ANTIOXIDANT COMPOUNDS IN SKIN AND PULP OF FRUITS CHANGE AMONG GENOTYPES AND MATURITY STAGES IN HIGHBUSH BLUEBERRY (*Vaccinium corymbosum* L.) GROWN IN SOUTHERN CHILE

A.E. Ribera[1*], M. Reyes-Díaz[1,2], M. Alberdi[1,2], G.E. Zuñiga[3], and M.L. Mora[1]

[1]Center of Plant-Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Casilla 54-D, Temuco, Chile. [2]Departamento de Ciencias Químicas y Recursos Naturales; Facultad de Ingeniería, Ciencias y Administración; Universidad de La Frontera, Casilla 54-D, Temuco, Chile. [3]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 40 Correo 33, Santiago, Chile. *Corresponding author: aribera@ufro.cl

ABSTRACT

We evaluated the genotype and maturity effects on antioxidant activity and phenolic compounds of whole, skin and pulp fruits from three highbush blueberry cultivars (cv. Brigitta, cv. Bluegold and cv. Legacy) grown in southern Chile. Total antioxidant activity (TAA) in ripe fruits varied among the cultivars in the order Legacy > Brigitta > Bluegold. We found that TAA in unripe green and fully ripe fruits was high and similar between them, whereas the lowest levels were found in intermediate ripe fruits. The same trend was observed for fruit total phenolic content. This could be attributed to the higher concentrations of phenolic acids (mainly chlorogenic acid) and flavonols (mainly rutin) at immature fruit stages; whereas the high TAA in mature fruits could be explained by the elevated amounts of anthocyanin. All antioxidant compounds were mostly located in the skin. High amounts of delphinidin aglycone were found. HPLC-DAD/MS revealed that the main contents of skin anthocyanins are petunidin-3-glucoside and petunidin-3-arabinnoside followed by malvidin-3-galactoside. It is noticeable that highbush blueberry fruits grown in southern Chile have exceptionally higher antioxidant activity and anthocyanins contents compared with those cultivated in the northern hemisphere.

Keywords: *Vaccinium corymbosum*, antioxidant compounds, maturity stages, phenolics and anthocyanins.

INTRODUCTION

Currently, evidence is accumulating from different fields of science, including human medicine and nutrition, to support that fruit antioxidants play an important role in the prevention of human degenerative diseases such as cancers, atherosclerosis, and cardiovascular and neurological disorders (Kalt *et al*., 1999). In this way, highbush blueberries (*Vaccinium corymbosum* L.) are considered a good source of antioxidant compounds (Baliga and Katiyar, 2006). Blueberry fruits rank high in antioxidant activity among fresh fruits and vegetables (Prior *et al*., 1998). Heinonen *et al*. (1998), Wang and Lin

(2000) and Moyer *et al*. (2002) have shown that blueberry fruits are rich in phenolic acids, flavonols and anthocyanins, which have high biological activities (Cho *et al*., 2005). Prior *et al*. (1998) highlighted that fruit phenolic compounds, and especially flavonols, have a high antioxidant capacity.

The United States is the most important blueberry producer and consumer in the northern hemisphere. In the southern hemisphere, Chile is the main blueberry producing and exporting country, covering an area of about a thousand hectares, and it is most intensively cultivated in southern Chile between the Regions of La Araucanía and Los Rios (Chilean Agropecuary Census, National Institute of Statistics, Chile, 2007).

In recent years, Argentina, New Zealand, South Africa and Australia have become significant competitors with evident repercussions on national export prices and they represent a threat to the commercial profit of this crop. We believe that for Chile to carry out research in relation to how the contents and antioxidant activity of blueberry grown in Chile can be maintained and even improved to provide added value and competitive advantages to the national blueberry supply. Phenolic content and antioxidant activity of blueberries are currently becoming target traits for plant breeders. Furthermore, the breeding objectives for blueberries have lately included research on the germplasm of wild species to identify phenolic rich genotypes and generate breed cultivars with improved bioactivity (Scalzo *et al*., 2005).

Factors that may impact fruit antioxidant activity and composition include preharvest environmental conditions, postharvest conditions and processing (Kalt *et al*., 2001; Connor *et al*., 2002a, b; Kalt, 2005). In addition, it is known that the content of phenolic compounds in berry fruits is also affected by genetic differences among species, within the same species and maturity at harvest (Prior *et al*., 1998; Kalt *et al*. 1999; 2001; Wang and Lin *et al*., 2000; Connor *et al*. 2002a; Zadernowski *et al*., 2005; Castrejón *et al*. 2008; Wang *et al*., 2009a, b). Additionally, Prior *et al*. (1998) reported that increasing maturity at harvest of blueberry cultivars from USA yielded fruits with higher antioxidant, anthocyanins, and total phenolic contents. By contrast, Castrejón *et al*. (2008) found that phenolic compounds concentration and antioxidant activity in highbush blueberry fruits decreased from unripe green to ripe blue stages of fruit maturity. These observations were consistent with the results previously reported by Kalt *et al*. (2003).

In Chile, little information is available about the genotype and maturity effects on antioxidant activity and phenolic composition of highbush blueberry. Therefore, the aim of the present study was to determine the genotype and maturity effects on antioxidant activity and phenolic compounds of skin and pulp of fruits from three highbush blueberry cultivars grown in southern Chile.

MATERIALS AND METHODS

Plant material and fruit sample collection

In this study, three commercially available cultivars of highbush blueberry (*V. corymbosum* L.) were studied: Legacy, Brigitta and Bluegold. All fruits were collected from eight-year-old bushes grown under field conditions on the commercial farm "Berries San Luis" (Lautaro, La Araucanía Region, Chile) at the same harvest date (January, 2009). For

each cultivar, fruits were harvested fresh at six different maturity stages based on their surface color at harvest: green; intermediate ripe stages: 25% red, 50% red, 75% red, 100% red; and blue (Figure 1).

Figure 1. Blueberry fruits at different maturity stages. Six different maturity stages for *V. corymbosum* fruits were selected: MS-1 (green), MS-2 (25% red), MS-3 (50% red), MS-4 (75% red), MS-5 (100% red) and MS-6 (blue).

Fruits at different stages of maturation were collected from three different field plots per cultivar. In each plot, three samples of ten fruits were randomly harvested from 20 bushes, given a total of nine samples per maturity stage for each cultivar. Fruit samples were placed in polyethylene bags and transported under refrigerated conditions to the laboratory of Universidad de La Frontera, and stored at -20ºC (no more than 1 month) until extraction and analysis of compounds. In order to evaluate fruit quality attributes, a subsample of 20 fresh fruits was separated from each fruit sample prior to freezing (Connor *et al*., 2002a).

Determination of fruit quality attributes

Physical parameters. As physical quality attributes, the average of weight, caliber (equatorial diameter) and height (polar diameter) of 20 fresh fruits per sample was determined using an analytical balance and calipers, respectively.

Chemical parameters. Total soluble solids (TSS), titratable acidity (TiA) and the maturity index were determined. TSS were analyzed in fruits juices with a manual thermo-compensated refract-tometer (Sudelab RHB-40, Santiago, Chile), standardized with distilled water and expressed as ºBrix. Fruit pH was estimated in 2 g of macerated fruits with a pH meter. The TiA was determined by the potentiometric titration method using a Metrohm E 349A automatic titrator equipped with a Jenway 3040 ion-analyser pH meter according to Castrejón *et al*. (2008). Acidity was expressed as percent (%) of total organic acid on basis of citric acid. Finally, the maturity index was calculated as the ratio between the TSS and TiA.

Fruit extract preparation

Before extracts were obtained, fruits were manually separated into whole fruits, skin and pulp fractions. Pulp extract included fruit seeds. For the antioxidant activity and total phenolic determinations, whole fruits and the different fruit fractions were subjected to a sequential extraction procedure described by Prior *et al*. (1998), modified using ethanol p.a. (80% v/v) as the extraction solvent. Aqueous mixtures with ethanol are one of the most common solvents for phenolic extraction for berries (Macheix *et al*., 1990).

To obtain the anthocyanin extract, samples of 0.1 g of whole fruits, skin or pulp were extracted with 1 mL of acidified ethanol (up to pH 1 with chlorhydric acid) in a mortar, boiled at 95ºC for 60 min, incubated at 4ºC in the dark for 24 hours and centrifuged at 3000 rpm for 10 min, as described earlier by Cheng and Breen (1991). According to

Harborne (1967), this solvent maximizes the extraction of anthocyanins. The pellets were eliminated and the supernatants were used for the determinations.

Antioxidant activity assay

Free radical scavenging activity in fruit extracts was assessed using the highly stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Chyau *et al*. (2006), with some modifications. Briefly, a 2 mL aliquot of ethanolic DPPH solution (80 µM) was added to 500 µL of diluted extracts. After 8 min incubation of the DPPH mixture sample at room temperature, the decrease in absorbance was measured at 515 nm with a spectrophotometer (model 2800 UV/VIS, UNICO, New Jersey, USA). Antioxidant activities of the samples were expressed in micromoles of Trolox Equivalents (TE) per 100 g of fresh weight (FW). Ethanol was used as the blank solution, and extract-free DPPH solution served as control.

Determination of total phenolic (TPH) contents

Total soluble phenolics were spectrophotometrically determined with Folin-Ciocalteu reagent according to Slinkard and Singleton's method (1977) using chlorogenic acid as the standard. Contents of TPH were calculated using a regression equation of chlorogenic acid and expressed as mg chlorogenic acid equivalents (CAE) per 100 g of FW.

Determination of total anthocyanin (TA) contents

Anthocyanin contents were estimated by the pH differential method as mentioned by Cheng and Breen (1991) with minor modifications. The absorbances of anthocyanin extracts were measured in a spectrophotometer at 530 nm and at 657 nm with a molar extinction coefficient for cyanidin-3-glucoside of 29,600. The contents of TA were expressed as milligrams of cyanidin-3-glucoside equivalent (c3g) per 100 g of FW.

HPLC-DAD analysis of phenolic compounds

Analytical HPLC system. Qualitative and quantitative analyses of blueberry phenolic compounds (non-anthocyanin- and anthocyanin-type metabolites) in whole fruit, skin and pulp extracts were conducted in a high performance liquid chromatography (HPLC) system Jasco (LC-Net II/ADC) using a Kromasil reversed-phase (RP)-18 column (250 x 4.6 mm i.d.) equipped with a photodiode array detector (DAD) (Jasco MD 2015 Plus).

