

ANTIOXIDANT CAPACITY, ANTHOCYANINS, AND TOTAL PHENOLS OF WILD AND CULTIVATED BERRIES IN CHILE

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ABSTRACT

It is possible to incorporate a lot of natural antioxidants into the human organism by consuming berries which can prevent diseases generated by the action of free radicals. Antioxidants neutralize free radicals and thus protect the organism from the oxidative damage of lipids, proteins, and nucleic acids. Berries stand out as one of the richest sources of antioxidant phytonutrients among various fruits and vegetables. The objective of this research was to determine antioxidant capacity (AC), total anthocyanins (TA), and total phenols (TP) of wild and cultivated berries in different localities of La Araucanía and Los Ríos Regions in Chile. These parameters were analyzed by using the free radical 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) method, pH-differential, and Folin-Ciocalteu method. Percentages of DPPH discoloration of different berries studied were between 67.8% and 95.3% for red sarsaparilla and rosehip, respectively. Maqui berries showed a significantly higher TA content (2240.2 and 1445.3 mg L⁻¹ cyanidin 3-glucoside) than other berries, and a mean for all berries of 335.5 mg L⁻¹. Higher phenol content levels were obtained in two cultivars of saskatoon (773.9 and 1001.9 mg L⁻¹ gallic acid) and wild rosehip (1457.0 and 1140.4 mg L⁻¹ gallic acid). We conclude that there are significant differences in antioxidant capacity of wild and cultivated Chilean berries in this study which show a strong correlation between AC and TP content.

Key words: 2,2-diphenyl-1-picrylhydrazyl, *Vaccinium corymbosum*, antioxidant potential.

INTRODUCTION

Plant tissues, especially fruits and vegetables, contain many different chemical compounds with different biological and pharmacological capacities and properties. The most common phytochemical antioxidants include ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids (provitamin A), and phenolic compounds such as phenolic and flavonoid acids (flavones, isoflavones, flavanones, anthocyanins, and catechins) (Hertog *et al.*, 1993; Cao *et al.*, 1996; Wang *et al.*, 1996; 1997; Prior *et al.*, 1998; Zheng *et al.*, 2003; Roberts and Gordon, 2003; Su and Chien, 2007; Seeram, 2008a; Speisky *et al.*, 2008).

Vegetable, fruit, and cereal consumption in the diet has been promoted because of its reported benefit to human health, especially in the prevention of degenerative diseases. Research studies carried out on this topic indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids (Prior *et al.*, 1998). It is also mentioned that they are associated to cancer, cardiovascular, and neurodegenerative diseases (Hollman and Katan, 1999). Accordingly, antioxidants are important in neutralizing these free radicals and thus prevent diseases (Gil *et al.*, 2002). The effective protection exerted by plants on degenerative diseases has been widely reported (Prior *et al.*, 1998; Seeram *et al.*, 2006; Halliwell, 2007; Seeram, 2008b).

Growing interest in the role of antioxidants in human health has triggered intense research in the field of agronomic and food sciences. Recently, studies have been devoted to determining how the content and activity of these compounds can be maintained or improved through cultivar development, production practices, postharvest storage, and plant processing. Moure *et al.* (2000) also maintain that it is a priority to replace these synthetic substances with natural ones, which is why the search

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for this type of new plant products has been intensified for their use in the food, pharmaceutical, and cosmetic industry.

Studies carried out by Kähkönen *et al.* (2001) in 26 samples of berries of the family *Ericaceae* genus *Vaccinium*; family *Rosaceae* genus *Rubus*; and family *Grossulariaceae* genus *Ribis* demonstrate a high variation in total phenol content among evaluated berry genera and species. Along the same line, in their study of antioxidant potential of five types of berries, Wada and Boxin (2002) indicate that the antioxidant capacity varied between 24 and 2 $\mu\text{mol Trolox eq g}^{-1}$ ($\mu\text{mol TE g}^{-1}$) of fresh weight among species, anthocyanins between 0.65 and 5.89 mg g^{-1} , and total phenols between 4.95 and 9.80 mg g^{-1} . They concluded that the high antioxidant capacity recorded for some of these berries was positively correlated with the high total anthocyanins and phenol content.

