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ORIGINAL ARTICLE

Bagging cv. Fuji, Raku Raku Apple Fruit Affects Their Phenolic Profile and Antioxidant Capacity

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Abstract
The objective of the work was to assess the effects of bagging cv. Fuji, Raku Raku apple fruit on the phenolic compounds and antioxidant capacity of the peel using five-year-old trees on M 9 rootstock at San Clemente, Chile (35°S). Total phenolic concentrations were lower in bagged apple fruit than in unbagged control fruit. The antioxidant activity increased once the bags were removed and fruit exposed to light. Bagging also decreased the incidence of sunburn. Chlorogenic acid, catechin and phloridzin in the apple fruit peel were not affected by the bagging treatment. After anthocyanin, quercetins were the phenolic compounds most sensitive to bagging. Bagging neither affected maturity parameters nor their mineralogical composition. In terms of physiological disorders, bagging showed a tendency of decreased incidence of internal browning, but with more rotting.

Keywords Apple (*Malus domestica* Borkh) · Bagged fruits · Phenolics · Antioxidant capacity · ORAC · Physiological disorders · Nutrition

Einfluss des Eintütens von Früchten der Apfelsorte 'Fuji, Raku Raku' auf deren Phenolgehalte und antioxidatives Potential

Schlüsselwörter Apfel (*Malus domestica* Borkh) · Fuji · Antioxidatives Potential · Fleischbräune · Nährstoffe · ORAC · Phenole · Physiologische Schäden · Sonnenbrand

Introduction

Chile is a major exporter of fresh fruit, with apples being the second most important fruit export in terms of volume (ODEPA 2015). Apple cv. Fuji, Raku Raku often bagged for the Taiwan market, which pays a significantly higher price than is paid by the general market. The practice of bagging usually begins at 60 days after foll bloom (DAFB) and continues until 30–15 days before harvest (DBH). Bagging increases the sensitivity to light (Chen et al. 2012) and modifies the composition of pigments in the peel, inhibiting chlorophyll synthesis, because of which the red color provided by anthocyanins develops on a whitish background (Feng et al. 2014), giving the apples a characteristic and unique colouring. Bagging also provides protection against pathogens, insects and sunburn (Zhang et al. 2015). Bagging can affect the composition of phenolic compounds and antioxidant capacity. As a widely consumed food product, apples are an important source of antioxidants (Petkovsek et al. 2010). Among the phenolic compounds are flavonoids, including 3-flavanols, anthocyanin, dihydrochalcone and flavonols (Tsao et al. 2005). The latter play an important role in photoprotection and it is generally considered that they act as protective agents against ultraviolet light (UV) and as radical scavengers (Zoratti et al. 2014). In addition to flavonoids, apples contain hydroxycinnamic acids that contribute to their quality (Awad et al. 2000).

The present study seeks to determine the effect of bagging on the peel of cv. Fuji, Raku Raku apples in terms of phenolic profile, antioxidant capacity, mineral content,

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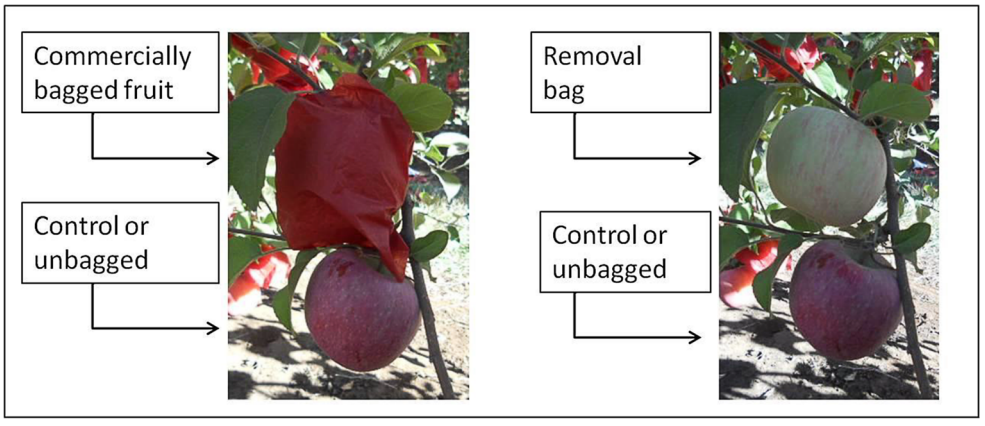

Fig. 1 Example of the treatments in orchard

maturity and physiological disorders at the different phenological states and after cold storage.