Phenolic acid and flavonol analyses. The HPLC-analyses of phenolic acids and flavonols were undertaken as described earlier by Ruhland and Day (2000) with minor modifications, at a flow-rate of 1.0 mL min^{-1}. Phenolic acids, chlorogenic, caffeic, ferulic, gallic and *p*-coumaric and the flavonols quercetin, myricetin, kaempferol and rutin were used as standards (Sigma Chemical Co. St. Louis, MO). These were dissolved in methanol for the preparation of calibration curves. Signals were detected at 320 nm. Acidified water (phosphoric acid 10%) (A) and 100% acetonitrile (B) was used as the mobile phase. The eluent gradient was: 0-9 min of 100% A, 9.1-19.9 min of 81%A and 19%B, 20-25 min of 100% B.

Anthocyanin analyses. The method based on anthocyanidin (anthocyanin aglycones) determination, with minor modifications was used for the anthocyanin analysis (Nyman and Kumpulainen, 2001). Delphinidin, malvidin, petunidin, cyanidin and peonidin provided by Sigma

Chemical Co. (St. Louis, MO) were used as anthocyanidin standards. For anthocyanin analyses, samples of 0.3 g of whole fruits, skin or pulp were extracted with 3 mL of acidified ethanol (up to pH 1 with chlorhydric acid) using a mortar and pestle. Then, 10 mL of the ethanolic extracts were placed into centrifugation tubes (Falcon (TM) type), which were sealed tightly and boiled at 95ºC in a water bath for 1 hour, incubated at 4ºC in the dark for 24 hours and centrifuged at 3000 rpm for 10 min. Signals were detected in the supernatants at 530 nm. The mobile phase was performed using acidified water (acetic acid 10%) (A) and 100% acetonitrile (B) with the following eluent gradient: 0-23.9 min of 90%A-10%B, 23.9-24.1 min of 80%A-20%B, 24.1-27 min of 20%A-80%B, and 27.1-37 min of 90%A-10%B.

HPLC Mass spectrometry (HPLC-DAD/MS) analyses of anthocyanins

Extracts were analyzed using an Agilent Technologies (Santa Clara, Ca) 1120 Compact LC consisting of a gradient pump with integrated degasser, Rheodyne 2-position, 6-port sample injection valve (20 µl loop), and EZChrom Elite Compact Software. HPLC was in-line with a 6400 Triple quadrupole (Agilent Technologies) with electrospray (ESI). Compounds were separated using a SUPELCOSIL LC-PAH RP-C18 column (15 cm x 4.6 mm; particle size 5µm) and a mobile phase of acetonitrile as eluent A and 0.1% formic acid as eluent B. All mobile phase solutions were filtered through a 0.45 µm filter and degassed for 15 min. Elution was performed using a linear gradient from 10:90 (A: B) to 70:30 over 20 min at a flow rate of 0.3 mL min^{-1}. The HPLC outlet was in-line with the ESI/MS. For analysis of the phenolic compounds, MS was operated in negative ion mode, capillary temperature 200 ºC, spray voltage 4 kV, and data were acquired in MS and MS/MS scanning modes.

Statistical analysis

Determinations are based on 3 replicates. Data statistical analyses were carried out by two-way ANOVA using SPSS 11.0 software (SPSS Inc., 2001). All data passed the normality and equal variance tests as per Kolmogorov-Smirnov normality test. Significantly different means were compared using Tukey's multiple comparisons test ($p \leq 0.05$). Additionally, Pearson correlations were used to test the relationships between two response variables using the same software mentioned above in some cases.

RESULTS AND DISCUSSION

Physical and chemical quality attributes are affected by maturity stages

In all cultivars, fresh weight (FW), caliber (equatorial diameter) and height (polar diameter) of blueberry fruits increased along the fruit maturity stages (Table 1). When compared with the unripe green stage, increases of these parameters during maturation were significantly different up to from 25% ($p \leq 0.05$). At the high maturity stage (blue), berries of the cv. Brigitta and cv. Bluegold showed higher average values of FW than cv. Legacy ($p \leq 0.05$). Moreover, fruits from Brigitta were the largest among the three cultivars, showing the highest values of caliber and height. Our ripe fruit FW data agreed well with the reports by Mackenzie (1997), Prior *et al*. (1998) and Ehlenfeldt and Prior (2001).

It is well known that highbush blueberry harvest is extensively based on the skin

Table 1. Physical quality attributes of fruits from three highbush blueberry cultivars harvested at different maturity stages. The average of weight, caliber and height of 20 fresh fruits per sample were determined.

Maturity stages	Fresh weight (g)			Caliber (cm)			Height (cm)		
	Bluegold	Legacy	Brigitta	Bluegold	Legacy	Brigitta	Bluegold	Legacy	Brigitta
Green	0.39 **Bc**	0.43 **Ad**	0.37 **Bd**	0.90 **Ab**	0.93 **Ab**	0.94 **Ab**	0.72 **Ab**	0.74 **Ab**	0.70 **Ab**
25% red	0.97 **Ab**	0.93 **Ac**	0.94 **Ac**	1.28 **Aa**	1.28 **Aa**	1.30 **Aa**	1.03 **Aa**	0.98 **Aa**	0.98 **Aa**
50% red	0.95 **Ab**	0.95 **Bc**	1.01 **Ac**	1.23 **Ba**	1.25 **Ba**	1.26 **Aa**	0.97 **Aa**	0.94 **Ba**	0.92 **Ba**
75% red	0.98 **Ab**	1.01 **Ab**	1.04 **Ac**	1.24 **Aa**	1.27 **Aa**	1.26 **Aa**	0.96 **Aa**	0.97 **Aa**	0.96 **Aa**
100% red	1.08 **Bb**	1.03 **Bb**	1.16 **Ab**	1.25 **Ba**	1.25 **Ba**	1.30 **Aa**	0.95 **Aa**	0.94 **Aa**	0.98 **Aa**
Blue	1.23 **Aa**	1.11 **Ba**	1.30 **Aa**	1.27 **Ba**	1.26 **Ba**	1.31 **Aa**	0.99 **Aa**	0.94 **Ba**	0.99 **Aa**

Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) between fruit maturity stages for the same cultivar and exposure time. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among cultivars for the same fruit maturity stage.

color of fruits, beginning 4 or 5 days after the first berries turn 100% blue to ensure a fruit size and sugar levels accepted by consumers (Banse, 2006). Nevertheless, other important blueberry harvest indicators for the assessment of fruit maturity are total soluble solid (TSS) and titratable acidity (TiA) of fruits (Mitcham *et al*. 2003). In general, high sugar contents and high acidity are required for good berry flavor. In contrast, high acidity with low sugar contents results in a tart berry, and high sugar contents and low acidity result in a bland taste, and when both parameters are low, fruit is tasteless (Kader, 1991). In the present study, highbush blueberry TSS and TiA values as well as maturity index (°Brix/acidity ratio) varied significantly among the different maturity levels, but no consistent differences were detected among the cultivars (Table 2). As expected, and in agreement with previous results (Castrejón *et al*., 2008; Wang *et al*., 2009b), in our study TSS content rose at increasing maturity stages concomitant with the maturity index, whereas TiA decreased with fruit maturity in the three cultivars. Practically no differences in these parameters were found among the cultivars. Castrejón *et al*. (2008) reported that during the course from unripe green to ripe blue fruit stages of several highbush blueberry berries, soluble sugars (SS) increased from 9 to 15 °Brix, respectively. Whereas, Prior *et al*. (1998) reported SS mean values of 13.9 °Brix for the mature berries from four highbush blueberry cultivars. According to Sapers *et al*. (1984), the SS contents for mature fruits in different highbush blueberry cultivars range from 11.2 to 14.3 °Brix.

In agreement with Prior *et al*. (1998), Connor *et al*. (2002a), Castrejón *et al*. (2008) and Wang *et al*. (2009b), our results indicated that TiA values varied significantly between the cultivars and through the maturity stages in the three cultivars. In general, a linear decrease in TiA was observed from the unripe to the mature stage, which was higher in Legacy and Brigitta (over 65%) than in Bluegold (53%). TiA levels (from 0.87 to 3.04) detected in the present study for ripe blueberry fruits were higher than those reported by Sapers *et al*. (1984) (from 0.40 to 1.31), but within the range observed by Connor *et al*. (2002a) for blueberry species (from 0.92 to 2.42%). No significant differences among cultivars for maturity index (MI) values were found, although this parameter tended to rise at increasing maturity stages. Galleta *et al*. (1971) indicated that MI equal to or less than 6.5, common in blue blueberry fruits are associated with better fruit postharvest quality. In each of the blueberry cultivars studied here, similar negative correlations (r= -0.9, $p \leq 0.05$) between TSS and TiA were found, whereas correlations between MI and TSS were positive (r= around 0.9, $p \leq 0.05$).

Fruit antioxidant activity as influenced by genotypes, fruit fraction and maturity stages

Total antioxidant activity (TAA) in ripe whole fruits varied significantly ($p \leq 0.05$) among the cultivars in the order Legacy> Brigitta>Bluegold (Figure 2). These results are agree with the observations of Connor *et al*. (2002a) in the same cultivars. In the leaves and roots of these genotypes, Reyes-Diaz *et al*. (2010) showed that the highest TAA levels were found in Legacy and the lowest in Bluegold. This feature was associated with a higher Al tolerance of Legacy growing under acidic soil conditions. Genotype variation for the TAA in blueberry fruits has been reported by Prior *et al*. (1998), Ehlenfeldt and Prior (2001), Connor *et al*. (2002a) and Castrejón *et al*. (2008).

Table 2. Chemical quality features of fruits from three highbush blueberry cultivars harvested at different maturity stages growing in southern Chile. The average of total soluble solids (TSS), titratable acidity (TiA) and maturity index (MI) of 20 fresh fruits per sample were determined.

Maturity stages	Total Soluble Solids (º Brix)			Titratable acidity (% citric acid)			Maturity Index		
	Bluegold	Legacy	Brigitta	Bluegold	Legacy	Brigitta	Bluegold	Legacy	Brigitta
Green	7.64 **Cd**	9.11 **Ad**	8.24 **Bd**	2.32 **Ba**	2.63 **Aa**	2.35 **Ba**	3.29 **Bd**	3.46 **Ad**	3.51 **Ae**
25% red	9.89 **Ac**	9.11 **Cd**	9.39 **Bc**	1.89 **Bb**	2.54 **Aa**	1.94 **Bb**	5.23 **Ac**	3.59 **Cd**	4.84 **Bd**
50% red	10.00 **Bbc**	10.33 **Ac**	10.00 **Bbc**	1.84 **Bbc**	2.11 **Ab**	1.66 **Cc**	5.43 **Bc**	4.90 **Cc**	6.02 **Ac**
75% red	11.00 **Bb**	11.72 **Abc**	10.53 **Cc**	1.79 **Ac**	1.76 **Ac**	1.66 **Bc**	6.15 **Bb**	6.66 **Ab**	6.34 **Ac**
100% red	11.70 **Bb**	12.32 **Ab**	11.79 **Bb**	1.78 **Ac**	1.81 **Ac**	1.63 **Bc**	6.57 **Cb**	6.81 **Bb**	7.23 **Ab**
Blue	13.72 **Ba**	14.72 **Aa**	13.31 **Ba**	1.10 **Ac**	0.95 **Bd**	0.87 **Cd**	12.47 **Ba**	15.49 **Aa**	15.30 **Aa**

Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) between fruit maturity stages for the same cultivar. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among cultivars for the same fruit maturity stage.

