Journal of Applied Botany and Food Quality 92, 288 - 297 (2019), DOI:10.5073/JABFQ.2019.092.040

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A new sunburned apple category browning under conventional and organic management: phenolic compounds and antioxidant capacity in cold storage

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(Submitted: November 23, 2018; Accepted: June 18, 2019)

Summary

The effect of mild sunburn damage on three apple cultivars: Brookfield®, Granny Smith and Fuji from conventional and organic orchards in the 6th Region of Chile were evaluated in 2012/2013. Total and specific phenols, antioxidant capacity and ripeness of the fruit were assessed at harvest and different time of conventional cold storage: after one, two and four months. According to the results, the peel of all the cultivars have their own property and the response to sunburn damage is independent of the type of management. Phenolic compounds content in the peel of sun-damaged apples at harvest was twice as high in comparison with the peel of healthy fruit. After four months, the apples with mild damage under this condition had higher phenolic compounds and antioxidant content than healthy fruit at harvest. Considering these successful results, a new category of "sunny – apple", we propose that has a higher content of antioxidant compounds.

Key words: *Malus domestica*, sunburn, phenolic compounds, antioxidants, alimentation, healthy human nutrition, "sunny-apple".

Introduction

Sunburn damage is a serious problem affecting apples produced in Chile, resulting in losses of up to 40% among susceptible cultivars like Fuji and Braeburn. Depending on the season, estimated commercial losses can exceed US$ 70 million per year owing to the impossibility of exporting fresh fruit due to their appearance defects (YURI et al., 2010). Numerous studies have been conducted to determine the causes and effects of sunburn damage, high temperatures being the main factor identified in the sunburn damage, high temperatures being the most important factor identified under the conditions of cultivation in Chile (YURI et al., 2009, 2012).

Sunburn damage has been reported in other fruits (raspberries and grapes) and vegetables (tomatoes and peppers) and accounts for significant economic losses in various regions of the world it (SCHRADER et al., 2003) owing to drastic reductions in yield and fruit quality.

While the main causes of physiological disorders are the prolonged exposure of the fruit to high temperatures and high levels of solar radiation, there are other factors that predispose the appearance of this defect, such as sensitive cultivars like Braeburn, Fuji and Jonagold, water stress, the orientation of the planting rows, the training system, the intensity of pruning and nutritional imbalances (CONTRERAS et al., 2008; WÜNSCHE et al., 2004 and YURI et al., 2000).

The initial symptom of sunburn damage is white, brown or yellow spots on the sun-exposed side of the fruit. With severe damage to the peel the affected areas can assume dark coloring in the tree. This damage not only represents an appearance problem but can also alter the characteristics of ripeness in the affected area, leading to softening and possible rotting during storage (YURI et al., 2010).

According to previous studies (CONTRERAS et al., 2008), sunburn damage is a defense mechanism developed by the fruit as a response to oxidative stress. The sensitivity of apple cultivars is related to the physiochemical properties of the peel of the fruit (YURI et al., 2009). The plants have developed antioxidant defense systems based on compounds like vitamin C (ascorbic acid), vitamin E (α-tocopherol), flavonoids, carotenoids and phenols, enzymes like peroxidase and polyphenol oxidase. The progressive rise in temperature in plant tissue increases respiration and consequently metabolic reactions. However, temperatures over a certain limit denaturalize enzymes and coagulate proteins, resulting in deteriorated tissue (YURI et al., 2000).

In addition to primary compounds like sugar and organic acids, apples contain a series of secondary metabolites like polyphenols, which together provide the characteristics and qualities of the fruit. Among the polyphenols are notably chlorogenic acid, phloridzin, anthocyanin, catechins and quercetin (KAHLE et al., 2005).

Along with their nutritional benefits, polyphenols contribute to the plant's resistance against numerous diseases. Flavonoids and phenolic acid affect the firmness of apples (MARTINEZ et al., 2000; LEE et al., 2003).

It is well known that polyphenols are beneficial for human health, contributing to the prevention of cancer, cardiovascular diseases, diabetes, pulmonary disorders, Alzheimer's and degenerative diseases (VRHOVSEK et al., 2004). Apples are an important dietary source of flavonoids. Compared to other fruits, apples have high levels of free phenolic compounds, which can present high levels of absorption in the blood (LEE et al., 2003).

The objective of the present work was to assess the content of total phenols, antioxidant capacity and ripeness in sun-exposed Brookfield®, Fuji and Granny Smith and apples from conventional and organic orchards and after conventional cold storage over four months in order to establish a new category of sun-burned apple.

Materials and methods

Plant Material

During the 2012/13 season a study was undertaken of the evolution of antioxidants in apples (*Malus doméstica* Borkh.) of the cultivars (cvs.) Brookfield®, Granny Smith and Fuji from conventional (CO) and organic orchards (OO) in the General Bernardo O'Higgins Region of Chile (Tab. 1).

The apples were collected from the commercial harvest of the orchards according to two categories: without sunburn damage (healthy) and with mild sunburn damage (damaged) (Fig. 1). Assessments were made at harvest and at 1, 2 and 4 months of conventional storage after one day of post-storage at room temperature. Specific phenolic compounds were quantified and antioxidant capacity and ripeness parameters were assessed.

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Tab. 1: Orchards and cultivars sampled for storage test. Season 2012/13.

Cultivars	Management	Orchard	Location	Harvest time
Brookfield®	Conventional	Chimbarongo	34°45' S / 71°03' O	February 7, 2013
	Organic	Tinguiririca	34°44' S / 70°57' O	
Granny Smith	Conventional	Codegua	34°40' S / 70°54' O	March 12, 2013
	Organic	Tinguiririca	34°44' S / 70°57' O	
Fuji	Conventional	Codegua	34°40' S / 70°54' O	March 28, 2013
	Organic	Tinguiririca	34°44' S / 70°57' O	

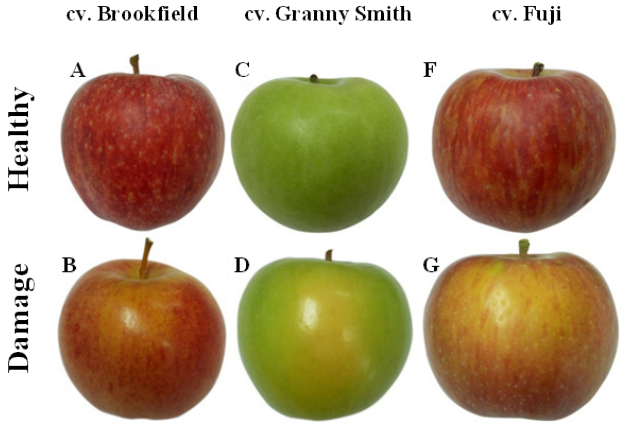

Fig. 1: Apples used in the test. Above healthy fruits. (A and B cv. Brookfield®; C and D cv. Granny Smith; F and G cv. Fuji).

