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CHANGES ON PHENOLIC COMPOUND CONTENTS UNDER DIFFERENT PRODUCTION SYSTEMS OF BLUEBERRIES

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ABSTRACT

Current trends in agriculture involve the use of farming practices conditions aimed at improving the fruit nutritional value by directly affecting the content of antioxidants. Chile is the second-largest blueberry-producing country in the world, and the biggest exporting country in the South hemisphere. Therefore, the purpose of this study was to evaluate if production systems (organic and conventional) in two consecutive seasons and two different agro-climatic zones (Andean foothill and Central Valley) have differences in the concentration of the main polyphenol and anthocyanin compounds, and antioxidant capacity, in three blueberry cultivars ('Brigitta', 'Duke' and 'Legacy') grown in andisol soils. The cultivars Legacy and Duke presented the greatest content of polyphenols and the highest antioxidant capacity, as well as important antioxidant compounds. Similarly, both polyphenol contents and antioxidant capacity reached superior values in organically grown blueberries, which means that this production system represents an important aspect to take into account. These results support an expansion of organic farming as strategy for the achievement on fruits quality in this kind of soils.
Additional key words: Berries, cultivars, delphinidin-3-galactoside, food safety, organic farming

RESUMEN

Cambios en el contenido de compuestos fenólicos bajo diferentes sistemas de producción de arándanos

Las tendencias actuales en agricultura implican el uso de prácticas agrícolas destinadas a mejorar el valor nutricional de la fruta al afectar directamente el contenido de antioxidantes. Chile es el segundo país productor de arándanos más grande del mundo y el mayor país exportador del hemisferio sur. Por lo tanto, el propósito de este estudio fue evaluar si los sistemas de producción (orgánicos y convencionales) en dos temporadas consecutivas y dos zonas agroclimáticas diferentes (Valle Central y Piedemonte Andino) presentan diferencias en la concentración de los principales compuestos de polifenoles y antocianinas, así como la capacidad antioxidante en tres cultivares de arándanos ('Brigitta', 'Duke' y 'Legacy') cultivados en suelos andisoles. Los cultivares Legacy y Duke presentaron el mayor contenido de polifenoles y la mayor capacidad antioxidante, así como de importantes compuestos antioxidantes. Del mismo modo, tanto el contenido de polifenoles como la capacidad antioxidante alcanzaron valores superiores en los arándanos cultivados orgánicamente lo que significa que este sistema de producción representa un aspecto importante a considerar. Los resultados apoyan una expansión de la agricultura orgánica como estrategia para el logro de la calidad de los frutos en este tipo de suelos.
Palabras clave adicionales: Agricultura orgánica, bayas, cultivares, delfinidina-3-galactósido, seguridad alimentaria

INTRODUCTION

Fruits, vegetables, nuts and seeds contain polyphenolic compounds that represent a great source of antioxidants for the human diet, and currently, nutrition-related health concerns have focused the attention on those compounds that can improve health conditions.

Many of the anthocyanins and other phenolic compounds found in the fruits have been tested and found to exert real health effects, although not all polyphenolic compounds present

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the same advantage (Yang et al., 2017; Tajik et al., 2017).

Blueberries (*Vaccinium corymbosum*) are one of the fruits with the highest antioxidant content consumed worldwide (Shiow et al., 2008). The main chemical groups responsible of this activity in blueberries are anthocyanins, which are a particular group of compounds belonging to flavonoid family, which are found in fruit skin and pulp, and contribute to the color of many flowers, fruits (especially berries), and vegetables (Ribera et al., 2010). Blueberry is a restoring, astringent and diuretic fruit, and also a great source of fiber, and vitamin C. In addition, blueberries also contain substances which all contribute to the fruit's antioxidant activity (Giovanelli and Buratti 2009). The synthesis of antioxidants in blueberry is determined by several factors, such as genetic factors, degree of fruit maturation, differences in the distribution of antioxidants in the different tissues of the fruit, and especially field conditions. In fact, there are studies that suggest that both environmental conditions and farming systems have a large impact on antioxidant levels (Howard et al., 2003). Some studies on the effect of farming systems have determined that organic fruits have higher antioxidant levels than conventionally grown fruits (You et al., 2011) though it has been vaguely demonstrated.