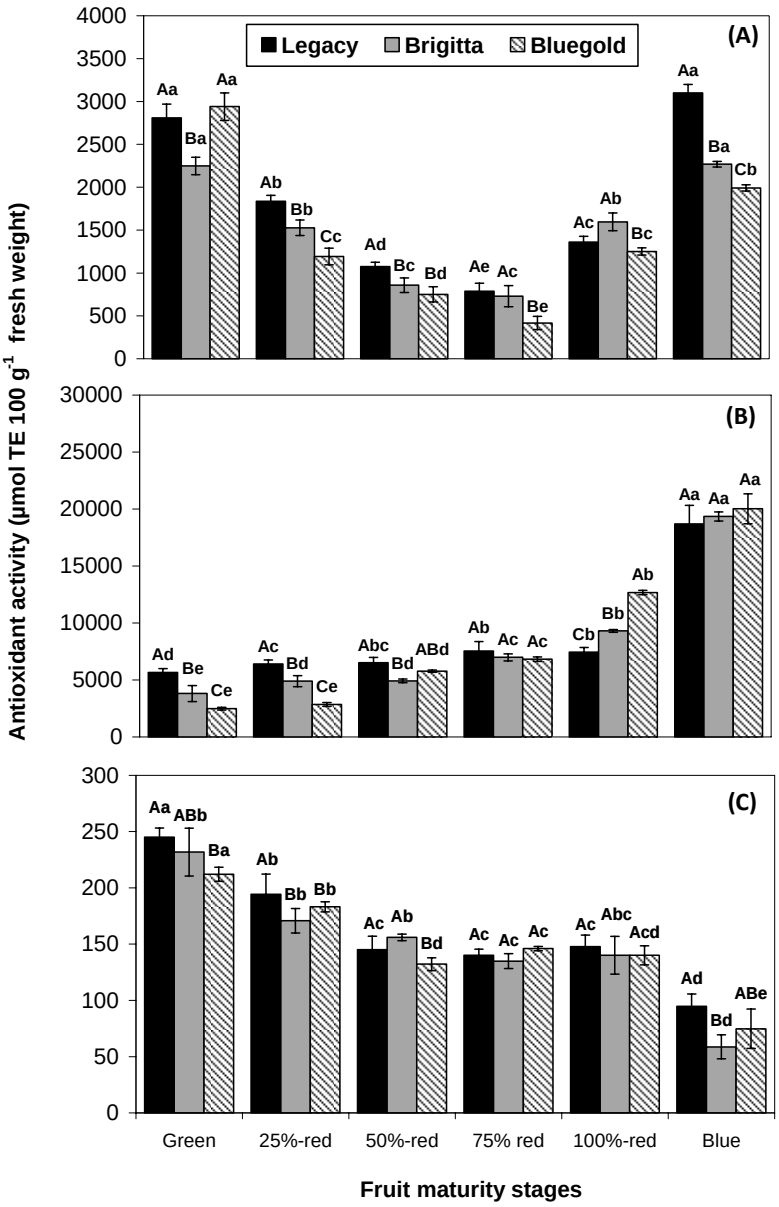

Figure 2. Total antioxidant activity (TAA) in blueberry fruits: A) whole fruit, B) skin and C) pulp; at different maturity stages. The values were expressed as µmol of TE (Trolox Equivalents) per mg of 100 g of fresh berries (A), per 100 g of fresh skin (B), or per 100 g of fresh pulp (C). Different lower case letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) among fruit maturity stages for the same cultivar and fruit fraction. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) between cultivars for the same fruit maturity stage and fruit fraction.

It is noteworthy that in the cultivars studied, skin extracts have significantly higher TAA than those detected for whole fruit and pulp (Figure 2). Thus, in ripe fruits TAA values in skin were around 7 and 192 times higher than in whole fruit and pulp, respectively ($p \leq 0.05$). In this regard, Mainland *et al*. (2000) showed that TAA in skin of ripe blueberry fruits were 4-fold higher than those found in whole fruits, whereas Guerrero (2006) reported only half for the Elliot blueberry cultivar. In general, TAA values for ripe whole blueberry observed here were slightly higher than those obtained in the same blueberry cultivars growing in the northern hemisphere (Connor *et al*., 2002a; Prior *et al*., 1998; Ehlenfeldt and Prior, 2001; Kalt *et al*., 2003; Castrejón *et al*., 2008). Such differences may be explained by several factors, including genotype variations, environmental conditions, geographical location, harvest year, and antioxidant extraction procedures (Kalt *et al*., 2001; Cho *et al*., 2004; Zadernowski *et al*., 2005). Moreover, we suggest that the high exchangeable Al concentrations in the volcanic acid soils of southern Chile, which cause oxidative stress in blueberry plants (Reyes-Diaz *et al*., 2009; 2010), could induce the production of the highest levels of fruit antioxidant compounds as a tolerance mechanism to overcome the negative effects of these metal stress conditions on plant growth.

Interestingly, we found that TAA of highbush blueberries fruits significantly changed during fruit maturation stages (Figure 2). Therefore, for all three blueberry cultivars our results indicated that the TAA of whole fruits decreased (~68 to 85%) at increasing maturity from unripe green to red stages, whereas after 75% red to ripe blue stages TAA increased steadily to 74% in Legacy, 68% in Brigitta, and 83% in Bluegold. Then, ripe blue and unripe green fruits have very similar TAA, except in cv. Bluegold, which showed higher TAA at the unripe stage than at the ripe stage. In fact, we observed that at the unripe green stage, fruit TAA varied among the cultivars in a different order than for ripe blue fruits, as follows: Bluegold > Legacy > Brigitta. According to these results, the lowest TAA levels were detected in intermediate ripe fruits (from 25% red to 100% red stages) for all cultivars. The noticeable variations in blueberry TAA through fruit maturation observed in the present study agreed well with the findings of Connor *et al*. (2002a), Kalt *et al*. (2003) and Castrejón *et al*. (2008) for highbush blueberry fruits, of Wang and Lin (2000) for strawberries, and of Wang *et al*. (2009a) for raspberries. Prior *et al*. (1998) and Kalt *et al*. (2003) showed that in cranberry, TAA increased linearly at increasing maturity stages. Similar observations have been reported for tomatoes (Cano *et al*., 2003) and peppers (Howard *et al*., 2000). Furthermore, according to Wang and Lin (2000), the highest TAA of strawberry and blackberry fruits have been observed at immaturity stages.

In addition, in the present study we found that TAA of pulp extracts decreased linearly as maturation progressed, whereas skin TAA increased linearly to 60% from the unripe green to ripe blue stages. It is remarkable that this is the first report about TAA variation in skin and pulp of highbush blueberry fruits through fruit maturation process.

Changes in total phenolic contents (TPH) of fruits fractions

In blue ripe fruits TPH content, expressed as mg CAE (Chlorogenic Acid Equivalent) x 100 g^{-1} FW, varied between cultivars in the order Legacy > Bluegold > Brigitta (Table 3). These

values are in accordance with those found for ripe blueberry fruits (190 to 390 mg) by Prior *et al*. (1998), although TPH was expressed as mg GAE (Gallic Acid Equivalent) x 100 g^{-1} FW. Using the same expression, Moyer *et al*. (2002) observed TPH variations from 171 to 868 mg GAE 100 g^{-1} FW. Fruit TPH contents detected in the present study were slightly higher than those reported earlier for other highbush blueberry cultivars (Ehlenfeldt and Prior, 2001; Connor *et al*., 2002a; Giovanelli and Buratti, 2009). However, it must be considered that in our study TPH content was determined using chlorogenic acid as the standard. This compound is the predominant phenolic acid in highbush blueberries (Gao and Mazza, 1994). Connor *et al*. (2002a) found that TPH determined on the basis of chlorogenic acid was 1.8 times higher than levels obtained using gallic acid as the standard. Moreover, our data showed that, in accordance with TAA variation in fruit tissues, TPH was significantly higher in fruit skin compared with those found in pulp extracts ($p \leq 0.05$). These observations agree with Allan-Wojtas *et al*. (2001), who reported that in ripe highbush blueberry fruits most antioxidant compounds are preferentially located in the skin. Nevertheless, no information is yet available regarding the antioxidant compound distribution during the fruit maturation process.

For all blueberry cultivars, we found that during fruit maturation TPH variations reflected the trend of TAA changes (Figure 2 and Table 3). Whole fruits harvested at intermediate ripe stages (from 75% red to blue stages) had lower TPH contents (average of 50%) than those detected in fruits at ripe or green stages. Nonetheless, blueberry fruits harvested at greener stages had higher TPH than those harvested as ripe blue fruits. Similar results have been previously reported by Connor *et al*. (2002a), Kalt *et al*. (2003) and Castrejón *et al*. (2008), who observed that TPH contents in highbush blueberry and cranberry ripe fruits decreased up to 30 and 50%, respectively, compared with the levels found in immature fruits. Moreover, similarly to our findings for TAA variation, TPH decreased linearly in pulp at increasing fruit maturation stages. By contrast, TPH increased linearly in skin extracts as fruit reached the highest fruit maturation stages. This means that the differences in TPH contents during maturity paralleled differences in the obtained TAA (Table 3). Therefore, for all three cultivars, in whole fruit and most markedly for skin and pulp, there was a high correlation between the TAA and TPH content during maturity stages (r = around 0.9 and 0.8 $p \leq 0.01$ for skin and pulp, respectively). However, in whole fruit correlations between the same parameters were lower (r= around 0.7, $p \leq 0.05$ for Bluegold; and r = around 0.5, $p \leq 0.05$ for both Brigitta and Legacy).

It is important to mention that there are no previous reports about the relationship of TPH variation in skin and pulp of highbush blueberry fruits at different maturity stages. High correlations between the TAA and TPH in ripe blueberry fruits have been previously reported by Prior *et al*. (1998), Kalt *et al*. (1999), Ehlenfeldt and Prior (2001) and Castrejón *et al*. (2008), who suggest that TPH be included as a selection trait in breeding study programs in order to improve the antioxidant activity of blueberry genotypes.