Material and Methods

Location and Plant Material and Treatment

The trial was conducted in the orchards of Frutícola El Aromo S.A. located in San Clemente (35°31'S; 71°26'W; at 224 m.a.s.l.), Chile. The apple trees were five-year-old cv. Fuji, Raku Raku planted at a spacing of 4.0 m × 1.2 m on M 9 rootstock.

The treatments assessed were:

1. Control or unbagged fruit that were not submitted to double bagging.
2. Commercially bagged fruit, with a double paper bag that was applied between 60 and 75 DAFB (1-15/12/2014). The outer grey bag was removed at 160 DAFB (11/03/2015) and the inner red paper was removed at 174 DAFB (24/03/2015).
3. Bagging until harvest, where fruit remained bagged until they were harvested (Fig. 1).

Samples for treatments 1 and 2 were collected at 158, 167, 173, 183 and 190 DAFB, the last being at commercial harvest. Treatment 3 was only assessed at harvest (190 DAFB). Five replications, each with three apples, were included in every sampling.

Extract Preparation to Determine Phenolic Compounds and Antioxidant Capacity

Peel was taken with a punch from both sides of the fruit. Then sample 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, 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.

Determining Anthocyanin Concentrations

Anthocyanin content was determined by the method of Fuleki and Francis (1968). A No. 7 hole punch was used to extract 0.95 cm^2 disks of apple peel. After homogenizing the samples, the fresh tissue was cut into 1 mm^2 squares that were then placed in Eppendorf tubes. A 500 µL HCI solution (1.5 *N*): technical ethanol at 96% (15:85) was added to the tubes, which were then agitated. The samples were incubated for 24 h at 4 ºC and then centrifuged for 3 min at 3000 rpm. The supernatant was transferred to a new Eppendorf tube and the resulting sediments were washed with a 500 µL HCl solution (1.5 *N*): technical ethanol at 96%, and left to stand for 24 h. Absorbance was quantified with a spectrophotometer (SpectroquantPharo 300, Merck) at 533 nm.

Determining Chlorophyll and Carotenoid Concentrations

Chlorophyll and carotenoid concentrations were determined by the method developed by Lichtenthaler (1987). Disks of apple peel 0.95 cm^2 in diameter were extracted with a No. 57 hole punch. Tissue was homogenized, cut into small squares and then placed in Eppendorf tubes. A 500 µL acetone solution at 80% was added to each tube, following which the tubes were incubated at 4 ºC for 24 h in darkness. The samples were centrifuged for three minutes at 3000 rpm and yielded a supernatant and a residue. The former was placed a new Eppendorf tube, and the latter was washed with 500 µL of acetone solution at 80%. The resulting solutions were combined in a single tube. Absorbance

was quantified at 663 nm, 647 nm and 470 nm with a spectrophotometer (SpectroquantPharo 300, Merck).

Determination of Phenolic Compound Concentrations

Phenolic compound content was determined by the Folin-Ciocalteu method (Coseteng and Lee 1987). Briefly, 0.1 mL of the extract was mixed with 0.5 mL of the Folin-Ciocalteu phenolic 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 concentration in the peel and flesh were expressed as mg of chlorogenic acid equivalents (CAE) g^{-1} fresh weight (FW).

Antioxidant Activity

Oxygen Radical Absorbance Capacity (ORAC) method: The method described by Huang et al. (2002) and Prior et al. (2003) was used with modifications. For the procedure (Trolox), 2.2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) and fluorescein sodium (FL) was used from Sigma-Aldrich. For preparation of solvent buffers and salts were used reactive from 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 in a spectrofluorometer with a Synergy HT microplate reader (1, 2).

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, an autosampler 3950, a column oven 4050, a PDA 2850 photodiode array detector and ChromGate Chromatography Data Software was used. 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 60 and 120 days of storage plus 1 and 7 days at room temperature (20 °C) to simulate shelf life. The following indices of ripeness were considered:

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 (model GS-14, Guss Manufacturing Ltd., South Africa) with an 11 mm plunger. The results are expressed in pound (lb).

Soluble solids were assessed with a digital refractometer (Refractec) with a range of 0–45% based using juice made from sample of fruit and the results expressed in %.

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 6 (maximum starch degradation) from the Export Association of Chile (ASOEX).

Mineral Analysis

The mineral elements determined in an accredited laboratory using a standard methodology were nitrogen (N), phosphorous (P), potassium (K), calcium, (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), copper (Cu), iron (F), and boron (B) in entire fruit at harvest for the control and commercially bagged treatments, with three replications per treatment.