The demand for organic food products has been progressively increasing in recent years, mainly due to the public recognition of the connection between the consumption of organic food and health benefits and the negative recognition of plant protection chemicals. Organic foods are grown according to certain production standards, with no use of conventional pesticides or artificial fertilizers, and processed without ionizing radiation or food additives. However, there is still little evidence on the real impact of organic food consumption on people's health.

Chile is the major producer of blueberries in the southern hemisphere and the most important supplier of off-season fruit for the northern hemisphere. Environmental conditions and farming systems seem to have a great influence on the antioxidant activity of this fruit, but there are very few studies on this respect. The objective of this study was to determine if there is any difference on polyphenol and anthocyanin contents, antioxidant capacity, and concentration of the main phenolic compounds among three blueberry cultivars grown under two different farming systems (conventional and organic) in an Andisol soil (Andosol in FAO soil classification). These are very fertile soils from relatively recent volcanic activity, and represent approximately 0.7 % of the Earth's land surface (Buol et al., 2011). Therefore, the objective of this research was to study the behavior of blueberries in this type of soil, particularly Brigitta, Duke and Legacy cultivars, which are the main varieties grown in Chile, one of the largest producer country of blueberries in the world.

MATERIALS AND METHODS

Material collection

Fruits of Brigitta (B), Duke (D), and Legacy (L) blueberry cultivars were organically (O) and conventionally (C) grown at two different sites: Central Valley (V) and Andean foothill (F), both in the Bio-Bio Region of Chile, from December to March during two consecutive seasons (2016-2017 and 2017-2018).

"Organic" refers to culture whose fertilization practices and pest control are based on natural resources and bioproducts. "Conventional" refers to culture with the use of synthetic fertilizers and pesticides. The soil in site V has lower organic matter content as compared to site F. The average temperature during growing season in site V (22.7 ºC) is higher than in site F (18.1 ºC).

For the Central Valley, fruit samples were collected from a blueberry orchard of El Nogal experimental station in Chillán (36º 35' S, 72º 50' W), while conventional samples were obtained from a blueberry orchard located in Bulnes (36 º 42' S, 72º 20' W), province of Ñuble. For the Andean foothill, organic samples were collected at "Orgánicos Brita" orchard located in Pinto (36º 42' S, 71º 40' W), while conventional samples were collected from a blueberry orchard in Pemuco (37º 00' S, 72º 00' W), province of Ñuble. After harvesting, all samples were stored at -80ºC until analysis.

Extraction of antioxidants

A hundred grams of blueberries were homogenized in a disperser Ultra-Turrax at room temperature. The extraction of antioxidants was performed using 0.5 g of blueberries from each

site and production system and treated with 20 mL of methanol/water acidified with 2 N HCl (50:50 v/v, pH 2). In each case, the mixture obtained was shaken with a magnetic stirrer for one hour and centrifuged at 3500 rpm for 15 minutes. The supernatant was collected. A volume of 20 mL of acetone/water (70:30 v/v) was added to the residue and the mixture was stirred again for 60 minutes. After stirring, the mixture was centrifuged for 15 additional minutes at 2000 G-force, and the supernatant was combined with the supernatant previously removed. The extracts were analyzed immediately after preparing them since the harvest for each cultivar was completed at different dates. These extracts were used to determine total polyphenols, total anthocyanins and antioxidant capacity. All the extracts were done in triplicate. The total polyphenol content and total anthocyanin content were analysed by spectrophotometry and antioxidant capacity was carried out by means of FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhidracyl radical) assays, two *in vitro* tests.

Total polyphenols content

Total polyphenols were determined using the Folin Ciocalteu method (Singleton and Rossi, 1965). Subsequently, the samples were prepared adding 750 µL of Folin Ciocalteu 1N reagent, 750 µL of 20 % sodium carbonate and 500 µL of the supernatant previously extracted. A blank was also prepared using distilled water to replace the sample. All of them were maintained for two hours in the dark and absorbance was measured by an Optizen 3220UV spectrophotometer at 760 nm.

Total anthocyanins content

Total anthocyanins were determined by a differential pH technique. A buffer of 0.025 M potassium chloride at pH 1 and another buffer of 0.4 M sodium acetate at pH 4.5 were used. Two samples of 0.3 mL extract of blueberry fruits were placed separately into two tubes. One of them with 2.7 mL potassium chloride buffer pH 1 and 2.7 mL sodium acetate buffer was added to the other. After mixing the preparations, the absorbance was recorded at 510 and 700 nm with the UV spectrophotometer.