Variations in total anthocyanin contents during fruit maturity

In accordance with previous reports (Prior *et al*., 1998; Kalt *et al*., 1999), here we found that total anthocyanin contents (AT) of blueberry fruits are present predominantly in skins ($p \leq 0.05$) and at

Table 3. Total phenolic (TPH) and total anthocyanin (TA) contents of fruits from three highbush blueberry cultivars harvested at different maturity stages. TPH contents were expressed as mg of CAE (Chlorogenic Acid Equivalent) per: [a] 100 g of fresh berries, [b] 100 g of fresh skin or [c] 100 g of fresh pulp. TA content were expressed as mg of c3g (cyanidin-3-glucoside) per: [d] 100 g of fresh berries, [e] 100 g of fresh skin or [f] 100 g of fresh pulp. n.d.: non detected.

Cultivar	Maturity stages	Total phenolic content			Total anthocyanins content		
		Whole fruit [a]	*Skin* [b]	*Pulp* [c]	*Whole fruit* [d]	*Skin* [e]	*Pulp* [f]
Bluegold	Blue	432.68 **Bb**	3109.19 **Aa**	52.91 **Af**	206.00 **Ba**	733.51 **Aa**	5.31 **Aa**
	100%-red	443.81 **Ab**	2842.01 **Ab**	86.65 **Ae**	22.99 **Bb**	207.36 **Ab**	1.43 **Ab**
	75%-red	165.28 **Be**	1308.70 **Ac**	63.65 **Bd**	12.33 **Ac**	60.12 **Ac**	n.d.
	50%-red	268.44 **Ad**	1428.00 **Ad**	135.93 **Bc**	5.76 **Bd**	36.18 **Ad**	n.d.
	25%-red	324.95 **Cc**	642.00 **Ce**	189.74 **Bb**	3.19 **Ae**	15.30 **Ae**	n.d.
	Green	543.05 **Ca**	812.45 **Cf**	265.69 **Aa**	n.d	n.d	n.d.
Brigitta	Blue	468.18 **Bc**	3099.49 **Aa**	47.64 **Af**	190.00 **Ca**	715.04 **Aa**	0.88 **Ba**
	100%-red	484.99 **Ac**	1875.50 **Bb**	66.03 **Be**	39.27 **Ab**	118.68 **Bb**	0.40 **Bb**
	75%-red	241.65 **Ad**	1564.24 **Ac**	44.95 **Cd**	14.81 **Ac**	53.13 **Bc**	n.d.
	50%-red	258.13 **Ad**	1340.57 **ABd**	107.16 **Cc**	6.01 **Bd**	17.09 **Cd**	n.d.
	25%-red	615.13 **Ab**	1010.83 **Be**	235.85 **Ab**	3.79 **Ae**	9.33 **Be**	n.d.
	Green	820.32 **Ba**	1466.00 **Bf**	268.09 **Aa**	n.d.	n.d	n.d.
Legacy	Blue	570.02 **Ab**	3168.55 **Aa**	42.70 **Af**	226.65 **Aa**	580.00 **Ba**	0.48 **Ba**
	100%-red	442.33 **Ac**	1639.00 **Bb**	55.91 **Be**	44.52 **Ab**	209.39 **Ab**	0.32 **Bb**
	75%-red	284.71 **Ad**	1680.39 **Ac**	86.31 **Ad**	9.88 **Ac**	44.61 **Bc**	n.d.
	50%-red	258.00 **Ad**	1265.52 **Bd**	189.20 **Ac**	8.01 **Ac**	28.30 **Bd**	n.d.
	25%-red	559.31 **Bb**	1870.00 **Ae**	133.49 **Bb**	3.43 **Ad**	13.40 **Ae**	n.d.
	Green	910.21 **Aa**	1624.00 **Af**	277.02 **Aa**	n.d.	n.d.	n.d.

Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) between fruit maturity stages for the same cultivar and fruit fractions. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among fruit fractions for the same fruit maturity stage and cultivar.

the high fruit maturity stages (Table 3). In fact, we observed that green fruits contained no anthocyanins, while the intermediate fruit stages had very low AT amounts. In addition, our results showed that ripe blue whole fruits of Legacy exhibited the highest AT levels compared with Bluegold and Brigitta. Genotype AT variations in ripe blueberry fruits have been previously reported (Prior *et al*., 1998; Kalt *et al*., 1999; Connor *et al*., 2002a, b; Cho *et al*., 2004). In whole fruits AT contents increased linearly at increasing fruit maturation stages, ranging on average from 2.7 to 226.3 mg c3g 100 g^{-1} FW among cultivars (Table 3). The highest AT increases occurred from 75% red to ripe blue stages, reaching 81, 80 and 90% for Legacy, Brigitta and Bluegold, respectively. These observations match those reported by Prior *et al*. (1998), Wang and Lin (2000), Moyer *et al*. (2002), Kalt *et al*. (2003) and Castrejón *et al*. (2008), who showed that highbush blueberry green fruits contain very low or undetectable amounts of anthocyanins, which strongly increased through fruit maturation. Fruit AT contents detected in this study are comparable to those reported for ripe blueberry fruits, which varied over a wide range: 25 to 495 mg 100 g^{-1} FW (Mazza and Miniati, 1993), 93 to 235 mg 100 g^{-1} FW (Prior *et al*., 1998) and 73 and 430 mg 100 g^{-1} FW (Moyer *et al*., 2002). Whereas, Cho *et al*. (2005) determined higher TA contents (up to 822 mg 100 g^{-1} FW) in blueberries.

Our results showed that during the stages of fruit maturity for each of the three cultivars, a high correlation between TAA and AT in skin (r= around 0.9, $p \leq 0.01$) was observed, while in whole fruits correlations were lower (r= around 0.5, $p \leq 0.05$). In this way, it is well documented that the correlation between the TAA and AT is lower than those detected for the TAA and TPH in ripe fruits (Prior *et al*., 1998; Ehlenfeldt and Prior, 2001; Connor *et al*., 2002a, b).

Phenolic acid and flavonol composition in blueberry fruits

In terms of phenolic composition, HPLC analyses revealed that for all three cultivars and at all maturity stages chlorogenic acid and rutin were the predominant phenolic acid and flavonol in highbush blueberry whole fruits. Chlorogenic acid was also the most abundant cinnamic derivative in highbush blueberry fruits studied by Kader *et al*. (1997), Gao and Mazza (1994) and Wang *et al*. (2009b). Also consistent with our results, Cho *et al*. (2004) found that rutin was the predominant flavonol in blueberry fruits, and Wang *et al*. (2009b) observed that the major flavonols in blueberry fruits include quercetin, myricetin and kaempferol glycosides.

We detected principally in skins high amounts of caffeic acid (mainly in cv. Legacy) and moderate quantities of ferulic acid (in average among the cultivars up to 96 and 20 mg 100 g^{-1} FW, respectively). Furthermore, we found differences among the cultivars with respect to other phenolic acids and flavonols present in the skin, such as gallic acid (in Bluegold up to 17.5 mg 100 g^{-1} FW), *p*-coumaric acid (in Bluegold and Legacy up to 10.2 and 13.4 mg 100 g^{-1} FW, respectively), quercetin (in all cultivars, varied up to 17.5 to 19.0 mg 100 g^{-1} FW) and kaempferol (in Bluegold up to 10.2 mg g^{-1} FW). All these compounds were noticeably located in fruit skins and at immature fruit stages. However, it must be considered that the amounts of these compounds were significantly lower compared with chlorogenic acid and rutin. It is important to mention that in our study we detected flavonols, opposite to reports indicating to reports indicating that these compounds

are absent in ripe blueberry fruits (Castrejón *et al*., 2008).

Phenolic acids and flavonol concentrations found in the present study were slightly lower than those reported for other highbush blueberry cultivars in previous works (Connor *et al*., 2002a; Cho *et al*., 2004; Riihinen *et al*., 2008; Wang *et al.*, 2009b). In this way, Heinonen *et al*. (1998) have indicated that methodological differences may contribute greatly to the variability in the flavonoid and phenolic acid concentrations reported for blueberry fruits. In addition, the reported discrepancies may also partly be due to some differences in the maturity stage of the fruits at harvest (Prior *et al*., 1998).

We also showed that the contents of both phenolic acid and flavonols significantly varied among the fruit fraction and fruit maturity stages ($p \leq 0.05$). The content and distribution of chlorogenic acid and rutin in whole fruit berries and in their skins and pulp during maturation are shown in Figures 3 and 4. In order to clarify how the phenolic compound contents of whole fruits is explained by skin or pulp phenolic concentrations; we expressed the results per 100 g basis and arithmetically converted to show the amounts in skin or pulp for a 100 g berry sample (as described earlier Lee and Wrolstad, 2004). In this context, we found that the skin percentages by weight in ripe blue fruits were as follows: 19% for Legacy 15% for Bluegold and 13% for Brigitta, corresponding to the differences needed to achieve 100% of the pulp percentages. Skin percentages in intermediate ripe and green unripe fruits were higher than for blue fruits because at these stages berry size is smaller (Ehlenfeldt and Prior, 2001; Moyer *et al*., 2002). Our results showed that in whole fruits chlorogenic acid and rutin concentrations changed among the cultivars in the order Legacy and Brigitta > Bluegold and Legacy > Bluegold and Brigitta, respectively. Additionally, as occurred with TPH in pulp we found that both chlorogenic acid and most obviously rutin decreased linearly at increasing fruit maturation stages. This pattern was also observed for whole fruits, and less markedly in skin (Figures 3, 4). We suggest that only the dominant chlorogenic acid appears to be responsible for the high TPH content detected in fruits at the lowest maturity stages. These results are consistent with the observations of Wang *et al*. (2009a) for raspberry fruits. Nevertheless, the contribution of flavonols (such as rutin) to the TAA cannot be underestimated, because this compound is considered an effective antioxidant due to the particular substitution pattern of free hydroxyl groups on the flavonoid skeleton (Roesch *et al*., 2003). For all cultivars, levels and variation of these compounds in whole fruits, mainly at the intermediate ripe and inmature stages, may be explained mostly by their concentrations and changes in fruit skin. It is important to point out that there was a loss (up to 30%) in chlorogenic acid and rutin when comparing the sum of the fruit fraction contents with values for whole berries. According to Lee and Wrolstad (2004), this loss of phenolic compounds may be due to the enzymatic oxidation that occurs during manual separating and weighing operations.