The assays applied were:
Assay 1: Total phenolics and ripeness parameters in healthy and damaged fruit at harvest:
Healthy and sunburn fruits from conventional and organic orchards, were analyzed on Brookfield, G. Smith and Fuji cvs.
Assay 2: Total phenolic compounds and antioxidant capacity in apple peel and flesh during storage: Healthy and sunburn fruits from conventional and organic orchards, were analyzed on Brookfield G. Smith and Fuji cvs.
Assay 3: Ripeness parameters during cold storage: the pulp firmness of Healthy and sunburn fruits from conventional and organic orchards, were analyzed on Brookfield, G. Smith and Fuji cvs.
Assay 4: Sunscald evaluation: damage assessment was carried out on apple from conventional and organic management orchards in G. Smith cv. at harvest and after 1, 2 and 4 months of cold storage. 5 repetitions of 50 fruits were used.
Assay 5: Comparing apples at harvest and after four months of cold storage.
Phenolic compounds and ORAC were analyzed in peel and flesh of healthy and sunburn apple in Brookfield, G. Smith y Fuji cvs. from conventional and organic management orchards, to harvest and after 4 month of cold storage
Assay 6: Quantification of specific phenols by high-pressure liquid chromatography (HPLC). Healthy and sunburn apple in Brookfield, G. Smith y Fuji cvs. from conventional and organic management orchards after 4 month of cold storage, were analyzed.

Extract preparation to determine phenolic compounds and antioxidant capacity
Peel and flesh were taken from the fruit. The sample were then frozen with liquid nitrogen, pulverized and homogenized in a mortar and pestle. The method for extraction was used with some modification. Briefly, tissue was extracted twice with a solution of ethanol at 80% (ethanol: water 80:20, v/v) for 10 and 5 min at 100 °C. Subsequently, the solution was filtered, graduated at 10 mL with ethanol at 80% and stored at -20 °C until use.

Determination of phenolic compound concentrations
Phenolic compound content was determined by the Folin-Ciocalteu method (Cosetenc et al., 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) was added and incubated for 15 (min) at room temperature (20 °C). Absorbance was measured at 640 nm with a spectrometer. Total phenolic concentrations in the peel and flesh were expressed as mg of chlorogenic acid equivalents (CAE) g^{-1} fresh weight.

Antioxidant activity
Oxygen Radical Absorbance Capacity (ORAC) method: The method previously described (Huang et al., 2002; Prior et al., 2003) was used with modifications. For the procedure (Trolox) 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) was used. Fluorescein sodium (FL) (Sigma-Aldrich) was used in the preparation of solvent buffers and salts (Merck S.A.). A stock solution of 500 uM of Trolox in a 75-mM phosphate buffer with a pH of 7.4. A calibration curve was prepared with concentrations between 6.25 and 100 uM. Fluorescein 4×10^{-6} mM was used as fluorescent compound, while the peroxyl radical AAPH was used in a concentration of 150 mM. Black 96-well polystyrene flat-bottomed plates (NUNC 237108) were used, in which 25 µL of each concentration of Trolox were placed, corresponding to the standard curve and of the samples in appropriate dilutions. Making use of the injectors of the equipment, 150 µL of a fluorescent compound and 25 µL of AAPH were added to each well. The assays were conducted at a temperature of 37 ºC, with an excitation wavelength of 485 nm and an emission of emission 520 nm, and a kinetic of one hour on a spectrofluorometer with a Synergy HT microplate reader (Yuri et al., 2010, 2012).

Determination of specific phenolic compounds by High-performance liquid chromatography (HPLC)
Specific phenol (chlorogenic acid, catechin, procyanidin B2, quercetins glycosides and phloridzin) in the samples were determined using a 100-5 C18 Kromasil column of 250 mm × 4.6 mm × 5 µm with a pre-column of the same characteristics, maintained at 25 °C. A Smartline HPLC-PDA system from Knauer (Germany)), equipped with a Manager 5050 (degasser module), a wp-1000 type water pump, a 3950 autosampler 3950, a 4050 column oven, a PDA 2850 photodiode array detector and ChromGate Chromatography Data Software. Automatic 20 µL 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 / 276 nm / 520 nm. The solvents of the mobile phase were: A: 1% formic acid in H_2O quality HPLC and B: 40% acetonitrile in H_2O, and C: acetonitrile. The elution parameters were: time 0-10 min: A (70), B (30), C (0) flow 1 mL min^1; time 45 min: A (25), B (75), C (0) flow 0.5 mL min^{-1}; time 46 min: A (5), B (75), C (20) flow 1.0 mL min^{-1}; time 50 min: A (0), B (70), C (30) flow 0.5 mL min^{-1}; time 52 min: A (0), B (50), C (50) flow 1 mL min^{-1}; time 55 min: A (70), B (30), C (0) flow 1 mL min^{-1}; time 58 min: A (70), B (30), C (0) flow 0.5 mL min^{-1}; The results were expressed in µg of samples in g of FW^{-1} (YURI et al., 2012).

Ripeness parameter

Ripeness was assessed at harvest and again at 30, 60 and 120 of storage plus one day at room temperature (20 °C) to simulate shelf life. The following indices of ripeness were considered:

Flesh firmness: Two cuts were made on either side sample fruit at the equatorial part to remove sufficient peel for an adequate reading with a digital penetrometer (GS14 model Fruit Texture Analyzer) with an 11-mm plunger (7/6''). The results are expressed in Newton (N).