Antioxidant capacity

The FRAP method was used to determine the antioxidant capacity of the blueberry samples (Benzie and Strain, 1996). A volume of 1800 µL of FRAP reagent was prepared and then mixed with 180 µL of distilled water and 60 µL of the sample. The mixture was incubated at 37 °C for 30 min and then the absorbance was measured at 595 nm with the spectrophotometer.

The free radical scavenging activities were also determined using the DPPH method as described by Mena et al. (2011). This method consists of determining the antioxidant activity by measuring the variation in absorbance at 515 nm after a reaction time of 30 min with the DPPH radical. Three replicates were performed per sample.

Identification and quantification of phenolic compounds by HPLC

HPLC with DAD (diode array detector) analyses for the identification and quantification of polyphenols were carried out on a Chromolith RP-18 column (Merck Millipore). Water : formic acid (99:1, v/v) and acetonitrile, named A and B, respectively, were used as the mobile phase with a flow rate of $1 mL \cdot min^{-1}$. The gradient started with 8 % of solvent B, reaching 15 % at 25 min, 22 % at 55 min, and 40 % at 60 min, and then maintained up to 70 min. The compounds were identified by comparison of the standards.

Quantitative analyses of blueberry phenols were conducted in a high performance liquid chromatography (HPLC) system, using a Kromasil reverse-phase (RP)-18 column (250-4.6 mm i.d.) equipped with DAD (Merk-Hitachi). Chromatograms were recorded at 320, 360 and 520 nm. Anthocyanins, such as cyanidin-3-glucoside, delphinidin-3-galactoside and malvidin-3-galactoside; hydoxycinnamic acids such as chlorogenic acid, and the flavonoids quercetin-3-rutinoside and quercetin dihydrate were used as standards (Sigma Chemical). The rest of compounds were tentatively identified using spectral data.

For the quantification of all compounds cyanidin 3-*O*-glucoside at 520 nm, flavonols as quercetin 3-*O*-rutinoside at 360 nm, ellagic acid derivatives as ellagic acid at 320 nm and cinnamic acids derivatives as 3-*O*-caffeoylquinic acid at 320 nm were used.

Experimental design and statistical analysis

The study was conducted under a completely randomized design with factorial arrangement of the treatments. Analysis of variance (Anova) was performed and the means were compared using

the LSD (least significant difference) test. Different years were considered as replications. The analyses were carried out with the statistical analysis software InfoStat Statistical version 2008.

RESULTS

Statistical analysis for total polyphenols, total anthocyanins and antioxidant capacity are shown in Table 1. It is evident that total polyphenols and FRAP were influenced by cultivars and production system, while no effect was found in total anthocyanins or DPPH. Similarly, there was not detected any interaction among the three factors under study (cultivars, production system and locations).

The cultivar Brigitta, with 2030.4 μg GAE·g^{-1} FW had the lowest content of polyphenols, being significantly inferior ($P \leq 0.05$) to 'Duke' and 'Legacy' (3015.1 and 3286.4 μg GAE·g^{-1} FW, respectively) (Table 2). On the other hand, the content of polyphenols was higher ($P \leq 0.05$) in the plants grown under organic production than in those under conventional system. As can be seen the content of polyphenols was clearly affected by the cultivar and by the production system.

Table 1. Probability (*P*) and least significant difference (LSD) of the effect of cultivar, location and production system on phenolics and antioxidant capacity of blueberries. Averages of two seasons

	Total Polyphenols		Total Anthocyanins		FRAP		DPPH	
	P	LSD	*P*	LSD	*P*	LSD	*P*	LSD
Cultivar	**0.007**	734.4	0.167	ns	**0.004**	10.39	0.198	ns
Prod. System	**0.029**	599.6	0.163	ns	**0.000**	8.48	0.299	ns
Location	0.908	ns	0.327	ns	0.254	ns	0.990	ns
R^2	0.73	-	0.56	-	0.81	-	0.39	-

Numbers in bold means significant effect

Similar to the response observed in the polyphenol contents, the antioxidant capacity, as assessed by the FRAP assay, was lowest in 'Brigitta' and highest under the organic production system (Table 2).