Macheix *et al*. (1990) and Wollenweber (1994) reported that the accumulation of phenolic compounds is greater in the external tissues of fleshy fruits (epidermal and subepidermal layers) than in the internal tissue (mesocarp and pulp). It is well known that, since the formation of phenolic compounds is a light-dependent process, it is not rare to find these compounds mainly in the berry skins. Indeed, in many fruits, flavonol glycosides are mostly, or even solely, located in the outer part of fruits (Price *et*

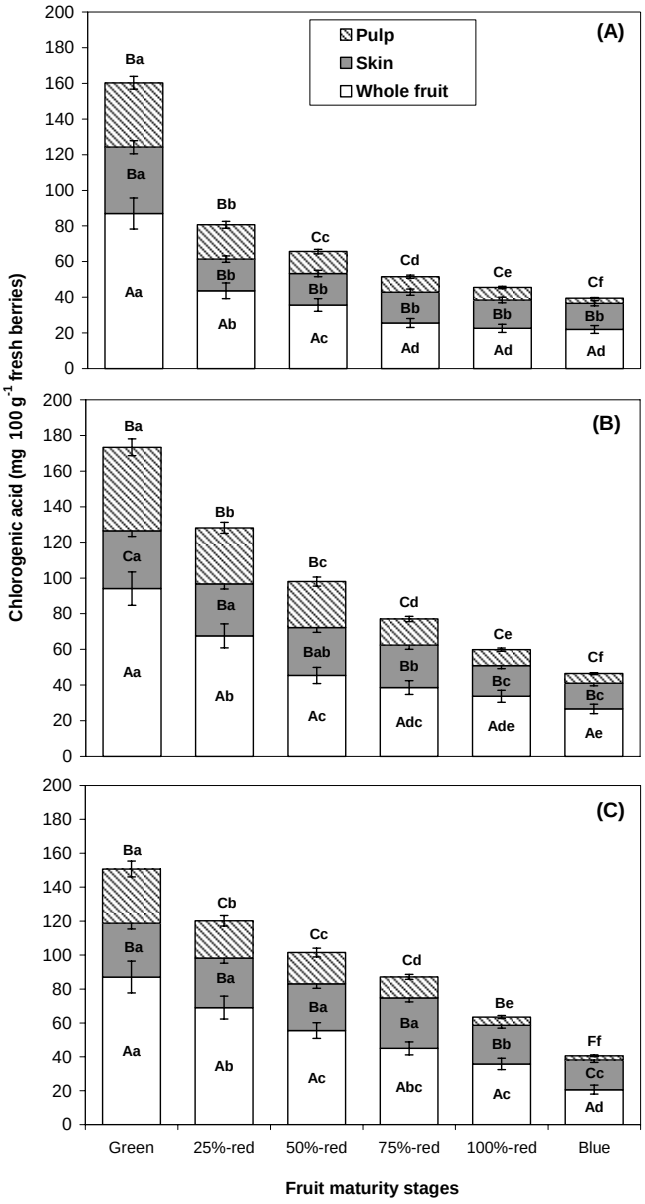

Figure 3. Chlorogenic acid concentrations in fruits (whole fruit, pulp and skin) from different cultivars of highbush blueberry cv. Bluegold (A), Brigitta (B) and Legacy (C), at different maturity stages. In order to clarify the relations between fruit fractions the chlorogenic acid concentrations in skin and pulp are expressed on a berry basis (mg 100 g^{-1} fresh berries). Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) among fruit maturity stages for the same fruit fractions. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among fruit fractions for the same fruit maturity stage.

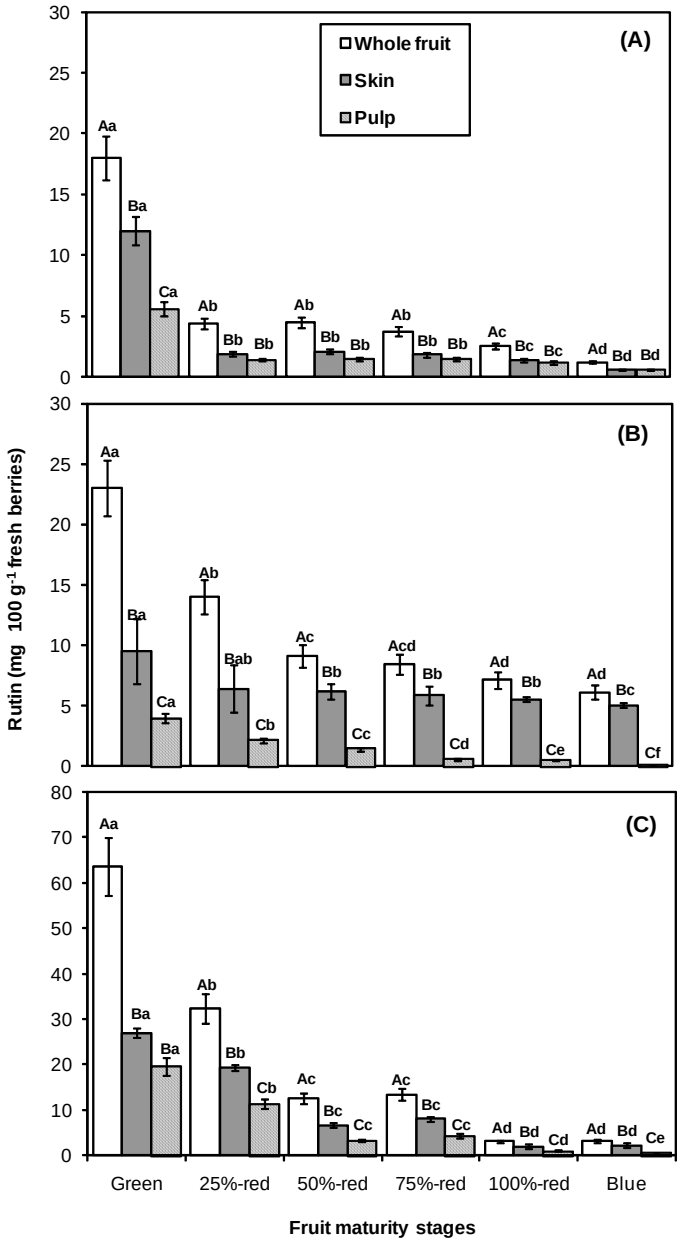

Figure 4. Rutin concentration in fruits (whole fruit, pulp and skin) from different cultivars of highbush blueberry cv. Bluegold (A), Brigitta (B) and Legacy (C), at different maturity stages. In order to clarify the relations between fruit fractions the rutin concentrations in skin and pulp are expressed on a berry basis (mg 100 g^{-1} fresh berries). Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) between fruit maturity stages for the same fruit fractions. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among fruit fraction for the same fruit maturity stage.

al., 1999). It has been also reported that the highest levels of hydroxycinnamic acids are often found in the external parts of the ripe fruit (Macheix *et al*., 1990).

Anthocyanin composition in blueberry fruits

Anthocyanins in fruits and vegetables are present in glycosylated forms. However, many of the analytical procedures convert the glycosides into aglycones (anthocyanidins) (Horbowicz *et al*., 2008). It has been reported that the number of anthocyanins is 15-20 times greater than the number of aglycon forms (Mazza and Mitani, 1993). To analyze an anthocyanin composition, the most reliable method is to release the anthocyanidins through acid hydrolysis, which reduces the number of peaks on the chromatogram to six (Nyman and Kumpulainen, 2001). We found that using a 60 min hydrolysis of extracts with acidified ethanol (pH 1.0) at 95°C was adequate to determine the anthocyanindin concentrations in all blueberry extracts.

As with almost all parameters, we found significant variations in anthocyanidin concentrations and compositions among the cultivars and during the fruit maturation process (Table 3, 4 and 5). Our results showed that at greener maturity stages both, whole fruits and skin contained anthocyanidins undetectable through the HPLC analysis. Despite the genotype and maturity effects, delphinidin (*Dp*) was the main anthocyanidin detected in all cultivars and at all fruit maturity stages. Delphinidin concentrations in blue ripe fruits significantly ($p \leq 0.05$) varied among the cultivars in the order Legacy (576 mg) > Brigitta (405 mg) > Bluegold (376 mg). Besides *Dp*, and in agreement with Kalt *et al*. (1999), Määttä-Riihinen *et al*. (2004) and Cho *et al*. (2004), we found that fruit from the blueberry cultivars also contained lower and variable amounts of malvidin (*Mv*), cyanidin (*Cy*) and petunidin (*Pet*). Our results also showed, in accordance with Määttä-Riihinen *et al*. (2004), that blueberry fruits have no detectable quantities of peonidin (*Peo*). On the contrary, Mazza and Miniati (1993), Gao and Mazza (1994) and Cho *et al*. (2004) indicated that added to *Dp*, *Cy*, *Pet* and *Mv*, the aglycone *Peo* is included within the major anthocyanins detected in blueberry fruits. Moreover, it is important to mention that our results differ from those obtained by Wang *et al*. (2009b), who found that *Mv* glycosides, but not *Dp* glycosides, were the most ubiquitous anthocyanins in blueberry fruits. This could be a different feature among blueberries from the northern and southern hemispheres.

The relative distribution of each aglycone also varied among genotypes in the order *Dp* > *Mv* > *Cy* > *Pet* for Bluegold; *Dp* > *Cy* > *Mv* > *Pet* for Brigitta; and *Dp* > *Mv* > *Pet* > *Cy* for Legacy. Moreover, total amounts of anthocyanidins (*Dp* + *Cy* + *Mv* + *Pet*) in all three blueberry cultivars were significantly different ($p \leq 0.05$). It is noteworthy that besides *Dp*, Bluegold fruits contained high amounts of *Cy* compared with those detected in Brigitta and Legacy. Wang *et al*. (1997) reported that the antioxidant activity of some common blueberry anthocyanidins is in the order *Cy* > *Mv* > *Dp* > *Pet* > *Peo*, indicating that information about anthocyanin compositions is very useful for predicting the antioxidant activity of berry species. The anthocyanidin concentrations found in the present study are exceptionally high for blueberry fruits when compared with those reported by Gao and Mazza (1994); Wang *et al*., (2009b) and Cho *et al*. (2004). This may explain t the high TPH content detected here compared with those reported in previous works.

Table 4. Anthocyanidin composition and content in fruits (whole fruit and skin) of highbush blueberry cv. Bluegold harvested at different fruit maturity stages. Anthocyanindin content of skin was expressed both, [a] on a fruit fraction basis as mg per 100 g of fresh skin and [b] on a berry basis as mg per 100 g of fresh berries. Determinations were performed by HPLC analysis.