Soluble solids were assessed with a digital refractometer (Refractec) with a range of 0 to 45 °Brix based using juice made from sample of fruit. The results are expressed in °Brix.

Starch: Cross-sections of apples were assessed after applying a solution of iodine at 0.1% dissolved in potassium iodide at 30%, highlighting the coloring pattern indicating the degree of starch degradation. Using a scale from 1 (no starch degradation) to 10 (maximum starch degradation) from the Centre Technique Interprofessionnel des Fruits et Légumes (CTIFL).

Statistical analysis

With the purpose to evaluate the experimental results, a statistical evaluation through variance analysis (ANOVA) was applied to healthy and sunburn damaged organically- and conventionally grown apples, with replicates per treatment. Depending of the assay, three to five repeats, per factor, were used. An ANOVA was applied for the statistical analysis. When significant different were observed means were compared with the LSD test ($p \leq 0.05$) using Statgraphics Centurion XV software (Warrenton, Virginia, USA).

Results and discussion

Total phenols and ripeness parameters in healthy and damaged fruit at harvest

No significant differences were observed at the time of commercial harvest between the types of orchards (conventional and organic) of the three cultivars under study, except in the peel of cv. Fuji.

Brookfield® and Fuji had similar phenolic compound content levels in the peel, which were higher than the levels in the peel of cv. Granny Smith. The three cvs., from CO and OO showed similar behaviors in response to sunburn damage, with phenolic compound content 1.8±0.2 and 1.7±0.3 as high, respectively for conventional and organic fruit in the peel of the sun-exposed area in damaged fruit than in undamaged fruit (Tab. 2).

The phenolic compound content in the peel of healthy fruit is on average 5.3±1.4 as high as in the flesh. This ratio increases to 8.3± 2.9 with sunburn damaged fruit. The flesh of healthy apples contains between 0.9 and 1.2 mg of phenolic compounds (chlorogenic acid equivalent (CAE)/gram of tissue), while the values for the flesh of damaged fruit is only slightly higher. This indicates the importance of consuming apple peel, as has been indicated in previous publications (HENRIQUEZ et al., 2010; PALOMO et al., 2009).

Comparing the two sides of healthy and sun-burned apples, we found that average phenol compound content was 30% higher on the exposed side than on the unexposed side for healthy examples of all three cultivars, while the difference rose to 60% with sunburn damaged apples, both OO and CO.

The results of the ripeness indices are shown in Tab. 3 respectively for the cultivars Brookfield®, Granny Smith and Fuji. Ripeness indices were not affected by the type of orchard management. Healthy apples generally had higher values for flesh firmness, soluble solids and starch degradation than damaged fruit. However, differences were generally not significant, which could due to the fact that only fruit with slight sunburn damage. OO fruit presented less significant differences between healthy and damaged examples.

Total phenolic compounds and antioxidant capacity in apple peel and flesh during storage

Both at harvest and after conventional cold storage the phenolic compound content and ORAC values were higher in the peel of sunburn damaged apples than in the peel of healthy apples of all three cultivars and with both OO and CO fruit (Fig. 2 and 3).

Tab. 2: Total phenolics compounds evaluation (mg (CAE)/g fresh weight) in three cultivars exposed to the sun from conventional and organic orchards.

Cultivar	Orchard	Peel		Flesh	
		Healthy	Damaged	Healthy	Damaged
Brookfield®	Conventional	7.8	14.2	1.2	1.6
	Organic	7.1	12.1	1.2	1.2
	Sign.(x)	***n.s.***	***n.s.***	***n.s.***	***n.s.***
	p-value	**0.29**	**0.10**	**0.66**	**0.22**
Granny Smith	Conventional	5.3	8.3	1.4[a]	1.5
	Organic	4.8	9.5	1.3[b]	1.5
	Sign.(x)	***n.s.***	***n.s.***	***n.s.****	***n.s.***
	p-value	**0.40**	**0.29**	**0.05**	**0.98**
Fuji	Conventional	7.4 a	14.4 a	1.0	1.1
	Organic	4.3 b	6.3 b	0.9	1.0
	Sign.(x)	*	**	***n.s.***	***n.s.***
	p-value	**0.01**	**0.00**	**0.31**	**0.15**

(x) Significance: n.s., no significant; *, $p \leq 0,05$; **, $p \leq 0,01$. Average separation by LSD Test.

Tab. 3: Maturity index in three cultivars at harvest from conventional and organic orchards.

Cultivar	Type fruit injury	Conventional orchard			Organic orchard		
		Flesh firmness (N)	Soluble solids (°Brix)	Starch degradation (1-10)	Flesh firmness (N)	Soluble solids (°Brix)	Starch degradation (1-10)
Brookfield®	Healthy	80.5 a	12.2	5.3 a	73.0	10.1 b	4.7
	Damaged	73.8 b	12.7	7.9 b	69.4	13.1 a	8.5
	Sign. $^{(x)}$	*	*n.s.*	*	*n.s.*	*	*n.s.*
	p-value	**0.04**	**0.56**	**0.01**	**0.13**	**0.03**	**0.10**
G. Smith	Healthy	95.6	11.1	2.1 a	86.3	10.1	4.0 a
	Damaged	97.9	11.1	3.7 b	88.1	9.8	4.7 b
	Sign. $^{(x)}$	*n.s.*	*n.s.*	*	*n.s.*	*n.s.*	*
	p-value	**0.40**	**0.89**	**0.02**	**0.39**	**0.36**	**0.01**
Fuji	Healthy	77.0 b	13.7 b	5.3	74.7	14.3	5.7
	Damaged	84.5 a	15.1 a	5.8	82.7	15.9	6.4
	Sign. $^{(x)}$	**	**	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	p-value	**0.00**	**0.00**	**0.66**	**0.07**	**0.10**	**0.57**

$^{(x)}$ Significance: n.s., no significant; *, $p \leq 0{,}05$; **, $p \leq 0{,}01$. Average separation by LSD Test.

