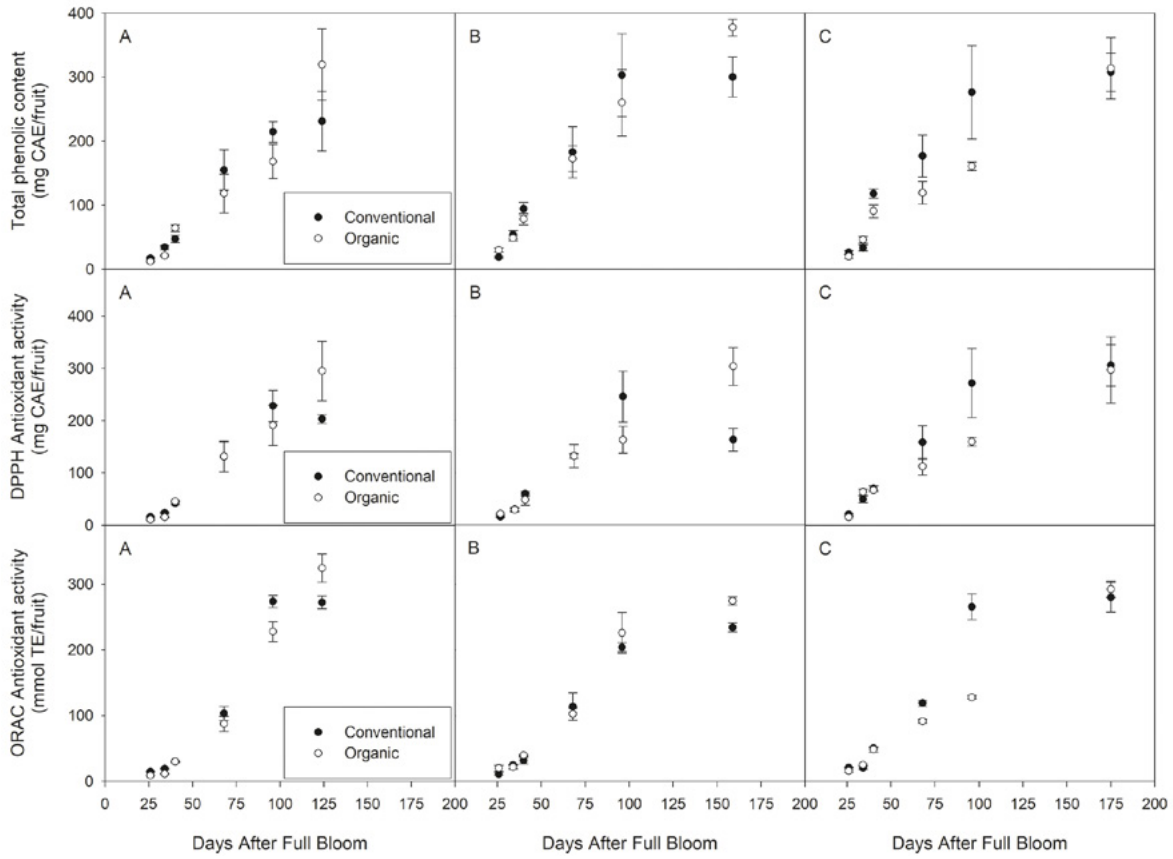

Figure 2. Evolution of total phenolic content per fruit, DPPH and ORAC antioxidant activity in Gala (A), Granny Smith (B) and Fuji (C) apples in conventional and organic management. Mean ± SD. DPPH: 2,2-diphenyl-1-picrylhydrazyl. ORAC: oxygen radical absorbance capacity.

ing from early developmental stages until harvest (Fig. 1), while total antioxidant activity per fruit progressively increased up to the end (Fig. 2). Similar results were found by many investigators (*e.g.* Awad *et al.,* 2001; Yuri *et al.,* 2012, 2014). Phenolic compounds and antioxidant activity are expected to act similarly during fruit growth and development, as their relationship is well documented in literature (Lee *et al.,* 2003). Antioxidant properties of phenolic compounds are directly linked to their structure, since their aromatic rings are potentially capable of scavenging free radicals, thus forming stable compounds (Rice-Evans *et al.,* 1996; Bors & Michel, 2002).

Concentrations of phenolic compounds and antioxidant activity in fruit from conventional and organic orchards have been of interest to many investigators. The observed reports are inconsistent in determining management practices effects on these compounds concentration (Peck *et al.,* 2009; Roussos & Gasparatos, 2009; Stracke *et al.,* 2009). Valavanidis *et al.* (2009) found no differences in phenolic content and antioxidant activity according to management in different apple cultivars. Also, no differences in polyphenol levels and antioxidant activity were observed by Peck *et al.* (2009) in apple cultivars produced in integrated *vs.* organic system. On the other hand, Stracke *et al.* (2009) and Petkovsek *et al*. (2010) found that fruit produced in orchards with conventional or integrated management had lower phenolic content and antioxidant activity than those found in organic orchards, which was associated with the organic orchards being more exposed to biotic and abiotic stress conditions. It is important to mention that the studies cited were carried out taking fruit at harvest, so the results obtained would not necessarily reflect the management practices used in the orchards, while our study was conducted at different season times. According to Weidner *et al*. (2011), depending on the intensity and duration of stress conditions, changes at cellular level can be reversible or irreversible. Stracke *et al*. (2009) found that the concentration of phenolic compounds and antioxidant activity varied more between seasons than between organic and conventional management.