Techniques based on spectrophotometry, high performance liquid chromatography (HPLC), and mass spectrometry (MS) have been developed to identify and quantify antioxidant molecules in plant species (Covey *et al.*, 1986). Kähkönen *et al.* (2001) indicate that there are many simple methods to extract fruit phenolic compounds (Escribano *et al.*, 2006). Therefore, the choice of method is very important so that results are reliable and representative.

Considering the above-mentioned information, we can infer that it is important to generate information about antioxidants in Chile. Accordingly, the objective of this research study was to determine antioxidant capacity (AC), total anthocyanins (TA), and total phenols (TP) in wild and cultivated berries in different localities of La Araucanía and Los Ríos Regions, Chile.

MATERIALS AND METHODS

The research study was carried out in the Biochemistry and Phytopathology Laboratories of the Instituto de Agroindustria, Universidad de La Frontera in Temuco.

Fruit samples. Fruit was picked from adult plants of eight commercial and eight wild berry cultivars. Commercial berries corresponded to the following cultivars: three highbush blueberry (*Vaccinium corymbosum* L.), two raspberry (*Rubus idaeus* L.), two saskatoon (*Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem.), and one strawberry (*Fragaria* \times *ananassa* Duchesne ex Rozier). The wild berries were sarsaparilla (*Muehlenbeckia chilensis* Meisn.), elderberry (*Sambucus nigra* L.), wild blackberry (*Rubus ulmifolius* Schott), Chilean guava (*Ugni molinae* Turcz.), rosehip (*Rosa* spp.), and maqui berry (*Aristotelia chilensis* (Molina) Stuntz). Table 1 shows the cultivars studied and the characteristics of the sampling sites.

Picking was carried out by direct harvesting of mature fruits in clamshell polyethylene terephthalate (PET) containers from plants with 40 to 60% fruit with optimal ripeness according to color. Picked fruit was transported to the Biochemistry Laboratory and stored according to use. Fruit analyzed before 7 d from harvest was maintained refrigerated at 4 ± 1 °C whereas fruit destined for further analysis was preserved according to the individual quick freezing, IQF, method at -20 °C. Thawing of the fruit in IQF was gradual over 24 h in laboratory conditions so as to avoid cell lysis.

Reagents. Reagents were: monohydrated gallic acid, ethanol, methanol, cyanidin 3-glucoside, Folin-Ciocalteu reagent (Sigma Chemical, St. Louis, Missouri, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphate buffer (75 mM, pH 7 phosphate), deionized, distilled, and sterilized water.

Extraction. Homogenization of 10 g of fruit was done through maceration in a ceramic mortar and deionized water was added in a ratio of 1:2 w/v. The homogenate was centrifuged in a Beckman GS centrifuge (Beckman GS, Beckman Instruments, California, USA) at 31 700 g for 15 min at 4 °C and the supernatant was separated (juice fraction). The pulp (insoluble fraction) was treated with 50 mL of pure ethanol solution (99%). The homogenate was maintained at a controlled temperature (25-30 °C) and shaken every 2 min for 20 min. It was then centrifuged at 31 700 g for 15 min at 4 °C. The supernatant was separated and the remaining insoluble fraction was washed again with 25 mL of pure methanol or ethanol solution which was combined with the previous extract and maintained at 4 °C \pm 1.

Analysis of antioxidant capacity. A method based on the discoloration test of the radical 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was employed to analyze AC in berries (Cao *et al.*, 1996). Since no references were found in the literature on the use of the DPPH method in fresh berries, the amount of reactants and reaction time were adapted to evaluate AC.

The chemical analysis protocol of extraction phases and determination of AC, TA, and TP was standardized prior to final extraction. Protocol standardization was carried out with fruit of diverse highbush blueberry cultivars from different locations which were kept frozen in IQF at -20 °C.

Determination of total anthocyanins. Total anthocyanin content of diluted fruit extract was estimated by the pH differential method proposed by Cheng and Breen (1991) and used by Prior *et al.* (1998), Wang and Lin

Table 1. Sampling and picking location background information for wild and cultivated berries.