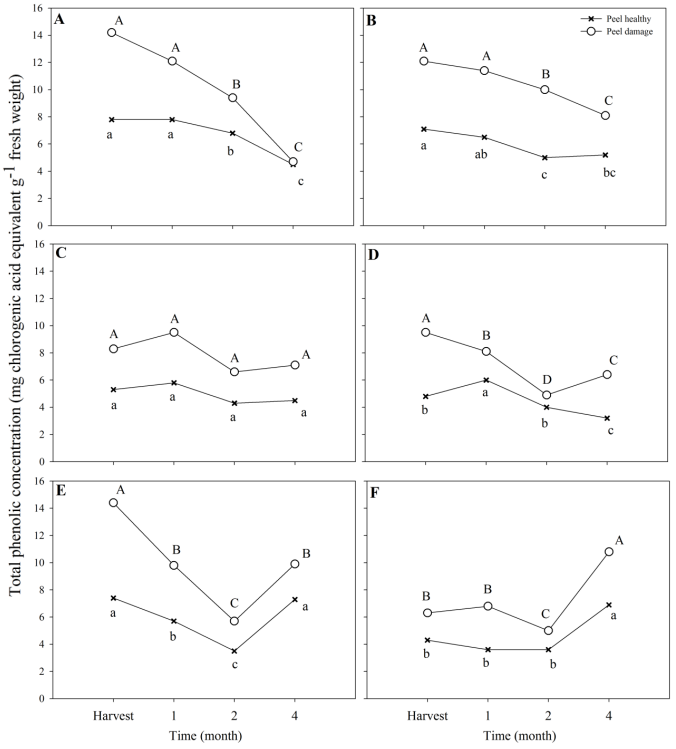

Fig. 2: Evolution of total phenolic compounds in apple peel with sunburned and healthy, from cv. Brookfield® (A and B), Granny Smith (C and D) and Fuji (E and F) of conventional (A, C and E) and organic (B, D and F) orchard, from harvest to 4 months of cold storage.

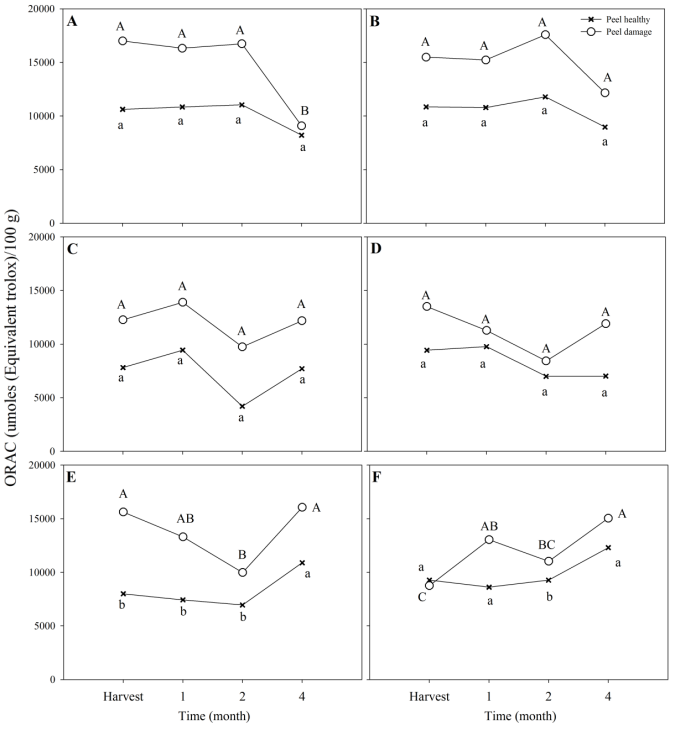

Fig. 3: Evolution of ORAC in apple peel from cv. Brookfield® (A and B), Granny Smith (C and D) and Fuji (E and F), from harvest to 4 months of cold storage from conventional (A, C and E) and organic (B, D and F) orchard, in healthy and sun-burned apple.

Phenolic compound content decreased in the peel of cv. Brookfield® (Fig. 2) during storage. ANOVA were made during the time, from harvest to 4 months of storage. Initially CO sunburn-damaged fruit had higher phenol content, while at the end of the storage period the highest phenol content was found in OO fruit. ORAC was observed to have increased at two months of storage in both OO and CO Granny Smith and Fuji apples, although the increased was only significant in cv. Fuji (Fig. 3).

Fig. 4 and 5 show the evolution of phenolic compounds and ORAC in the flesh of OO and CO apples of the cvs. from harvest to four months of storage. In general terms, phenolic content did not vary with storage time for all three cultivars, both organic and nonorganic. Phenol content was also similar in the flesh of healthy and sunburn damaged fruit. Content values remained the same from harvest to

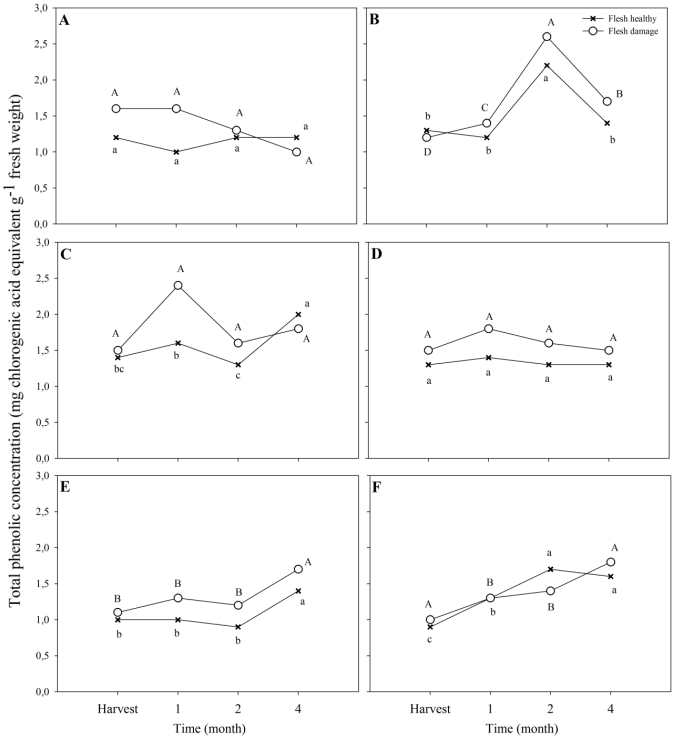

Fig. 4: Evolution of Total Phenols in apple flesh from cv. Brookfield® (A and B), Granny Smith (C and D) and Fuji (E and F), from harvest to 4 months of cold storage from conventional (A, C and E) and organic (B, D and F) orchard, in healthy and sun-burned apple.