Skin surface area and fruit weight were variables incorporated into the models, since literature shows that there is a decrease in phenol concentration over time. The latter seems to be linked to these metabolites dilution due to the increase in fruit size and skin surface area. Yet, results did not show a negative association of fruit weight with phenolic content and antioxidant activity, but rather a positive correlation between both, excluding the antioxidant capacity expressed in ORAC units.

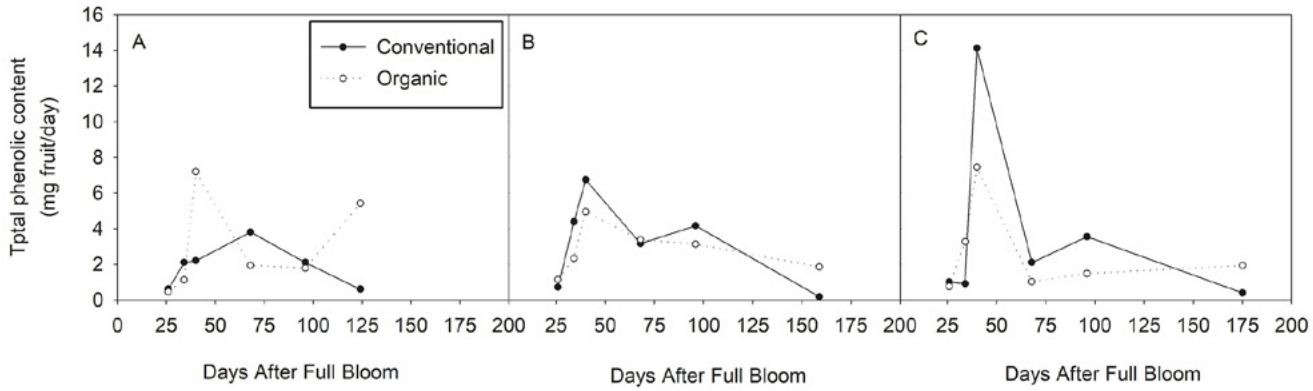

Figure 3. Evolution of total phenolic content accumulation per day in Gala (A), Granny Smith (B) and Fuji (C) apples in conventional and organic management.

Stopar *et al*. (2002) reported that a significant decrease in fruit number per plant in cv. Jonagold greatly increased fruit weight and phenol concentration. According to Lux-Endrich *et al.* (2000), an increase in sucrose and a decrease in macronutrients, in a growing medium with apple shoots, increased accumulation of glycosidic quercetins, catechins, procyanidins and phenolic acids.

Skin surface area showed a negative association with phenolic content and antioxidant activity, although antioxidant activity expressed in ORAC units showed opposite trends. Awad *et al.* (2001) found similar results, observing that skin phenol concentration in cvs. Elstar and Jonagold decreased as fruit grew and skin surface area increased.

Conclusions

The content of phenolic compounds, glycosidic quercetins and antioxidant activity are variables that are conditioned by several factors. In the study, the cultivar factor was highly determinant in apples, decreasing its content throughout the season, given by a dilution effect as its volume increased. This was shown in the fruit growth stage and skin surface factors. No significant differences were found for fruit weight. Both conventional and organic management practices had a minor relevance in the variables studied, being significant only for quercetin glycoside concentration, which is the main polyphenol in apples.

Authors' contributions

Conceptualization: J.A. Yuri.
Data curation: F. Maldonado.
Formal analysis: F. Maldonado, J.A. Yuri, A. Neira, I. Razmilic.
Funding acquisition: J.A. Yuri.
Investigation: F. Maldonado.
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Project administration: J.A. Yuri.
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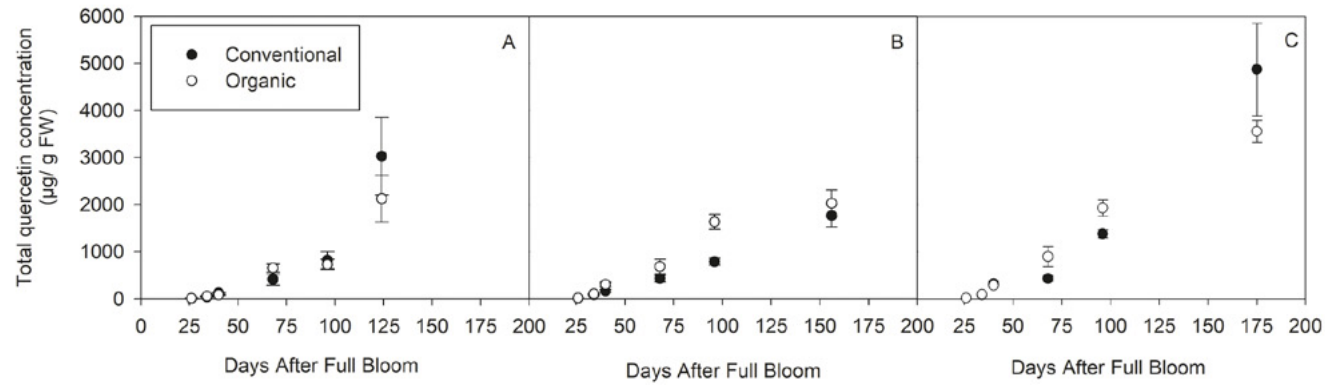

Figure 4. Evolution of total quercetin concentration during fruit development in Gala (A), Granny Smith (B) and Fuji (C) apples in conventional and organic management. Mean ± SD.

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