When the pool of anthocyanins were evaluated as a whole (total anthocyanins), no differences were found between the treatments (Table 1). However, when the evaluation was performed individually, significant differences were detected in five out of the eight anthocyanins under study (Table 3). The chlorogenic acid, and four of the five flavonols, were affected as well.

Table 2. Mean value of phenolics and antioxidant capacity of blueberries as affected by the cultivar, location and production systems. Averages of two seasons

Factor	Level	Total polyphenols (μg GAE· g^{-1} FW)	Total anthocyanins (μg cyanidin-3-glucoside·g^{-1})	FRAP (μmol Trolox equiv·g^{-1})	DPPH (% of antioxidant activity)
Cultivar	Brigitta	2030.4 b	284.3 a	27.8 b	49.7 a
	Duke	3015.1 a	579.0 a	38.3 a	36.6 a
	Legacy	3286.4 a	542.3 a	47.6 a	40.7 a
Production system	Organic	3117.0 a	563.9 a	46.7 a	45.3 a
	Convencional	2437.6 b	373.1 a	29.2 b	39.4 a
Location	Foothill	2761.1 a	402.9 a	35.6 a	42.3 a
	Valley	2793.5 a	534.1 a	40.2 a	42.5 a

Means not followed by the same letters are significantly different according to LSD test ($P \leq 0.05$)

It should be highlighted that the concentration of the three anthocyanins not affected by the treatments (delphinidin-3-pentoside, cyanidin-3-glucoside, and petunidin-3-pentoside), were always higher in the second year (Table 4).

Petunidin-3-hexoside was another anthocyanin affected by the cultivar. 'Brigitta' showed the lowest values in both first and second year (22.5 and 26.5 μg.g^{-1} fresh fruit), surpassed by 'Duke', and in turn, by 'Legacy' (Table 5). The latter cultivar surpassed 'Brigitta' by at least 2 or 3 fold its concentration, and reached at the second year a value of 81.25 μg.g^{-1} fresh fruit. Again, the concentrations at the second year were consistently higher than in the first year. The other antioxidants affected by the cultivar (malvidin-3-arabinoside and syringetin-derivative) showed much lower concentrations in 'Brigitta' as compared to 'Duke' and 'Legacy'.

The antioxidant malvidin-3-galactoside did not show cultivar effect, but varied according to the production system and performed better when plants were grown under organic conditions respect to conventional production, i.e. 131.5 vs. 81.66 μg.g^{-1} fresh fruit in the first year, and 203.33 vs. 88.33 μg.g^{-1} in the second (Table 6). At this opportunity, the concentrations at the first year were notoriously lower than those at the second year.

The concentration of the remaining six main antioxidant compounds showed interaction effects (Table 7). The interaction cultivar x production system (C x PS) was found in four of the antioxidants (delphinidin-3-acetylhexoside, chloro genic acid, quercetin-3 rutinoside and quercetin-3-glucoside) and the interaction production system x location was found in the two remaining antioxidants (delphinidin-3-galactoside and quercetin-derivative). Some of the former antioxidant compounds presented more than one interaction.

In the case of delphinidin-3-galactoside, it can be seen that in the foothill its concentration is lower than in the valley (193 vs. 222 μg.g^{-1}); however, in this location the compound concentration is almost triple than that in the foothill (352 vs. 132 μg.g^{-1}), thus showing an evident effect of interaction (Table 7). It is concluded that the highest concentration was obtained when plants were grown under organic conditions in the valley. Similarly, quercetin-derivative was highest (18.3 μg.g^{-1}) when plants were grown under conventional conditions in the valley.

When considering interaction cultivar x production system (C x PS), the highest concentration of delphinidin-3-acetylhexoside was found in 'Duke' and 'Legacy' grown under organic conditions (20 μg.g^{-1}). In the interaction cultivar x locality, the chlorogenic acid showed its maximum concentration (500 μg.g^{-1}) in 'Brigitta' grown in the foothill. Also, the maximum concentration of quercetin-3 rutinoside (125 μg.g^{-1}) was reached with 'Legacy' in the foothill, and finally, quercetin-3-glucoside reached its highest concentration (40 μg.g^{-1}) in 'Duke' grown in the valley (Table 7).