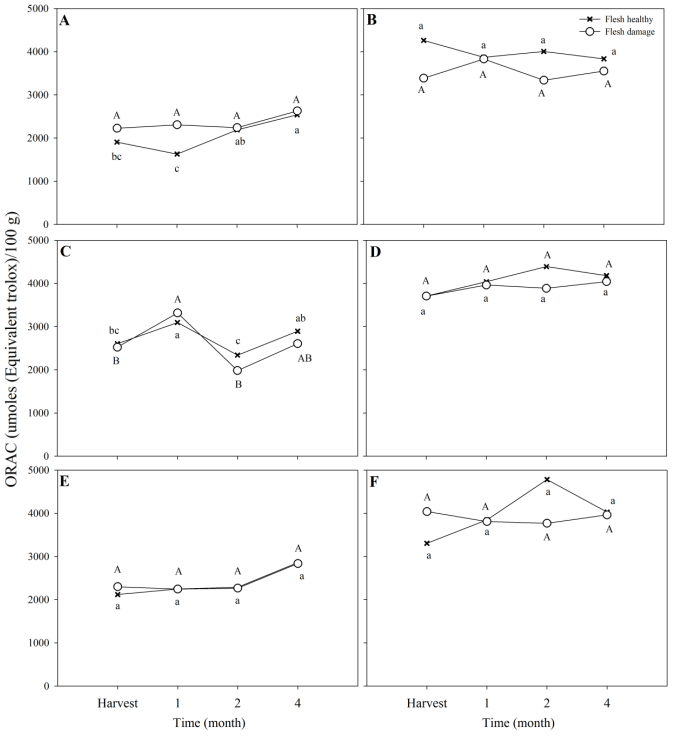

Fig. 5: Evolution of ORAC in apple flesh from cv. Brookfield® (A and B), Granny Smith (C and D) and Fuji (E and F), from harvest to 4 months of cold storage from conventional (A, C and E) and organic (B, D and F) orchard, in healthy and sun-burned apple.

four months of cold storage. The flesh of the three cultivars from the OO had higher ORAC values than samples the orchard under conventional management.

Ripeness parameters during cold storage

The evolution of flesh firmness from harvest to four months of cold storage (plus one day of exposure to room temperature) was analyzed statistically. Flesh firmness was observed to decrease with storage time.

There was a tendency for flesh firmness to decrease with more storage time, which coincides with previous reports (SALDIAS, 2011), where fruit storage for a period of four months showed similar trend. Sun-damaged Granny Smith and Fuji apples had higher flesh firmness values than their healthy counterparts, similar results were reported (RASKÓ et al., 2005). While the opposite was the case with cv. Brookfield®, in which case healthy apples had higher flesh firmness values.

Previous investigation (SALDIAS, 2011), observed that in cv. Granny Smith fruit with sunburn had higher firmness values when compared with healthy fruit, similar results reported previously (SCHRADER et al., 2009) Differences were minimal in the majority of the comparisons made (Fig. 6).

Physiological disorders

Physiological disorders were assessed each time samples were removed from cold storage (at 1, 2 and 4 months of storage), along with assessment of phenolic compounds and ORAC.

Overall, no significant alterations were observed in examples of cv. Brookfield®. Granny Smith apples presented a high incidence of sunscald at four months of storage, reaching 85% among CO apples and 55% in OO samples (Fig. 7). Several authors (CONTRERAS et al., 2008; LURIE et al., 2009) found incidences of sunscald around 95% in fruit storage for two months. The incidence of sunburn damage among Fuji apples was minimal (<4.0%).

According to the above, cv. Granny Smith cannot be stored for prolonged periods with a view to exporting as a new category of apple (sun-exposed) that is richer in antioxidants.

Comparing apples at harvest and after four months of cold storage

We studied the feasibility of storing apples with mild sunburn damage that are otherwise usually designated for the internal market, given that sunburn damage increases phenolic compounds and ORAC in apple peels.

We compared the levels of phenolic compounds and ORAC at harvest and after four months of convention cold storage and found levels were higher in apple peels after harvest than at the end of the assessment period. The quantity of phenolic compounds in the peel of fruit with mild sunburn damage tended to be significantly higher than in the peel of healthy fruit, which was observed in OO samples of all three cultivars (Tab. 6 and 7), but only in Granny Smith and Fuji apples grown under CO (Tab. 4 and 5).

Phenolic compound content in CO sunburn-damaged Brookfield® apples was the same as that of healthy fruit at the end of the storage period. A similar determination of phenolic compound content was made by high pressure liquid chromatography (HPLC) (Fig. 8).

Quantification of specific phenols by high-pressure liquid chromatography (HPLC)

Fig. 8 shows that apple peels contain different phenolic compounds, among them are notably chlorogenic acid, catechin, procyanidin, phloridzin and quercetins, the latter belonging to the flavonoid group. It is worth noting that some of these compounds are also found in