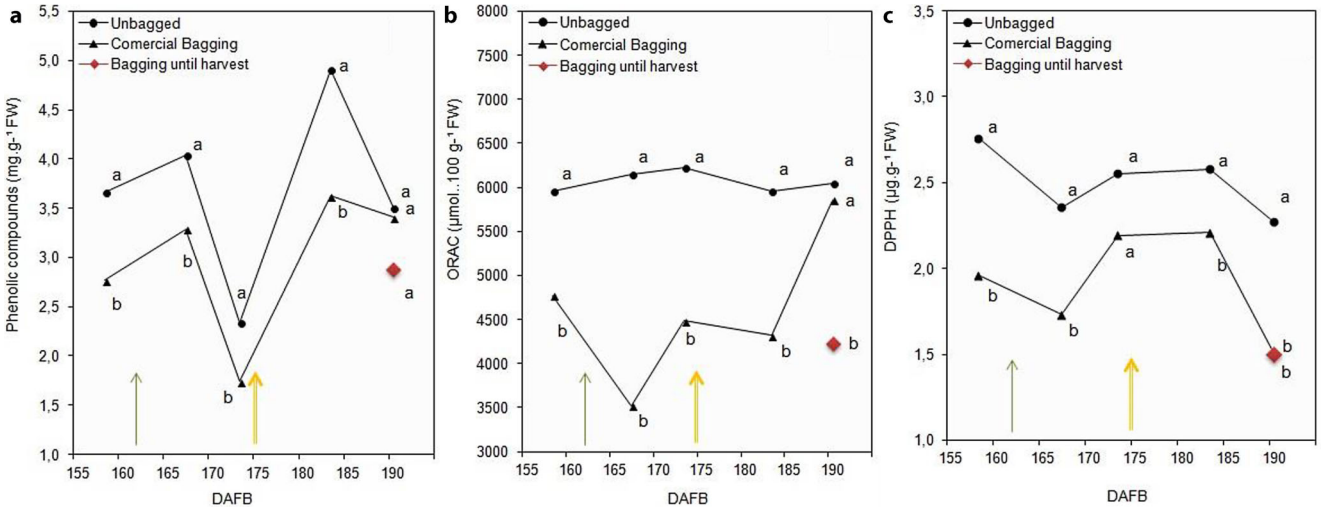

Fig. 2 Total phenolic compunds (**a**) and antioxidante capacity (ORAC (**b**)) and DPPH (**c**) at 158, 167, 173 and 190 DAFB in the peel of cv. Fuji, Raku Raku apples. (*green*↑: Elimination of external bag. *orange*↑: Elimination of internal bag)

Physiological Disorders and Other Alternations

Physiological disorders were assessed at 120 days of cold storage plus 7 days at ambient temperature (20 °C). The assessment consisted of visual appreciation of the fruit externally and determination of any alteration in the fruit pulp.

Statistical Analysis

The experimental design was completely random given the homogeneity of the trees. Five replications were used to determine phenolic compounds, DPPH (1, 1diphenyl-2-picryl hydrazyl) and ORAC, four replications to determine specific phenols and pigments, three replications per treatment to determine maturity indices and eight replications with 20 fruit each were used to determine physiological disorders and other alterations. A variance analysis was carried out with statistical program Statgraphics Centurion XVII (Warrenton, Virginia) and means were separated with the LSD test (p value ≤ 0.05), which verified the variances in the analysis.

Results and Discussion

Phenolic Compounds

In the first evaluated stages, total phenolic compounds were lower in commercially bagged fruit than in control fruit. However, the three treatments did not present significant differences at harvest (190 DAFB) (Fig. 2a). Jakopic et al. (2009) assessed the effect of the position of cv. Fuji, Raku Raku apples in the canopy and found total phenolic content was higher in fruit on the edges and in the upper parts of trees. These results suggest that removing the bags and exposing the fruit to light induces the production of phenolic in the peel of cv. Fuji, Raku Raku apples. However, Ju (1998) and Yuri et al. (2014) argue that some factors that induce phenolic synthesis could be transferred to the fruit or that the genes or promoters that control phenolic synthesis are not regulated by light, as the treatment with bagging until harvest evidences.

Antioxidant Capacity

ORAC

Antioxidant capacity (ORAC) was significantly higher in control fruit than in commercially bagged fruit in the samplings prior to harvest (Fig. 2b), however no differences in antioxidant capacity were observed between control and bagged fruit at harvest. This is in contrast to the phenolic compounds content, where no differences were found between treatments prior to or at harvest. This could be due to the different phenolic compound composition in the tissue of the different treatments.

DPPH

Antioxidant activity, as determined by the DPPH methodology, was higher in the peel of apples without bagging than in that of bagged apples in the samplings prior to harvest. The level of antioxidant activity at harvest was significantly higher in control fruit than in fruit from the commercial bagging and bagging until harvest treatments (Fig. 2c).