Anthocyanidins in fruit tissues		**Fruit maturity stages**				
		Blue	100% red	75% red	50% red	25% red
Whole fruit (mg 100 g^{-1} berry)	*Delphinidin*	375.68 **Aa**	29.02 **Ab**	4.32 **Ac**	1.54 **Ad**	1.08 **Be**
	Cyanidin	284.70 **Ba**	4.51 **Cb**	1.84 **Bc**	1.55 **Ad**	1.39 **Ae**
	Petunidin	5.65 **Da**	2.01 **Db**	1.11 **Cc**	0.71 **Cd**	0.20 **Ce**
	Malvidin	45.38 **Ca**	11.99 **Bb**	1.81 **Bc**	1.27 **Bd**	0.19 **Ce**
Skin[a] (mg 100 g^{-1} skin)	*Delphinidin*	2189.65 **Aa**	179.70 **Ab**	28.48 **Ac**	9.50 **Ad**	6.83 **Be**
	Cyanidin	1746.40 **Ba**	24.80 **Cb**	10.70 **Bc**	9.60 **Ac**	11.20 **Ac**
	Petunidin	35.68 **Da**	12.38 **Db**	6.47 **Cc**	4.23 **Cd**	1.21 **Ce**
	Malvidin	293.04 **Ca**	66.43 **Bb**	10.22 **Bc**	8.18 **Bc**	1.02 **Cd**
Skin[b] (mg 100 g^{-1} berry)	*Delphinidin*	306.46 **Aa**	26.95 **Ab**	3.67 **Ac**	1.42 **Ad**	1.02 **Be**
	Cyanidin	244.49 **Ba**	3.72 **Cb**	1.61 **Bc**	1.44 **Ad**	1.48 **Ad**
	Petunidin	4.99 **Da**	1.85 **Db**	0.97 **Cc**	0.63 **Cd**	0.18 **Ce**
	Malvidin	41.02 **Ca**	9.96 **Bb**	1.53 **Bc**	1.23 **Bc**	0.15 **Cd**

Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) between fruit maturity stages for the same cultivar and anthocyanidin. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among anthocyanidins for the same fruit maturity stage and fruit fractions. Statistical differences among the cultivars are indicated in the text.

Table 5. Anthocyanidin composition and content in fruits (whole fruit and skin) of highbush blueberry cv. Brigitta harvested at different fruit maturity stages. Anthocyanindin content of skin was expressed both, [a] on a fruit fraction basis as mg per 100 g of fresh skin and [b] on a berry basis as mg per 100 g of fresh berries. Determinations were performed by HPLC analysis.

Anthocyanidins in fruit tissues		**Fruit maturity stages**				
		Blue	100% red	75% red	50% red	25% red
Whole fruit (mg 100 g^{-1} berry)	*Delphinidin*	404.75 **Aa**	7.39 **Ab**	1.38 **Ac**	0.85 **Ad**	0.59 A**e**
	Cyanidin	4.61 **Ca**	1.67 **Bb**	1.12 **Bc**	0.75 **Ad**	0.41 **Be**
	Petunidin	1.14 **Da**	1.58 **BCb**	0.74 **Dc**	0.58 **Bd**	0.31 **Ce**
	Malvidin	7.34 **Ba**	1.20 **Cb**	0.73 **Cc**	0.37 C**d**	0.24 **De**
Skin[a] (mg 100 g^{-1} skin)	*Delphinidin*	2859.50 **Aa**	47.14 **Ab**	8.95 **Bc**	5.31 **Ad**	3.45 A**e**
	Cyanidin	37.26 **Ca**	9.89 **Bb**	6.70 **Ac**	4.60 **Bd**	2.20 **Be**
	Petunidin	28.46 **Da**	9.42 **Bb**	4.75 **Dc**	3.44 **Cd**	1.82 **Be**
	Malvidin	51.78 **Ba**	7.18 **Bb**	4.42 **Cc**	2.35 **Dd**	1.24 **Ce**
Skin[b] (mg 100 g^{-1} berry)	*Delphinidin*	343.08 **Aa**	6.59 **Ab**	1.25 **Ac**	0.74 **Ad**	0.48 **Ae**
	Cyanidin	4.47 **Ca**	1.38 **Bb**	0.93 **Bc**	0.64 **Ad**	0.30 **Be**
	Petunidin	1.01 **Da**	1.31 **Bb**	0.66 **Cc**	0.48 **Cd**	0.25 **BCe**
	Malvidin	6.21 **Ba**	1.01 **Cb**	0.61 **Cc**	0.33 **Cd**	0.17 **Ce**

Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) between fruit maturity stages for the same cultivar and anthocyanidin. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among anthocyanidins for the same fruit maturity stage and fruit fractions. Statistical differences among the cultivars are indicated in the text.

Table 6. Anthocyanidin composition and content in fruits (whole fruit and skin) of highbush blueberry cv. Legacy harvested at different fruit maturity stages. Anthocyanindin content of skin was expressed both, [a] on a fruit fraction basis as mg per 100 g of fresh skin and [b] on a berry basis as mg per 100 g of fresh berries. Determinations were performed by HPLC analysis.

Anthocyanidins in fruit tissues		**Fruit maturity stages**				
		Blue	100% red	75% red	50% red	25% red
Whole fruit (mg 100 g^{-1} berry)	*Delphinidin*	576.36 **Aa**	9.22 **Ab**	6.10 **Ac**	1.85 **Ad**	1.02 **Ae**
	Cyanidin	4.27 **Da**	2.72 **Cb**	2.31 **Cb**	1.70 **Ac**	0.46 **Bd**
	Petunidin	18.48 **Ca**	6.98 **Bb**	1.21 **Dc**	0.61 **Cd**	0.13 **Ce**
	Malvidin	27.09 **Ba**	7.45 **Bb**	4.53 **Bc**	0.80 **Bd**	0.60 **Be**
Skin[a] (mg 100 g^{-1} skin)	*Delphinidin*	3120.00 **Aa**	43.80 **Ab**	30.60 **Ac**	8.23 **Ad**	4.74 **Ae**
	Cyanidin	20.40 **Da**	14.04 **Cb**	11.70 **Cc**	8.10 **Ad**	2.16 **Be**
	Petunidin	95.44 **Ca**	35.64 **Bb**	6.14 **Dc**	2.97 **Cd**	0.59 **Ce**
	Malvidin	131.57 **Ba**	38.10 **Bb**	20.10 **Bc**	3.60 **Bd**	2.70 **Be**
Skin[b] (mg 100 g^{-1} berry)	*Delphinidin*	561.60 **Aa**	8.32 **Ab**	5.80 **Ac**	1.56 **Ad**	0.90 **Ae**
	Cyanidin	3.67 **Da**	2.66 **Cb**	2.10 **Cc**	1.52 **Ad**	0.42 **Be**
	Petunidin	17.17 **Ca**	6.77 **Bb**	1.16 **Dc**	0.56 **Cd**	0.11 **Ce**
	Malvidin	23.68 **Ba**	7.24 **Bb**	3.82 **Bc**	0.68 **Bd**	0.51 **Be**

Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) between fruit maturity stages for the same cultivar and anthocyanidin. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among anthocyanidins for the same fruit maturity stage and fruit fractions. Statistical differences among the cultivars are indicated in the text.

As we discussed above, despite colorless phenolics being found in skin and pulp of blueberry fruits at levels dependent on cultivar and maturity, all anthocyanidins analyzed here were detected mainly in the skins, which increased dramatically during fruit maturation. In fact, for all cultivars almost all anthocyanidin concentrations found in whole fruits (~80%) may be explained by the high concentration of anthocyanins in the skins (Table 4, 5 and 6). Indeed, for all cultivars, the results showed that anthocyanidin concentrations detected in skin were almost 4 times higher than those detected in whole fruits. Interestingly enough, there was a loss (up to 18%) in anthocyanidin content when comparing the sum of the fruit fraction concentrations with values for whole berries. Furthermore, the pulp of blueberry cultivars evaluated by spectrophotometer contained very low amounts of total anthocyanins (< 1.0% of skin contents), which was consistent with the results of Lee and Wrolstad (2004) and Riihinen *et al*. (2008), but were undetectable via HPLC analysis.

In order to confirm the identification of anthocyanidins detected through HPLC-DAD analyses in hydrolyzed extracts and to clarify the situation with respect to the presence of anthocyanidin glycosides in non-hydrolyzed samples, skin extracts were also analyzed by HPLC-DAD/MS by comparing the m/z of each anthocyanin molecule and its fragmentation to the values in available published works. In this study, HPLC-DAD/MS made it possible to positively identify anthocyanin previously detected through HPLC-DAD. Moreover, the LC-MS analysis shows that the extracts contained higher amounts of glycosides than aglycones. Figure 5 shows the relative content of some anthocyanins detected in fruits at the ripe stage. The main contents of skin anthocyanins are in the form of petunidin (petunidin-3-glucoside and petunidin-3-arabinnoside) followed by malvidin- 3-galactoside. HPLC-DAD/MS spectra of the major anthocyanins are shown in Figure 6. These observations match those reported by Lee and Wrolstad (2004) for blueberry skin anthocyanins. Anthocyanin relative contents varied among the cultivars in the order Bluegold > Legacy > Brigitta. The same genotype variations are in accordance with those obtained through HPLC-DAD. The variable anthocyanidin recovery percentages obtained before the acid hydrolysis of blueberry extracts (Nyman and Kumpulainen, 2001) could make the differences clear among the major anthocyanin molecules detected through HPLC-DAD in hydrolyzed extract or through HPLC-DAD/MS in non-hydrolyzed extracts.

The contribution of the different phenolic compound groups to blueberry fruit TAA may vary. In this context, Kalt *et al*. (2001) suggested that in lowbush and highbush blueberries anthocyanins contributed more to TAA than other phenolics. However, our results showed that decreasing contents of some phenolic acids and flavonols during fruit maturation (which also explained the decrease in TPH at the same maturity stage) was strongly associated with the trend observed for TAA, independent from the increase in anthocyanin contents detected during the maturation process. Therefore, in the present study we demonstrate and confirm (Connor *et al*. 2002, Castrejón *et al*. 2008) that phenolic compounds (other than anthocyanins), such as chlorogenic acid and rutin, may contribute significantly to TAA of highbush blueberry fruits grown in southern Chile. Indeed, our results indicated that the ripe fruits of cv. Legacy, which contained the highest levels of phenolic acids and flavonols, exhibited the highest antioxidant capacity.