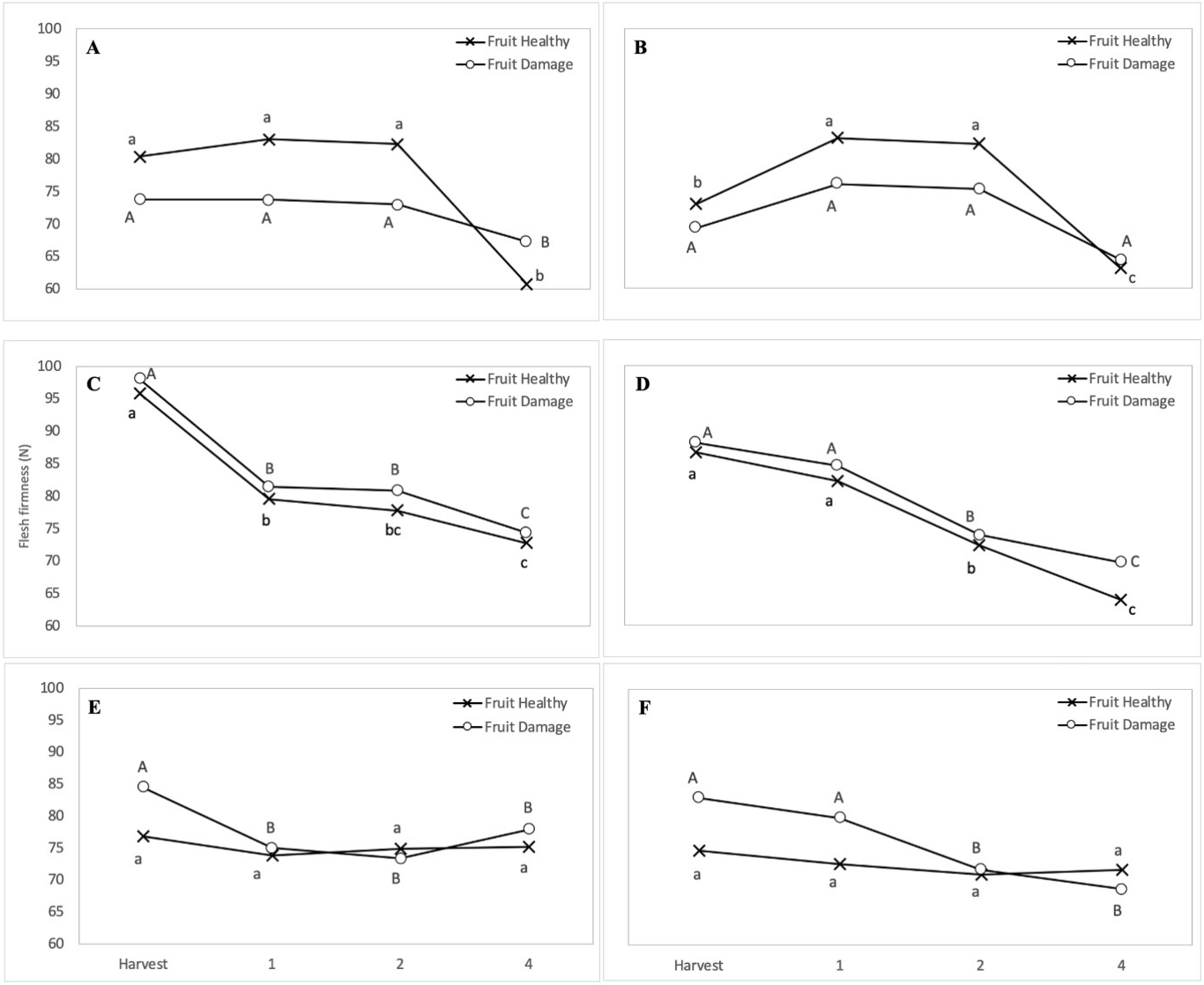

Fig. 6: Evolution of flesh firmness (newton) in apple from cv. Brookfield® (A and B), Granny Smith (C and D) and Fuji (E and F), from harvest to 4 months of cold storage from conventional (A, C and E) and organic (B, D and F) orchard, in healthy and sun-burned apple.

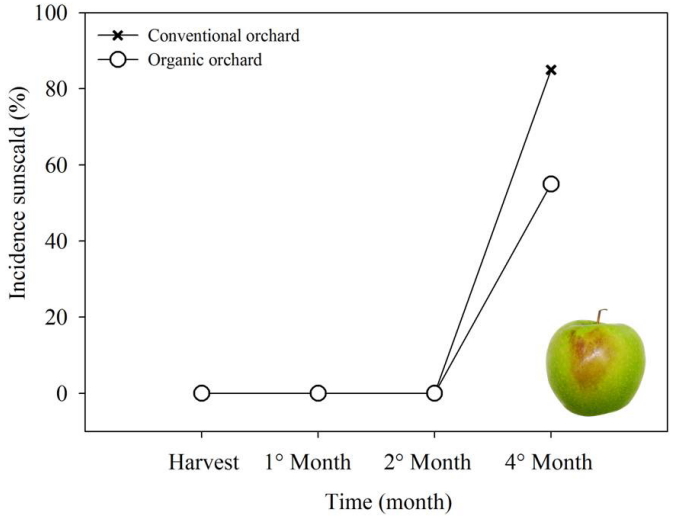

Fig. 7: Incidence of sunscald in apples cv. Granny Smith, after 4 months of cold storage.

apple flesh. However, flavonols are almost exclusively present in apple peel, as is also the case with anthocyanins, which are responsible for the red coloring of apples. Owing to the presence of double bonds, hydroxiphenol groups and ketone, the quercetin molecule is an important antioxidant and constitutes the antioxidant compound in apple peel (LEE et al., 2003; MARTINEZ et al., 2002).

Quercetin glycosides contribute the highest percentage of antioxidant activity in the peel, with 35% versus 1.8% in the flesh in relation to total phenols in each type of tissue (KHANIZADEH et al., 2008). These molecules can prevent the oxidation of low-density lipoproteins (LDL) by eliminating free radicals and transition metal ions. As a result, they can aid in preventing diseases like cancer and chronic inflammation (PALOMO et al., 2010).

Considering the importance of antioxidants for human health, we determined the total presence of quercetin glycosides in healthy and sunburn-damaged fruit from both systems of management. We observed that the level of flavonols in damaged fruit after four months of storage, although lower than the level at harvest, was still higher than the level in healthy fruit at harvest. In both organically and conventionally grown Granny Smith and Fuji apples, levels were 9 and 5 times as high at harvest, respectively, and three times as high for

Tab. 4: Quantification of total phenolics compounds (mg Chlorogenic acid/100 g) in peel and flesh of healthy and sun-burned apple, of conventional orchards, from harvest to 4 months of cold storage, 2012/2013 season. Season 2012/2013.