According to Sun et al. (2014), first bagging and then re-exposing fruit to solar radiation can be an effective method to improve the antioxidant capacity of cv. Golden Delicious

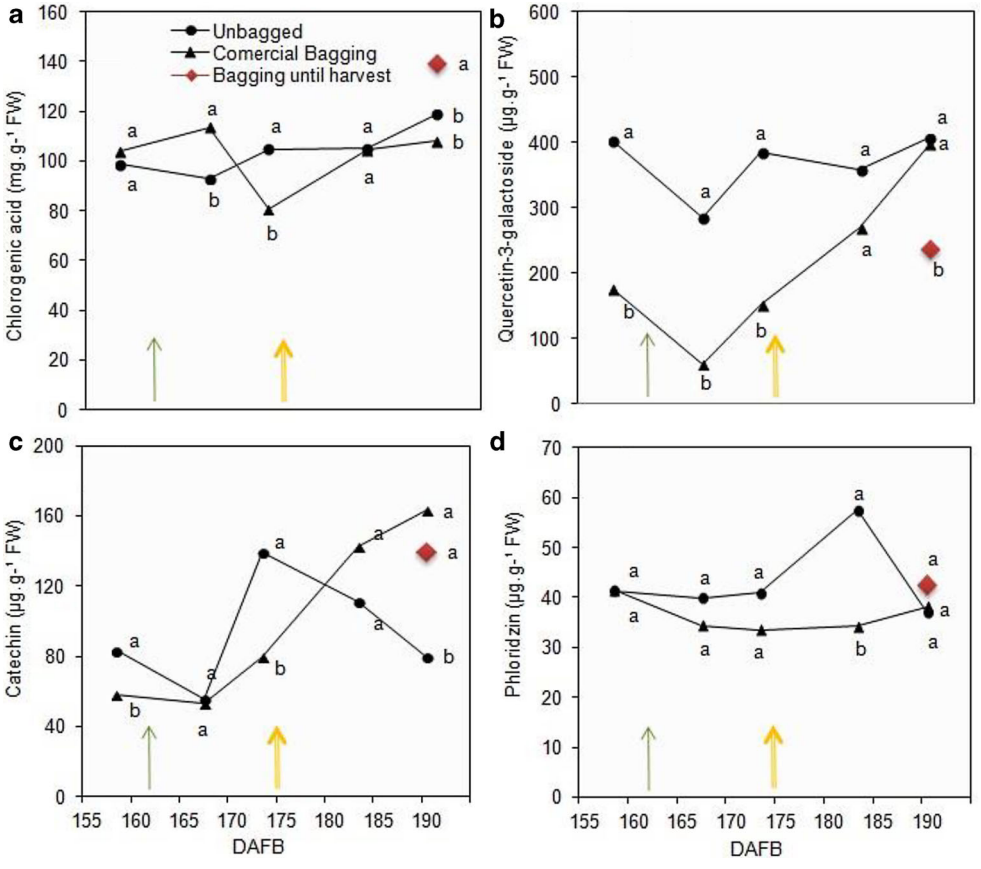

Fig. 3 Content chlorogenic acid (**a**), quercetin galactoside (**b**), catechin (**c**) and phloridzin (**d**) to 158, 167, 173 and 190 DAFB in the peel of fresh apples cv. Fuji, Raku Raku apples. (*green* ↑: Elimination of external bag. *orange* ↑: Elimination of internal bag)

apples, thus contributing to their nutritional values. This concurs with the data obtained via ORAC with the treatment with commercial bagging, where antioxidant activity at harvest was similar to that of the control fruit. However, as determined by the DPPH methodology, antioxidant activity was lower in commercially bagged fruit than in control fruit. The differences between the results obtained by ORAC and by DPPH could be because the former is based on inhibiting the peroxyl radical through the thermal decomposition of the AAPH (2.2'-Azobis(2-amidinopropane) dihydrochloride) compound, which is source of biologically significant radicals (Prior et al. 2003). While DPPH is a compound that differs from the oxidation reactions that occurs in biological systems (Huang et al. 2005).

Specific Phenolics

Chlorogenic Acid

There were no major variations in chlorogenic acid concentrations between the control and commercially bagged fruit at the different sampling dates. Chlorogenic acid content at 190 DAFB in apples bagged until harvest was significantly higher than in apples from the other two treatments (Fig. 3a). This concurs with the results obtained by Sun et al. (2014) with cvs. Red Delicious and Golden Delicious and suggests that light regulation of the synthesis of chlorogenic acid is dependent on the genotype. Awad et al. (2000) also reported that the genes that control the synthesis of chlorogenic acid, as well as catechins and phloridzin, are not light dependent in cvs. Jonagold and Elstar. This indicates that the use of bagging does not affect chlorogenic acid content in apple peel.