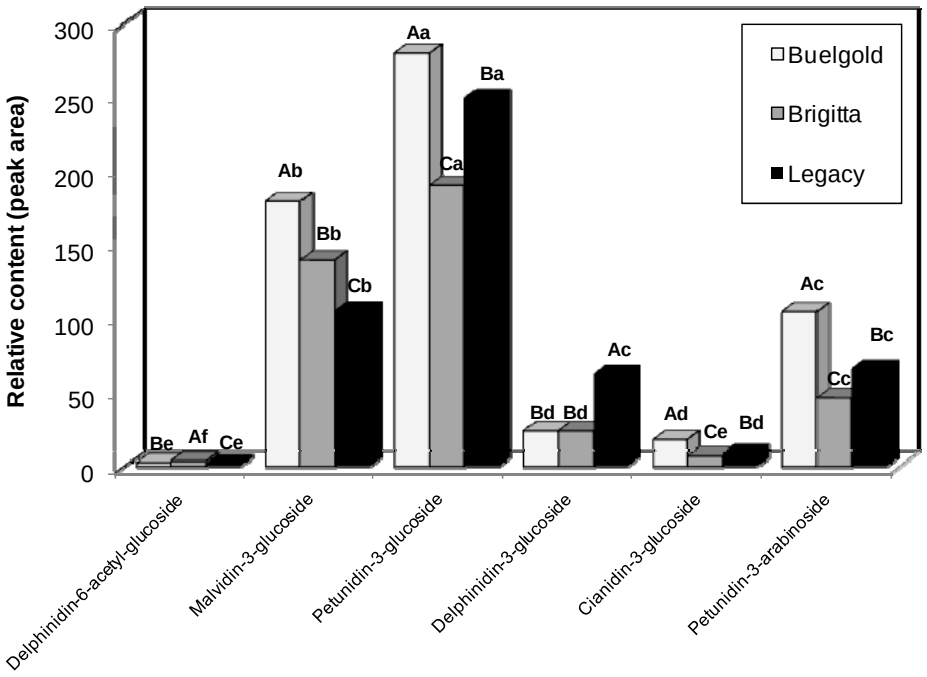

Figure 5. Anthocyanin relative concentrations in skin extracts of ripe blue fruits from different cultivars of highbush blueberry (Bluegold, Brigitta, Legacy) determined by HPLC equipped with a photodiode array detector - mass spectroscopy (HPLC-DAD/MS). Different lowercase letters indicate statistically significant differences (Tukey's HSD at $p \leq 0.05$) among fruit maturity stages for the same fruit fractions. Different uppercase letters indicate differences (Tukey's HSD at $p \leq 0.05$) among fruit fraction for the same fruit maturity stage.

This work also revealed that, whereas anthocyanins were the most ubiquitous phenolic compounds found in ripe fruits, the high antioxidant activity found in blueberry fruits harvested at greener maturity stages may be due to the high levels of the same compounds, mainly located in fruit skin, which decreased during fruit maturation. Castrejón *et al*. (2008) and Wang *et al*. (2009b) reported that in blueberry fruits after the unripe green stages until ripeness, a number of antioxidant compounds are synthesized, which may explain the high TAA levels detected in ripe blue fruits compared with those of fruits at intermediate ripe stages. Thus, there are several findings showing that flavonoid biosynthesis is closely associated with the fruit maturation process. Jaakola *et al*. (2002) found a coordinated expression of genes involved in flavonoid biosynthesis in developing bilberry (*Vaccinium myrtillus*) fruits. Moreover, Halbwirth *et al*. (2006) demonstrated that in strawberry (*Fragaria* x *ananassa*) flavonoid biosynthesis, two key flavonoid enzymes activity peaks are identified during fruit maturation, one at early and the other at late development stages (Halbwirth *et al*., 2006).

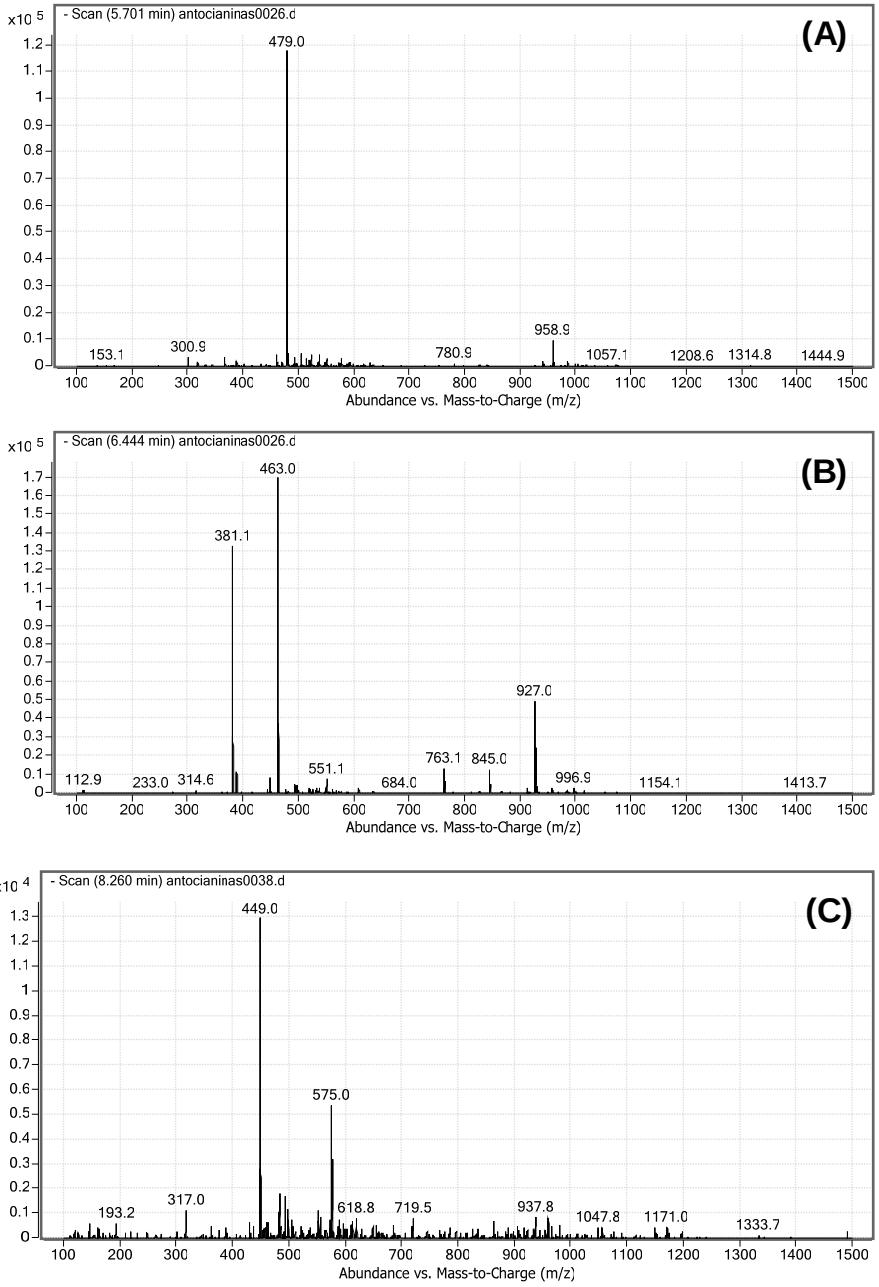

Figure 6. Mass spectra of main anthocyanins detected in skin blueberry extracts. Spectra were obtained in negative mode as indicated in the Materials and Methods section. (A) Petunidin-3-glucoside, (B) Malvidin-3-glucoside and (C) Petunidin-3-arabinoside.

Castrejón *et al*. (2008) proposed that during blueberry fruit maturation the first flavonoid biosynthesis peak may be associated with flavonols and hydroxycinnamic acid biosynthesis and that the second peak may be related to anthocyanin accumulation. According to Kalt *et al*. (2003), during maturation of blueberry fruits there is a shift in the pool of total phenolics toward anthocyanin synthesis, which should be associated with a decrease in the other phenolic components in the fruit. In the same regard, Mazza and Miniati (1993) suggest that the lower ATi levels found in fruits at increasing fruit maturity should be associated with higher carbon skeletons available for the synthesis of both anthocyanin-type or non-anthocyanin-type phenolic compounds, which would contribute to the increase in TAA in ripe maturity stages with respect to TAA in intermediate ripe fruits.

These findings may explain why, after the decrease in fruit TPH content detected here from green fruits to intermediate ripe stages, we observed an increase in TPH content at the highest maturity stages. These antecedents may also illustrate why green and ripe fruits exhibited a very similar TAA and why we found that during the fruit maturation process, fruit TAA correlated better with TPH than with AT.

In recent years, phenolic compounds have received increasing interest from consumers mainly because epidemiological studies have suggested that the consumption of polyphenol-rich foods can prevent several human diseases associated with oxidative stress (Steinmetz and Potter *et al*., 1996). Indeed, according to Chun *et al*. (2007), flavonoids constitute the most abundant antioxidants in our diets.

A limited number of studies on the estimation of flavonoid intake have been documented around the world, with mean values ranging from 20 to 180 or 200 mg per day (mg day^{-1}) (Kyle and Duthie, 2006; Chun *et al*., 2007), whereas recent estimates for anthocyanin intake are 12.5 mg day^{-1} in the US (Wu *et al*., 2006). The recommended daily dose of flavonoids ranges from 300 mg to over 1000 mg daily (Horvathova *et al*., 2001). Given this information, one cup of ripe blueberry fruits of the Chilean cultivars investigated here (~100 mg of fresh fruit), containing flavonoid concentrations (including some flavonols and anthocyanins) ranging from 700 to 900 mg 100 g^{-1} FW, should be sufficient to supply the daily flavonoid intakes recommended.

CONCLUSIONS

The evaluation of the total antioxidant activity (TAA) of highbush blueberry grown in southern Chile during the fruit maturation process revealed that fruit TAA varied among different blueberry cultivars. We also found a strong relation of TAA and total phenolic content (TPH) throughout maturation and in different fruit fractions. In general, TAA was higher in unripe green and fully ripe berries, while intermediate ripe fruits showed the lowest TAA levels. Higher concentrations of phenolic acids and flavonols at greener fruit stages explain high antioxidant capacity of fruits at this maturity stage. Whereas, the high TAA in mature fruit is related to the elevated quantities of anthocyanin. In the three cultivars, antioxidant compounds (phenolic acids, flavonols and anthocyanins) were mostly located in fruit skin; thus, skin showed considerably higher TAA and TPH content compared with those detected in the pulp. TAA of the fruits evaluated here was higher than those detected in fruits from the same

genotypes cultivated in the northern hemisphere. In addition, this study demonstrated that in highbush blueberry cultivars grown in southern Chile, not only anthocyanins but also phenolic acids and some flavonols contribute to TAA of blueberry fruits. We suggest that all the findings obtained in this work should be considered in processing technologies with aimed to aiming at maximizing the antioxidant extraction for food, nutraceutical or pharmaceutical industry as well as for further blueberry breeding programs.

ACKNOWLEDGEMENTS

This work was supported by the Postdoctoral FONDECYT 3090074 and Regular FONDECYT 1080372 Grants. We thank "Berries San Luis" farm, Lautaro, La Araucanía Region, Chile. We also thank the Agronomist Water Lobos (Fruticulture specialist) for his collaboration.

REFERENCES

Allan-Wojtas, P.M, Forney, C.F., Carbyn, S.E. and Nicholas, K.U.K.G. 2001. Microstructural Indicators of Quality-related Characteristics of Blueberries—An Integrated Approach. Lebensm. Wiss. Technol. 34(1), 23-32.

Baliga, M.S., Katiyar, S.K. 2006. Chemoprevention of photocarcinogenesis by selected dietary botanicals. Photochem. Photobiol. Sci. 5, 243-53.

Ballinger, W., Kushman, L. 1970. Relationship of stage of ripeness to composition and keeping quality highbush blueberries. J. Amer. Soc. Hort. Sci. 95 (2), 239-242.