DISCUSSION

The three main anthocyanins found were cyanidin-3-glucoside, delphinidin-3-galactoside and malvidin-3-galactoside, whose concentrations were higher than those of hydroxicinamic acids or flavonols from the three cultivars of blueberry. In general, the main anthocyanins presented differences by the cultivar and/or production system. On the other hand, chlorogenic acid presented significant differences by interactions between cultivar and location, cultivar and production system and location and production system. Also, many flavonols were affected by cultivar, location, production system and some interactions.

The total polyphenol values observed in HPLC were lower than those obtained by the Folin-Ciocalteau assay to determine total polyphenols. This could be mainly due to the fact that the Folin-Ciocalteau assay is susceptible to the interference of ascorbic acid, protein and sugar (Bastola et al, 2017). Nevertheless, the method is useful since it can provide approximate values thanks to low equipment costs. Also, it could be due to another circumstance as well, as that not all the peaks found in the chromatograms were quantified. In the case of anthocyanins values obtained by HPLC and spectrophotometric assay, these marked differences were not observed.

Our results showed that cv Duke and cv Legacy presented better antioxidant properties. The values obtained in polyphenols in this work are somewhat lower than those reported by Moyer et al. (2002), who found levels that ranged from 1710 to 8680 μg GAE·g^{-1} FW. These differences may be due to variations in polyphenol content depending on the cultivar (Mikkonen et al., 2001), and the degree of fruit maturation (Wang and Jiao

2001). A study conducted by Giongo et al. (2006) on the same cultivars under different conditions showed that the highest polyphenol content was found in 'Legacy', followed by 'Duke' and then 'Brigitta' in accordance with our results.

Regarding to anthocyanins, the values look lower than those found by Chiabrando and Giacalone (2015), who reported an amount of 213 µg of cyanidin-3-glucoside·g^{-1} FW. Our results also differ from those obtained by Sellappan et al. (2002), who reported an average value of 841.2 µg of anthocyanins·g^{-1} FW and those by You et al. (2011), who found a value of 1680 µg of cyanidin-3-glucoside.g^{-1} FW. Giongo et al. (2006) conducted a study in northern Italy and determined an amount of 1080, 2576 and 1386 µg·g^{-1} FW of total anthocyanins for 'Brigitta', 'Duke' and 'Legacy', respectively. As pointed out Cacace and Mazza (2003), differences in concentration of phytocehmicals in berries may be mainly due to cultivar, although environmental conditions and location might affect as well, since the plants, being more stressed by abiotic factors, synthesizes a higher content of polyphenols. Also, anthocyanin content depends on environmental temperature and solar radiation for the same reasons. In addition, it is also influenced by the cultivar. Kalt et al. (2001) found differences in the polyphenol content of cv. Rabbiteye compared to highbush cultivars, reporting differences of up to 30 % in anthocyanin content in two different seasons.

It has been demonstrated that UV irradiation can stimulate the expression of the genes involved in the anthocyanin biosynthesis, and therefore could increase or enhance anthocyanin accumulation (Azuma et al., 2012). Rodarte-Castrejón et al. (2008) found that the content of anthocyanins in ripe berries correlated significantly with fruit weight, the smallest fruits would contain a higher concentration of anthocyanin.

Table 3. Probability (*P*) values of the effect of cultivar (C), location (L) and production system (PS) on the main antioxidant compounds in blueberries

	C	L	PS	C x L	C x PS	L x PS
Anthocyanins						
Delphinidin-3-galactoside	**0.044**	0.431	**0.041**	0.720	0.166	**0.011**
Delphinidin-3-pentoside	0.179	0.336	0.265	0.664	0.872	0.158
Cyanidin-3-glucoside	0.472	0.574	0.202	0.278	0.857	0.597
Petunidin-3-hexoside	**0.001**	0.731	0.908	0.777	0.099	0.731
Petunidin-3-pentoside	0.262	0.952	0.859	0.803	0.868	0.518
Malvidin-3-galactoside	0.261	0.855	**0.010**	0.114	0.848	0.670
Delphinidin-3-acetylhexoside	0.503	0.677	**0.001**	0.836	**0.025**	0.677
Malvidin-3-arabinoside	**0.000**	0.477	0.254	0.550	0.308	0.392
Hydroxycinnamic acid						
Chlorogenic acid	0.072	0.422	0.127	**0.010**	**0.010**	**0.025**
Flavonols						
Quercetin-3-rutinoside	0.050	**0.022**	**0.038**	**0.036**	**0.030**	0.333
Quercetin dehydrate	0.262	0.999	0.999	0.262	0.262	0.999
Quercetin-3-glucoside	**0.000**	**0.030**	**0.000**	0.262	**0.005**	**0.030**
Quercetin-derivative	**0.002**	0.850	0.202	0.963	0.629	**0.013**
Syringetin-derivative	**0.000**	0.270	**0.001**	0.300	0.119	0.952

Numbers in bold means significant effect

The antioxidant capacity values are similar to data obtained by Moyer et al. (2002), who reported a capacity between 18.5 and 74.6 µmol Trolox.g^{-1} FW in blueberry.