Treatment	Brookfield®		Granny Smith		Fuji	
	Peel	Flesh	Peel	Flesh	Peel	Flesh
Healthy to harvest	780 b	120	530 bc	140 c	740 c	100 c
Healthy 4 months	450 c	120	450 c	200 a	730 c	140 b
Damaged to harvest	1420 a	100	830 a	150 bc	1440 a	110 c
Damaged 4 months	470 c	160	710 ab	180 ab	990 b	170 a
***Sign.*[(x)]**	**	***n.s.***	*	*	**	**
p-value	***0.00***	***0.13***	***0.03***	***0.03***	***0.00***	***0.00***

[(x)] Significance: n.s., no significant; *, $p \leq 0{,}05$; **, $p \leq 0{,}01$. Average separation by LSD Test.

Tab. 5: Quantification of ORAC (µmol of Trolox equivalent/100g) in peel and flesh of healthy and sun-burned apple, of conventional orchard, from harvest to 4 months of cold storage. Season 2012/2013.

Treatment	Brookfield		Granny Smith		Fuji	
	Peel	Flesh	Peel	Flesh	Peel	Flesh
Healthy to harvest	10624 b	1908	7817 b	2605	8005 c	2121 b
Healthy 4 months	8207 b	2540	7774 b	2894	10894 b	2861 a
Damaged to harvest	16992 b	2227	12268 a	2525	15631 a	2301 b
Damaged 4 months	9088 a	2630	12180 a	2606	16068 a	2839 a
Sign.[(x)]	**	**n.s.**	*	**n.s.**	**	*
p-value	**0.00**	**0.06**	**0.03**	**0.57**	**0.00**	**0.01**

[(x)] Significance: n.s., no significant; *, $p \leq 0{,}05$; **, $p \leq 0{,}01$. Average separation by LSD Test.

Tab. 6: Quantification of total phenolic compounds (chlorogenic acid mg/100 g) in peel and flesh of healthy and sun-burned apple, from organic orchard, from harvest to 4 months cold storage. Season 2012/2013.

Treatment	Brookfield		Granny Smith		Fuji	
	Peel	Flesh	Peel	Flesh	Peel	Flesh
Healthy to harvest	710 b	120 bc	480 c	130	430 c	90 b
Healthy 4 months	520 c	140 b	320 d	130	690 b	160 a
Damaged to harvest	1210 a	120 c	950 a	150	630 b	100 b
Damaged 4 months	810 b	170 a	640 b	150	1080 a	180 a
***Sign.*[(x)]**	**	**	**	**n.s.**	**	**
p-value	**0.00**	**0.00**	**0.00**	**0.51**	**0.00**	**0.00**

[(x)] Significance: n.s., no significant; *, $p \leq 0{,}05$; **, $p \leq 0{,}01$. Average separation by LSD Test.

Tab. 7: Quantification of ORAC (µmol of Trolox equivalent/100g) in peel and flesh of healthy and sunburned apple, of organic orchard, from harvest to 4 months of cold storage, Season 2012/2013.

Treatment	Brookfield		Granny Smith		Fuji	
	Peel	Flesh	Peel	Flesh	Peel	Flesh
Healthy to harvest	10850 bc	4262	9433	3708	9280 c	3306
Healthy 4 months	8976 c	3835	7019	4181	12301 ab	4027
Damaged to harvest	15942 a	3388	13502	3708	8756 b	4040
Damaged 4 months	12156 b	3553	11913	4042	15053 a	3965
***Sign.*[(x)]**	**	**n.s.**	**	**n.s.**	*	**n.s.**
p-value	**0.00**	**0.20**	**0.05**	**0.13**	**0.01**	**0.08**

[(x)] Significance: n.s., no significant; *, $p \leq 0{,}05$; **, $p \leq 0{,}01$. Average separation by LSD Test.