Banse, G. 2006. Revista Agrícola especial de arándanos II. Temuco. El Diario Austral. Sociedad Periodística Araucanía S.A. 31 p.

Cano, A., Acosta, M., Arnao, M.B. 2003. Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum* Mill.). Postharvest Biol. Tec. 28 (1), 59-65.

Castrejón, A.D.R., Eichholz, I., Rohn, S., Kroh, L.W., Huyskens-Keil S. 2008. Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. Food Chem. 109, 564-572.

Cheng, G.W., Breen, P. J. 1991. Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. J. Am. Soc. Hort. Sci. 116, 865–869.

Cho, M.J., Howard, L.R., Prior, R.L., Clark, J.R. 2004. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. J. Sci. Food Agric. 84, 1771-1782.

Cho, M.J., Howard, L.R., Prior, R.L., Clark, J.R. 2005. Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high- performance liquid chromatography/mass spectrometry. J. Sci. Food. Agric. 85, 2149-2158.

Chun, O.K., Floegel, A., Chung, S.J., Chung, C.E., Song, W.O., Koo, S.I. 2010. Estimation of antioxidant intakes from diet and supplements in U.S. adults. J. Nutr. 140(2), 317-24.

Chyau, C.C., Ko, P.T., Mau, J.L. 2006. Antioxidant properties of aqueous extract from *Terminalia catappa* leaves. Zeitschrift fur Naturforschung (LWT) 39, 1099-1108.

Connor, A.M., Luby J.J., Tong B.S., Finn, C.E., Hancock, J.F. 2002a. Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. J. Am. Soc. Hort. Sci. 127, 89-97.

Connor, A.M., Luby, J.J., Hancock, J.F., Berkheimer, S., Hanson, E.J. 2002b. Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage J. Agric. Food Chem. 50 (4), 893- 898.

Ehlenfeldt, M.K., Prior, R.L. 2001. Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit

and leaf tissues of highbush blueberry. J. Agric. Food Chem. 49, 2222-2227.

Galleta, G., Ballinger, W., Monroe, R., Kushman, L. 1971. Relationships between fruit acidity and soluble solids levels of highbush blueberry clones and fruit keeping quality. J. Amer. Soc. Hort. Sci. 96 (6), 758-762.

Gao, L., Mazza, G. 1994. Quantification and distribution of simple and acylated anthocyanins and other phenolics in blueberries. J. Food Sci. 59: 1057–1059.

Guerrero, J. 2006. Capacidad antioxidante, fenoles y antocianinas totales e inhibición de Botrytis cinerea Pers. Ex Fr. por extractos crudos de fruta de cultivares de arándano alto (*Vaccinium corymbosum* L.) según localidades de la zona sur de Chile. Tesis Doctor en Ciencias Agrarias. Universidad Austral de Chile. Valdivia, Chile. 121 p.

Halbwirth, H., Puhl, I., Hass, U., Jezik, K., Treutter, D., Stich, K. 2006. Two-phase flavonoid formation in developing strawberry (*Fragaria* x *ananassa*) fruit. J. Agric. Food Chem. 54, 1479n -1485.

Harborne, J.B., Williams, C.A. 1976. Flavone and flavonol glycosides. In: the flavonoids. Harborne, J. B., Mabry, T. J. and Mabry, H. (eds). Chapman & Hall. London, U.K. pp. 376-443.

Heinonen, I.M., Meyer, A.S., Frankel, E.N. 1998. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J. Agric. Food Chem. 46, 4107-4112.

Horbowicz, M., Grzesiuk, A., Dębski, H., Koczko-daj, D., Saniewski, M. 2008. Methyl jasmonate inhibits anthocyanins synthesis in seedlings of common buckwheat (*Fagopyrum esculentum* Moench). Acta Biol. Crac. Series Bot. 50: 71-78.

Horvathova, K., Vachalkova, A., Novotny, L. 2001. Flavonoids as chemoprotective agents in civilization diseases. Neoplasma 48, 435–441.

Howard, L.R., Talcott, S.T., Brenes, C.H., Villalon, B. 2000. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. J. Agric. Food Chem. 48(5), 1713-1720.

Instituto Nacional de Estadística. 2007. Censo Agopecuario y Forestal. Available in: http://www.ine.cl/canales/chile_estadistico/censos_agropecuarios/censos_agropecuarios.php

Jaakola, L., Määttä, K., Pirttilä, A. M., R., Kärenlampi, S., Hohtola, A. 2002. Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. Plant Physiol. 130, 729–739.

Kader, A.A. 1991. Quality and its maintenance in relation to the postharvest physiology of strawberry. In: J. J. Luby & A. Dale (Eds.). The strawberry into the twenty-first century, pp. 145–152. Timber Press, Portland, OR.

Kader, F., Rovel, B., Girardin, M., Metche, M. 1997. Mechanism of browning in fresh highbush blueberry fruit (*Vaccinium corymbosum* L). Role of blueberry polyphenol oxidase, chlorogenic acid and anthocyanins. J. Sci. Food. Agric. 74, 31-34.

Kalt, W., McDonald, J.E. Ricker, R.D., Lu, X. 1999. Anthocyanin content and profile within and among blueberry species. Can. J. Plant Sci. 79, 617-623.

Kalt, W., Howell, A., Duy, J. C., Forney, C. F., McDonald, J.E. 2001. Horticultural factors affecting antioxidant capacity of blueberries and other small fruits. Horttechnology 11, 523–528.

Kalt, W., Lawand, C., Ryan, D. A. J., McDonald, J. E., Donner, H., Forney, C. F. 2003. Oxygen radical absorbing capacity, anthocyanin and phenolic content of highbush blueberries (*Vaccinium corymbosum* L.) during ripening and storage. J. Am. Soc. Hort. Sci. 128, 917–923.

Kalt, W. 2005. Effects of production and processing factors on major fruit and vegetable antioxidants. J. Food Sci. 70, R11–R19.

Kyle, J.A.M. and Duthie, G.G. 2006. Flavonoids in foods. In. Flavonoids; Chemistry, Biochemistry and Health Implications, pp. 219–263.

Lee, J., Wrolstad, R.E. 2004. Extraction of anthocyanins and polyphenolics from blueberry processing waste. J. Food Sci. 69(7), C564-C573.

Määttä-Riihinen, K.R., Kamal-Eldin A., Mattila P.H., Gonzales-Paramas A.M., Törrönen R. 2004. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. J. Agric. Food Chem. 52, 4477-4486.

Macheix, J.J., Fleuriet, A., Billot, J. 1990. Fruit Phenolics. CRC Press, Boca Raton, Florida, USA, 378 p.

Mackenzie, K. 1997. Pollination requirements of three highbush blueberry (*Vaccinium corymbosum* L.) cultivars. J. Amer. Soc. Hort. Sci. 122 (6), 891-896.

Mainland, C., Tucker, J., Hepp, R. 2000. Blueberry health information-some new mostly review. Acta Hort. 574, 39-43.

Mazza, G., Miniati, E. 1993. Anthocyanins in Fruits, Vegetables, and Grains. CRC Press, Boca Raton, Florida, USA, 362 p.

Mitcham, E., Crisosto, C., Kader, A. 2003. Bushberry: blueberry, cranberry, raspberry. In: Recommendations for maintaining postharvest quality. Department of Pomology, University of California. Davis. Available in: http://postharvest.ucdavis.edu/Produce/ProduceFacts/Fruit/berry.shtml

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., Wrolstad, R.E. 2002. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. J. Agric. Food. Chem. 50 (3), 519-25.

Nyman, N.A., Kumpulainen, J.T. 2001. Determination of anthocyanidins in berries and red wine by high-performance liquid chromatography. J. Agric. Food Chem. 49, 4183-4187.

Price, K.R., Prosser, T., Richetin, A.M.F., Rhodes, M.J.C. 1999. A comparison of the flavonol content and composition in dessert, cooking and cider-making apples; distribution within the fruit and effect of juicing. Food Chem. 66: 489-494.

Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., Mainland, C.M. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J. Agric. Food Chem. 46, 2686-2693.

Reyes-Díaz, M., Alberdi, M., Mora, M.L. 2009. Short-term aluminum stress differentially affects the photochemical efficiency of photosystem II in Highbush Blueberry genotypes. J. Am. Soc. Hort. Sci. 134, 1-8.

Reyes-Díaz, M., Inostroza-Blancheteau, C., Millaleo, R., Cruces, E., Wulff-Zottele, C., Alberdi, M., Mora, M.L. 2010. Long-term aluminium exposure effects on physiological and biochemical features of Highbush Blueberry cultivars. J. Am. Soc. Hort. Sci. 135 (3), 1-11.

Riihinen, K., Jaakola, L., Karenlampi, S., Hohtola, A. 2008. Organ-specific distribution of phenolic compounds in bilberry (*Vaccinium myrtillus*) and 'northblue' blueberry (*Vaccinium corymbosum* x *V. angustifolium*). Food Chem. 101 (1), 156-160.

Roesch, D., Bergmann, M., Knorr, D., Kroh, L.W. 2003. Structure–antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. J. Agric. Food Chem. 51, 4233-4239.

Ruhland, C.T., Day, T.A. 2000. Effects of ultraviolet-B radiation on leaf elongation, production and phenylpropanoid concentrations in *Deschampsia antarctica* and *Colobanthus quitensis* in Antarctica. Physiol. Plantarum 109, 244-251.

Sapers, G., Burgher, A., Phillips, J., Jones, S. 1984. Color and composition of highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 109 (1), 105-111.

Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., Battino, M. 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition 21, 207–213.

Slinkard, K., Singleton, V.L. 1997. Phenol analyses: automation and comparison with manual methods. Am. J. Enol. Viticult. 28, 49-55.

Steinmetz, K., Potter, J. 1996. Vegetables, fruit, and cancer prevention: a review. J. Am. Diet. Assoc. 96, 1027–1039.

Wang, H., Cao, G., Prior, R.L. 1997. Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem. 45, 304-309.

Wang, S.Y., Lin, H.S. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. 1: J. Agric. Food. Chem. 48 (2), 140-6.

Wang, S.Y., Chen, C.T., Wang, C.Y. 2009a. The influence of light and maturity on fruit quality and flavonoid content of red raspberries. Food Chem. 112, 676–684.

Wang, C.Y., Chen C.T., Wang, S.Y. 2009b. Changes of flavonoid content and antioxidant capacity in blueberries after illumination with UV-C. Food Chem. 117 (3), 426-431.

Wollenweber, E. 1994. In: The Flavonoids Advances in Research since 1986 (Harborne, J. B., Ed.). Chapman and Hall, London.

Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., Prior, R.L. 2006. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J. Agric. Food Chem. 54, 4069-4075.

Zadernowski, R., Naczk, M. and Nesterowicz, J. 2005. Phenolic acid profiles in some small berries. J. Agric. Food Chem. 53, 2118–2124.