Comparing polyphenol content, anthocyanins and antioxidant capacity, the differences found in 'Legacy' and 'Duke' could be, at least partially, due to the particular growing conditions or seasons. The lowest level of polyphenols were always observed in the first year (tables 4, 5 and 6). Also, significant differences were observed by cultivar and production system, with 'Legacy' reaching the highest polyphenol content.

Table 4. Mean values of four of the main antioxidant compounds not affected by the treatments in blueberry (Mean ± SD)

Year	Main antioxidant compound (μg.g^{-1} fresh fruit)			
	Delphinidin-3-pentoside	Cyanidin-3-glucoside	Petunidin-3-pentoside	Quercetin Dehydrate
1	98.33 ± 4.57	75.01 ± 5.55	8.66 ± 0.92	10.83 ± 2.03
2	125.00 ± 5.66	137.50 ± 11.97	10.75 ± 1.75	9.16 ± 1.01

A seasonal effect is clearly observed in anthocyanins since the values found in the first year were always lower than those of the second year. There are authors who claim that the phenolic composition of the fruit is influenced by environmental parameters, such as relative humidity, radiation or temperature (Mitler, 2006). In the year 1, the radiation was higher in the Central Valley (averaging 26.0 MJ·m^{-2}) than that in the foothill (23.7 MJ·m^{-2}). Photoinhibition is a phenomenon that can be generated when high-radiation occurs, providing rise to the production of reactive oxygen species. Antioxidant compounds are particularly involved in the detoxification of reactive oxygen species absorbing excess radiation and finally protecting plants against excess radiation (Cardeñosa et al., 2016).

No differences between cultivars were observed for cultivar and production system in terms of antioxidant capacity using DPPH, but differences were observed in antioxidant capacity when using FRAP. It is important to note that 'Brigitta' always showed the lowest concentrations related to anthocyanin and polyphenol contents, and the lowest for antioxidant activity (FRAP).

Table 5. Mean values of three of the main antioxidant compounds as affected by the cultivar (C) of blueberry (Mean ± SD)

Year	Cultivar	Main antioxidant compound (μg·g^{-1} fresh fruit)		
		Petunidin-3-hexoside	Malvidin-3-arabinoside	Syringetin-derivative
1	C1	22.5 ± 2.65	3.75 ± 0.70	5.00 ± 1.00
	C2	38.75 ± 3.25	21.25 ± 2.37	15.00 ± 2.37
	C3	52.50 ± 5.47	20.00 ± 2.82	15.00 ± 1.50
2	C1	26.25 ± 4.01	4.00 ± 0.97	7.5 0± 1.80
	C2	41.25 ± 2.12	16.25 ± 2.42	14.75 ± 1.95
	C3	81.25 ± 9.02	20.00 ± 2.30	15.00 ± 1.65

C1: Brigitta, C2: Duke, C3: Legacy

Considering that all cultivars were grown in Andisols, the variations in the antioxidant capacity could be attributed to the climate characteristics of the site. Therefore, temperature, relative humidity and radiation of the site may have influenced the antioxidant capacity of blueberries, as other authors have described for other species (Liu et al., 2016). Regarding to anthocyanins and other phenolic compounds, differences were observed mainly by cultivar and production system. Considering Chile as a major blueberry producing country and the fact that the use of organic farming systems is incipient, it has to be pointed out that this system resulted in fruit with somewhat higher nutritional quality compared to traditionally grown fruit. Some anthocyanins showed higher concentrations in organic blueberries compared to conventional ones. In general, delphinidin-*3*-galactoside was the anthocyanin found in the highest amount in the cultivars, and 'Legacy' and 'Duke' showed the highest values for this compound in both years. Regarding other phenolic compounds, which were also quantified, chlorogenic acid were the most interesting in blueberry as also described by You et al. (2011), and its concentration showed total interactions with cultivars, production systems and locations (Table 7).