Quercetin Galactoside

Concentrations of quercetin galactoside were significantly lower in commercially bagged fruit than in control fruit up to when the bags were removed, after which concentrations increased and by harvest were at the same level in previously bagged fruit as in control fruit. Concentrations in the fruit that were bagged until harvest were significantly lower than in fruit from the other two treatments (Fig. 3b). According to Jakopic et al. (2009), this indicates that the synthesis of the compounds depends on exposure to light. Quercetin galactoside concentrations in the treatment with bagging at harvest decreased at 190 DAFB, which concurs with what Chen et al. (2012) found with cvs. Golden Delicious, Red Delicious and Royal Gala.

Table 1 Quantification of pigments at 167, 173, 183, and 190 DAFB in fresh peel of cv. Fuji, Raku Raku apples

Date (DAFB)	Treatments	Pigments (μg/cm^{-2} FW)				
		Chlorophyll b	Chlorophyll a	Total Chlorophyll	Carotenoids	Anthocyanin
167	Unbagged	0.243 a	0.234 a	0.476 a	0.062 a	3.953 a
	Comercial Bagging	0.345 a	0.199 a	0.544 a	0.043 a	0.358 b
	Sign.[a]	n. s.	n. s.	n. s.	n. s.	n. s.
	p-value	0.142	0.214	0.435	0.270	0.000
173	Unbagged	0.663 a	0.940 a	1.603 a	0.457 a	5.478 a
	Comercial Bagging	0.497 a	0.472 b	0.969 b	0.395 a	1.008 b
	Sign. [a]	n. s.	***	**	n. s.	***
	p-value	0.122	0.000	0.007	0.401	0.000
183	Unbagged	0.749 a	0.888 a	1.637 a	0.597 a	3.414 a
	Comercial Bagging	0.392 b	0.556 a	0.948 b	0.276 b	3.729 a
	Sign. [a]	**	n. s.	*	*	n. s.
	p-value	0.002	0.059	0.015	0.019	0.977
190 Harvest	Unbagged	0.474 a	0.693 a	1.167 a	0.442 a	6.035 a
	Comercial Bagging	0.413 a	0.706 a	1.118 a	0.345 a	7.068 a
	Bagging until harvest	0.552 a	0.770 a	1.322 a	0.458 a	0.878 b
	Sign.[a]	n. s.	n. s.	n. s.	n. s.	***
	p-value	0.538	0.861	0.738	0.190	0.000

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$. Different letters in columns represent statistically significant differences among treatments at a $p \leq 0.05$

[a] *Sign.* Significance; *n. s.* no significant

Catechins

Catechin concentrations increased significantly in apples from the commercial bagging treatment after the bags were removed to double the concentration in control fruit, which concurs with Chen et al. (2012) who indicated that the accumulation of catechins could be affected by light during the development of the fruit. However, catechin concentrations in fruit from the treatment of bagging until harvest was significantly higher than concentration in control fruit (Fig. 3c). This concurs with what Jakopic et al. (2009), who assessed the effect on cv. Fuji, Raku Raku apples of their position on the tree and found no significant differences in catechin or in chlorogenic acid and epicatechin content relating to the position of the fruit, which suggests that the synthesis of these compounds is not dependent on light. This study indicates that using bags has not effect of catechin concentrations in the peel of cv. Fuji, Raku Raku apples.

Phloridzin

Phloridzin levels were stable throughout the assay and did not differ among treatments (Fig. 3d). Chen et al. (2012) found that phloridzin content in fruit bagged until harvest was significantly lower than in control fruit with cvs. Golden Delicious, Red Delicious and Royal Gala, which is contrary to our results.

Pigments

Chlorophylls and Carotenoids

Neither chlorophyll (a, b and total) nor carotenoids were affected by commercial bagging in the majority of the samplings (Table 1). Jakopic et al. (2009) reported that chlorophyll and carotenoids did show significant differences in relation to the position of the cv. Fuji, Raku Raku fruit on the tree, which suggests that re-exposing the fruit to sunlight after removing the bags does not affect the concentrations of these pigments in the peel. Feng et al. (2014) noted that the use of bags decreases the synthesis of chlorophyll in the peel, resulting in anthocyanin developing on a whitish background, which does not concur with the results obtained at harvest, where there were no differences in chlorophyll content.