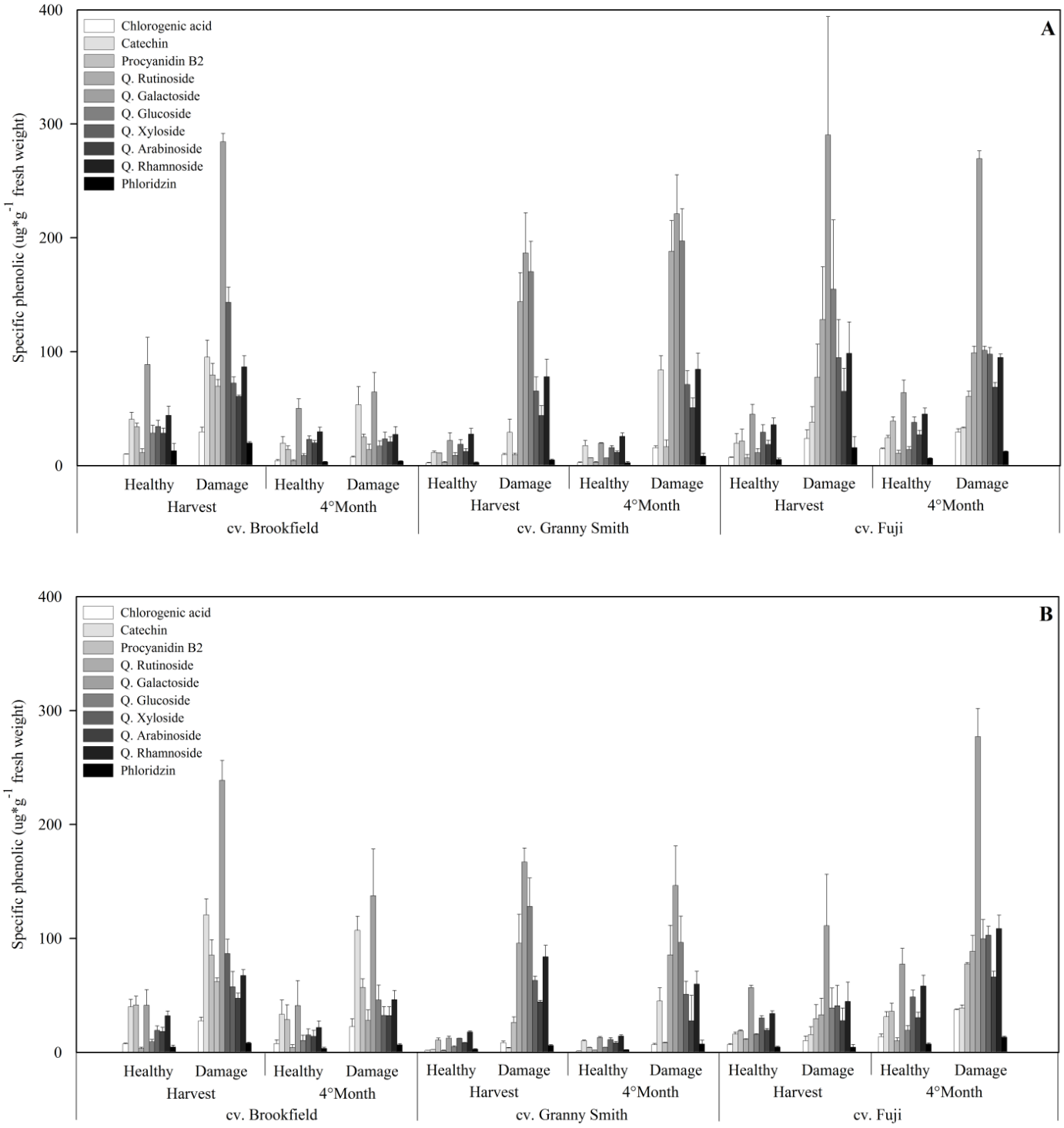

Fig. 8: High-performance liquid chromatography (HPLC). Quantification of specific phenolic compounds in conventional (A) and organic (B) orchard.

organically-grown Brookfield® apples.

Another antioxidant in apples, phloridzin, behaves similarly to quercetins, although it has less effect than that observed for flavonols.

Analysis of the differences in the percentage of each type of quercetin (% in sunburn-damaged apples minus the % in healthy apples, Fig. 9) showed that the percentages of the quercetins rutinoside, galactoside and glucoside increase, while the percentage of xyloside, arabinoside and rhamnoside decrease. This behavior was similar among the three cultivars under study, independent of the type of management and was found both at harvest and after storage, as was described previously for cv. Fuji (YURI et al., 2012). The greatest variation was found in the peel of Granny Smith apples, followed by Fuji. Both cultivars are recognized as susceptible to sunburn.

Conclusions

Phenolic compounds and antioxidant capacity were always higher in the peels of apples with sunburn damage than in the peel of healthy

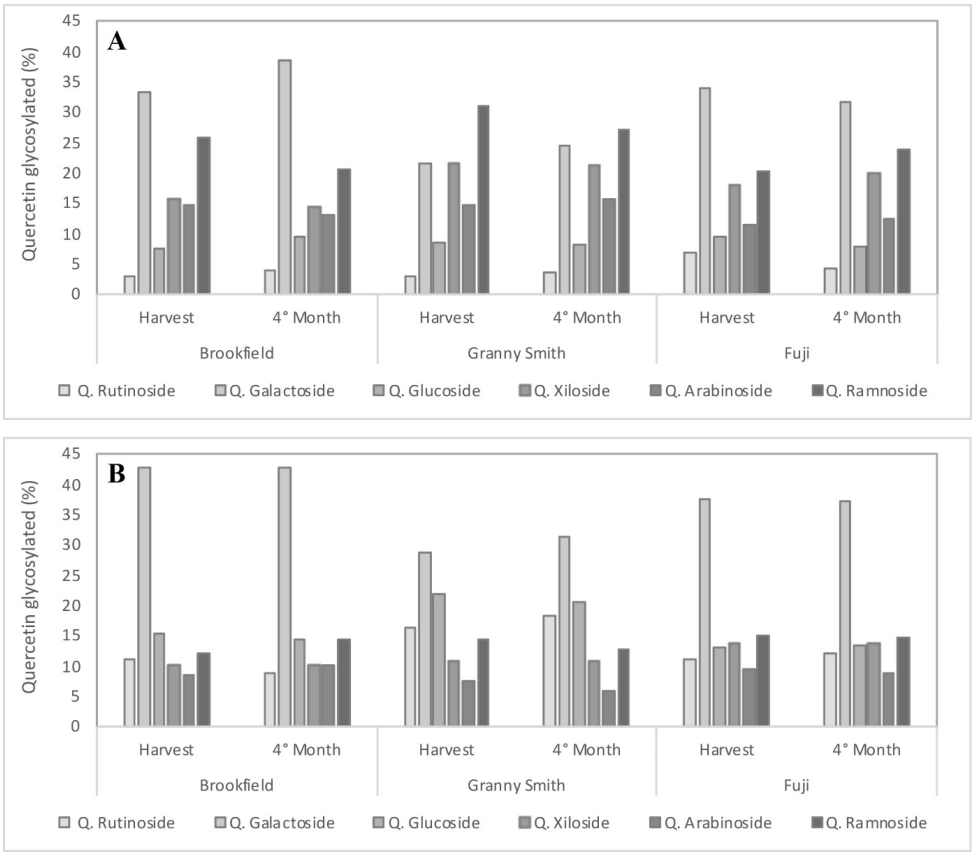

Fig. 9: Quercetin glycosylated between healthy and sun-burned fruit from conventional (A) and organic (B) orchard.

fruit independent of the type of management (conventional or organic). Phenolic compounds and antioxidant capacity decreased in both organically and conventionally grown apples during cold storage. However, apples with sunburn damage had higher phenolic compound content than those healthy at harvest.

Excluding cv. Granny Smith, which evidenced a high incidence of sunscald, we propose that consuming apples with mild sunburn damage could presents benefits for human health given the higher polyphenol content. In these context, we propose a new category of "sunny-apple" with mild sunburn damage, the acceptance of which in target markets should be assessed.

Acknowledgements

This paper is part of the project AF10I1022 supported by "Fondo de Fomento al Desarrollo Científico y Tecnológico" (FONDEF), Chile.

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