Table 6. Mean values of one of the main antioxidant compounds as affected by the production system (PS) in blueberry (Mean ± SD)

Year	Production system	Main antioxidant compound (μg·g^{-1} fresh fruit)
		Malvidin-3-galactoside
1	PS1	131.5 ± 7.38
	PS2	81.66 ± 3.53
2	PS1	203.33 ± 19.61
	PS2	88.33 ± 4.06

PS1: Organic, PS2: Conventional

The antioxidant composition varied considerably between cultivars. 'Brigitta' presented the lowest content of anthocyanins, hydroxicinnamic acids or flavonoids, while 'Duke' and 'Legacy' showed the highest content of phenolic compounds, which is directly related to total polyphenols, total anthocyanins and antioxidant activity in this study.

It was demonstrated that total polyphenols and antioxidant activity (FRAP) were directly affected by the cultivar and the production system. The former refers to the fact that 'Brigitta' always showed the lowest values, and the latter refers to the finding that total polyphenols and antioxidant activity were highest under the organic production system. Likewise, the effect of cultivar was shown in two main antioxidant compounds (petunidin-3-hexoside and malvidin-3-arabinoside) (Table 5), and the effect of the production system can be observed in one compound (malvidin-3-galactoside) (Table 6). On the other hand, however, the location did not show any clear direct effect.

Table 7. Concentration of six of the main antioxidant compounds in blueberry as affected by the interactions among cultivars (C), locations (L) and production systems (PS). (Mean ± SD)

Interaction		Main antioxidant compound (μg.g^{-1} fresh fruit)					
		Delphinidin-3-galactoside	Delphinidin-3-acetylhexoside	Chlorogenic acid	Quercetin-3 rutinoside	Quercetin-3-glucoside	Quercetin-derivative
C x L	C1xL1	-	-	500 ± 11.25	10 ± 0.80	-	-
	C2xL1	-	-	97 ± 3.62	57 ± 2.80	-	-
	C3xL1	-	-	325 ± 2.32	125 ± 2.82	-	-
	C1xL2	-	-	125 ± 10.47	17 ± 0.90	-	-
	C2xL2	-	-	287 ± 7.52	40 ± 4.03	-	-
	C3xL2	-	-	125 ± 4.62	15 ± 0.92	-	-
C x PS	C1xPS1	-	12.5 ± 1.17	525 ± 12.25	10 ± 0.35	10.0 ± 2.45	-
	C2xPS1	-	20.0 ± 1.40	125 ± 3.82	45 ± 2.62	37.5 ± 5.20	-
	C3xPS1	-	20.0 ± 2.72	125 ± 4.82	15 ± 1.37	40.0 ± 4.62	-
	C1xPS2	-	4.0 ± 1.07	100 ± 9.47	35 ± 1.35	20.0 ± 3.80	-
	C2xPS2	-	5.0 ± 0.77	260 ± 7.32	43 ± 3.20	62.5 ± 4.47	-
	C3xPS2	-	10.0 ± 2.07	100 ± 2.12	125 ± 2.37	47.5 ± 5.45	-
PS x L	PS1xL1	193 ± 11.7	-	367 ± 7.98	-	35 ± 4.43	15.8 ± 1.55
	PS2xL1	222 ± 16.1	-	98 ± 3.48	-	43 ± 3.26	12.5 ± 1.28
	PS1xL2	352 ± 28.2	-	150 ± 5.95	-	25 ± 3.75	9.1 ± 1.38
	PS2xL2	132 ± 16.1	-	208 ± 9.13	-	45 ± 5.90	18.3 ± 3.45

C1: Brigitta, C2: Duke, C3: Legacy; L1: Foothill, L2: Valley; PS1: Organic, PS2: Conventional

CONCLUSIONS

Brigitta cultivar recorded levels of polyphenols, anthocyanins and antioxidants that were within the normal range for blueberries, but these values were always lower compared to the

other cultivars. In contrast, 'Duke' and 'Legacy' blueberries showed high antioxidant capacity and a high content of polyphenolic compounds, such as anthocyanins.

Blueberries grown under an organic farming system had higher antioxidant properties which indicates that blueberry quality may be better when traditional fertilization and chemical protection are not used.

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