Anthocyanin

Removing the bags in the commercial bagging treatment had the expected effect of increasing anthocyanin concentrations in the peel by 7 to 10 fold. Anthocyanin concentration was significantly lower at 190 DAFB in fruit from the treatment of bagging until harvest than in fruit from the other two treatments (Table 1). These results concur with those of Sun et al. (2014), who argue that anthocyanin is the most sensitive phenolic compound to bagging. Antho-

Table 2 Maturity index at harvest (190 DDPF) and cold storage (120 + 1 and 120 + 7 days (storage plus 1 and 7 days to 20 °C)) in apple cv. Fuji, Raku Raku

Date (DAFB)	Treatments	Ripeness parameter		
		Firmness (lb)	Soluble Solids (ºBrix)	Starch Index (1–6)
190 Harvest	Unbagged	14.1 a	16.5 a	4.5 a
	Comercial Bagging	15.1 a	18.2 a	4.7 a
	Bagging until harvest	15.1 a	17.5 a	5.8 b
	Sign.[a]	n. s.	n. s.	***
	p-value	0.062	0.084	0.000
120 + 1	Unbagged	13.9 b	16.8 a	6.0 a
	Comercial Bagging	14.9 a	17.2 a	6.0 a
	Sign.[a]	**	n. s.	n. s.
	p-value	0.008	0.480	1.000
120 + 7	Unbagged	15.0 a	17.7 a	6.0 a
	Comercial Bagging	15.7 a	16.7 a	6.0 a
	Sign.[a]	n. s.	n. s.	n. s.
	p-value	0.363	0.349	1.000

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$. Different letters in columns represent statistically significant differences among treatments at a $p \leq 0.05$

[a] *Sign.* Significance; *n. s.* no significant

Table 3 Mineralogical analysis of cv. Fuji, Raku Raku apples at harvest

Treatments	Mineral content [a] (mg/100 g DW)									
	N	P	K	Ca	Mg	Mn	Zn	Cu	Fe	B
Unbagged	46.8	10.3	58.8	4.2	5.1	0.03	0.03	0.02	0.2	0.35
Comercial Bagging	57.3	10.3	58	4.3	5.1	0.04	0.03	0.03	0.2	0.29
Sign.[a]	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
p-value	0.216	0.921	0.815	0.808	0.882	0.116	0.643	0.519	0.894	0.089

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$. Different letters in columns represent statistically significant differences among treatments at a $p \leq 0.05$

[a] *Sign.* Significance; *n. s.* no significant

cyanin synthesis is catalyzed by an enzymatic complex that includes the light-dependent enzymes phenylalanine ammonia-lyase (PAL), chalcone synthase and dihydroflavonol-reductase (Treutter 2001).

Parameters of Maturity

In general, there were no effects of bagging on pulp firmness, soluble solids and the starch index. The latter was the only indicator to show any effect, with a higher degree of starch degradation in the treatment of bagging until harvest. There were post-harvest (120 + 1 days) differences in pulp firmness in commercially bagged apples (Table 2). Fallahi et al. (2001) reported that there were no differences in pulp firmness between cv. Fuji, Raku Raku control fruit and fruit bagged until harvest. Their results also evidenced higher concentrations of soluble solids in apples from the control treatment, which is corroborated by some measurements.

Minerals at Harvest

No differences were found between control and commercially bagged fruit in terms of concentrations of minerals in entire fruit (Table 3). Potassium levels in fruit from both treatments were around 50% less than what is considered normal. In a study with cv. Delicious apples, Sharma et al. (2013) found that fruit submitted to bagging until three days before the estimated harvest date had higher levels of calcium (6.0 mg/100g FW) than control fruit (4.5 mg/100g FW) at harvest.

The ratios among mineral elements did not show any effect of bagging cv. Fuji, Raku Raku apples. However, N/Ca, N/K and P/Ca ratios were not in the normal range, indicating that the fruit has a propensity for post-harvest alterations. The risk index (IR) of the control and commercially bagged fruit were the same, indicating that the two treatments present the same probability of having post-harvest physiological disorders (Table 4).

Table 4 Mineral ratios in cv. Fuji, Raku Raku apples at harvest

Treatments	Relations between mineral elements [a]								
	N/Ca	K/Ca	Ca/Mg	Mg/Ca	N/K	K/P	P/Ca	(K + Mg) /Ca	*Risk Index*
Unbagged	11.2	14	0.8	1.2	0.8	5.8	2.5	15.3	*2*
Comercial Bagging	13.5	13.7	0.8	1.2	1	5.6	2.4	14.8	*2*
Sign.[a]	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	*n. s.*
p-value	0.392	0.448	0.922	0.844	0.331	0.729	0.785	0.456	*1,000*

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$. Different letters in columns represent statistically significant differences among treatments at a $p \leq 0.05$
[a] *Sign.* Significance; *n. s.* no significant

Table 5 Quantification of physiological disorders in cv. Fuji, Raku Raku apples at 120 + 7 days of conventional cold storage

Treatments	Physiological disorders (%)		Other damages (%)	
	Bitter pit	Internal browning	Sunburn	Decay
Unbagged	14.2 a	10.7 b	12.6 b	9.1 a
Comercial Bagging	7.9 a	2.1 a	1.9 a	13.2 a
Sign. [a]	n. s.	*	***	n. s.
p-value	0.216	0.046	0.000	0.347

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$. Different letters in columns represent statistically significant differences among treatments at a $p \leq 0.05$
[a] *Sign.* Significance; *n. s.* no significant

Physiological Disorders

Bitter pit

While there were no statistically significant differences among treatments in terms of bitter pit, from a commercial perspective, the disorder was reduced by bagging, despite a *p* value of 0.216 (Table 5). This result corroborates what is observed with local producers, where bagged fruit generally present a lower incidence of bitter pit. In a study with cv. Royal Delicious, Sharma et al. (2013) found a higher incidence of bitter pit and brown heart in unbagged than in bagged apples, even after 6 months of conventional cold storage.

Internal Browning

The results show a positive effect of the use of commercial bagging on the incidence of internal browning, which was significantly reduced (Table 5). However, Chung et al. (2005) did not find differences in internal browning between control and bagged fruit that were assessed after storage at different temperatures.

Alterations of the Fruit

Sunburn

Bagging fruit significantly reduced damage by sunburn (Table 5), which concurs with what was found by Reyes (2009) with cv. Fuji, Raku Raku apples.

Bagged fruit generally does not suffer burning, despite the fact that they experience higher temperatures than do fruit exposed to solar radiation. This could be because although the threshold for thermal damage is reached, the accumulation of heat is not sufficient to induce sunburn damage (Yuri 2010). Yuri et al. (2000) also argued that fruit that develop in the shade are more susceptible to damage than fruit that receive sunlight throughout their development, as the latter generate resistance mechanisms. Thus, commercially bagged apples are more susceptible to sunburn after the bags are removed.

Rotting

Bagging had no effect on the incidence of post-harvest rotting (Table 5), which concurs with what Amarante et al. (2002) found with cv. Doyenne du Comice pears after six months of storage. Sharma et al. (2014) indicated that bagging prevents pathogenic agents and pests from reaching the fruit. In contrast, in our study a higher incidence of rotting was observed among commercially bagged fruit than among control fruit, although the difference was not statistically significant. This could be due to reduced development of the cuticle of the fruit owing growth in a more humid environment, as well as being due to the absence of fungicides, which could increase the plant pathogen inoculum.

Conclusions

The results obtained in this research indicate that

1. Using the bagging during the development of the fruit, then removing and exposing the fruit to light induces the production of phenolic in the peel of cv. Fuji, Raku Raku apples.
2. The bagging does not affect chlorogenic acid and catechin content in apple peel of cv. Fuji, Raku Raku apples.
3. From a commercial perspective, the fruit disorder could reduced by bagging.
4. The results obtained, corroborates what is observed with local producers, where bagged fruit generally present a lower incidence of bitter pit. Also, could reduce the internal browning and damage by sunburn.
5. Bagging could affect the rotting, because reduces development of the cuticle of the fruit in a more humid environment plus with the absence of fungicides could increase the plant pathogen inoculum.

Conflict of interest J.A. Yuri, A. Neira, M. Fuentes, I. Razmilic, V. Lepe and M.F. González declare that they have no competing interests.

References

Amarante C, Banks NH, Max S (2002) Effect of preharvest bagging on fruit quality and postharvest physiology of pears (Pyrus communis). N Z J Crop Hortic Sci 30:99–107

Awad MA, de Jager A, van Westing L (2000) Flavonoid and chlorogenic acid levels in apples fruit: characterisation of variation. Sci Hortic 83:249–263

Chen C, Zhang D, Wang Y, Li P, Ma F (2012) Effects of fruit bagging on the contents of phenolic compounds in the peel and flesh of 'Golden Delicious', 'Red Delicious', and 'Royal Gala' apples. Sci Hortic 142:68–73

Chung H-S, Moon K-D, Chung S-K, Choi J-U (2005) Control of internal browning and quality improvement of 'Fuji' apples by stepwise increase of CO2 level during controlled atmosphere storage. J Sci Food Agric 85(5):883–888

Coseteng M, Lee C (1987) Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. J Food Sci 52(4):986–989

Fallahi E, Colt M, Baird C, Fallahi B, Chun I-J (2001) Influence of nitrogen and bagging on fruit quality and mineral concentrations of 'BC-2 fuji' apple. horttech 11(3):462–466

Feng F, Li M, Ma F, Cheng L (2014) The effects of bagging and debagging on external fruit quality, metabolites, and the expression of anthocyanin biosynthetic genes in 'Jonagold' apple (Malus domestica Borkh.). Sci Hortic 165:123–131

Fuleki T, Francis FJ (1968) Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. J Food Science 33:72–77

Huang D, Ou B, Hampsch-Woodill M, Flanagan JA, Deemer EK (2002) Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated beta-cyclodextrin as the solubility enhancer. J Agric Food Chem 50:1815–1821

Huang D, Ou B, Prior L (2005) Reviews the chemistry behind antioxidant capacity assays. J Agric Food Chem 53:1841–1856

Jakopic J, Stampar F, Veberic R (2009) The influence of exposure to light on the phenolic content of cv. Fuji, Raku Raku Apple. Sci Hortic 123:234–239

Ju ZG (1998) Fruit bagging, a useful method for studing anthocyanin synthesis and gene expression in apples. Sci Hortic 77:155–164

Lichtenthaler HK (1987) Cholorophylls and carotenoids: pigments of photosynthetic biomembranes. Meth Enzymol 148:350–382

ODEPA (2015) Superficie plantada nacional, regional, número de huertos e infraestructura frutícola. www.odepa.gob.cl. Accessed 12 Nov 2015

Petkovsek MM, Slatnar A, Stampar F, Veberic R (2010) The influence of organic/integrateed production on the content of phenolic compounds in Apple leaves and fruits in four different varieties over a 2-years period. J Food Science 90:2366–2378

Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, Hampsch-Woodill M, Huang D, Ou B, Jacob R (2003) Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. J Agric Food Chem 51:3273–3279

Reyes M (2009) Respuestas de las ceras epicuticulares de manzanas al daño por sol. Memoria Doctor en Ciencias Agrarias. Facultad de Ciencias Agrarias, Universidad de Talca, Talca, p 7

Sharma RR, Pal RK, Asrey R, Sagar VR (2013) Pre-hervest bagging influences fruit color and quality of apple cv. Delicious. Agric Sci 4(9):443–448

Sharma RR, Pal RK, Sagar VR, Parmanick KK, Paul V, Gupta VK, Kumar K, Rana MR (2014) Impact of pre-harvest fruit-bagging with different coloured bags on peel color and the incidence of insect pests, disease and storage disorders in 'Royal Gala' apple. J Hortic Sci Biotechnol 89(6):613–618

Sun S, Xin L, Gao H, Wang J, Li P (2014) Response of phenolic compounds in 'Golden Delicious' and 'Red Delicious' apples peel to fruit bagging and subsequent sunlight re-exposure. Sci Hortic 168:161–167

Treutter D (2001) Biosynthesis of phenolic compounds and its regulation in apple. Plant Growth Regul 34:71–89

Tsao R, Yang R, Xie S, Sockovie E, Khanizadeh S (2005) Wich polyphenolic compounds contribute to the total antioxidant activities of apple? J Agric Food Chem 53:4989–4995

Yuri JA (2010) Daño por sol en manzanas. Rev Fruticult 8:3–9

Yuri J, Maldonado F, Razmilic I, Neira A, Quilodrán A, Palomo I (2012) Concentrations of total phenols and antioxidant activity in apple do not differ between conventional and organic orchard management. J Food Agric Environ 10(2):207–216

Yuri JA, Neira A, Maldonado F, Quilodrán A, Simeone D, Razmilic I, Palomo I (2014) Total phenol and quercetin content and antioxidant activity in apples in response to termal, light stress and to organic management. J Appl Bot Food Qual 87:131–138

Yuri JA, Torres C, Bastías R, Neira A (2000) Golpe de sol en manzanas. II. Factores inductores y respuestas bioquímicas. Agro Ciencia 16(1):23–32

Zhang J, Niu J, Duan Y, Zhang M, Liu J, Li P, Ma F (2015) Photoprotectionmechanism in the cv. Fuji, Raku Raku apple peel at different levels of photoocidative sunburn. Physiol Plantarum 154:54–65

Zoratti L, Karppinen K, Luengo A, Häggman H, Jaakola L (2014) Light-controlled flavonoid biosynthesis in fruits. Front Plant Sci 5(534):1–16