Species	Cultivar	Fruit picking month (2004)	Soil series	Order	Texture	Classification ¹
Cultivated berries						
Blueberry	Elliot	January	Villarrica	Andisol	Sandy loam	Acrudoxic Melanudands
Blueberry	Brighitta	February	Villarrica	Andisol	Sandy loam	Acrudoxic Melanudands
Blueberry	Brightwell	January	Collipulli	Alfisol	Silt clay loam	Typic Rhodoxeralfs
Raspberry	Meeker	February	La Unión	Inceptisol	Sandy loam to silt clay loam	Andic Dystrudepts
Raspberry	Heritage	February	La Unión	Inceptisol	Sandy loam to silt clay loam	Andic Dystrudepts
Saskatoon	Honeywood	March	Loncoche	Andisol	Silt loam	Acrudoxic Hapludands
Saskatoon	Martin	March	Loncoche	Andisol	Silt loam	Acrudoxic Hapludands
Strawberry	--	February	Collipulli	Alfisol	Silt clay loam	Typic Rhodoxeralfs
Wild berries						
Sarsaparilla	Wild	March	La Unión	Inceptisol	Sandy loam to silt clay loam	Andic Dystrudepts
Elderberry	Wild	February	La Unión	Inceptisol	Sandy loam to silt clay loam	Andic Dystrudepts
Wild blackberry	Wild	March	Villarrica	Andisol	Sandy loam	Acrudoxic Melanudands
Chilean guava	Wild	March	Villarrica	Andisol	Sandy loam	Acrudoxic Melanudands
Rosehip	Wild	March	Collipulli	Alfisol	Silt clay loam	Typic Rhodoxeralfs
Rosehip	Wild	March	Villarrica	Andisol	Sandy loam	Acrudoxic Melanudands
Maqui berry	Wild	February	Freire	Andisol	Silt loam	Typic Placudands
Maqui berry	Wild	February	Villarrica	Andisol	Sandy loam	Acrudoxic Melanudands

¹Soil classification according to CIREN (1999) and US Soil Taxonomy (Soil Survey Staff, 2003).

(2000), Wang and Stretch (2001), as well as Zheng and Wang (2003). Absorbance was measured with a UV-visible spectrophotometer at 510 and 700 nm at pH 1 and 4.5 where $A = (A_{510} - A_{700}) \text{ pH } 1 - (A_{510} - A_{700}) \text{ pH } 4.5$. Data were calculated with the extinction coefficient for cyanidin-3-glucoside (29 600) and expressed as mg cyanidin 100 g⁻¹ fresh weight.

Determination of total phenolic compounds. Total phenolic content in diluted fruit extract was measured by the Folin-Ciocalteu colorimetric procedure based on the formation of a blue molybdenum-tungsten complex with a gallic acid standard (Singleton and Rossi, 1965 as cited by Wang and Lin, 2000; Asami *et al.*, 2003). Diverse authors (Heinonen *et al.*, 1998a; 1998b; Prior *et al.*, 1998; Kähkönen *et al.*, 2001; Wang and Stretch, 2001; Asami *et al.*, 2003; Zheng and Wang, 2003) have used this methodology to study TP in berries.

From 0.5 to 1 mL of the extract was mixed with 5 mL of deionized water and 1 mL of Folin-Ciocalteu reagent in a 25 mL volumetric flask and maintained for 5 to 8 min at room temperature. Subsequently, 10 mL of a 7%

sodium carbonate solution was added to complete 25 mL. The solution was mixed again and maintained for 2 h in laboratory conditions. Aliquots of this sample were filtered through a 0.45 µm polytetrafluoroethylene filter (Whatman), and then total sample phenols were evaluated with a UV-visible spectrophotometer monitoring at 765 nm every 5 min. Total phenol content was standardized with gallic acid and expressed as mg L⁻¹ of gallic acid equivalent (GAE). The concentration correlation was established as a function of gallic acid for equal absorbance in a range of 0.5 to 5 mg L⁻¹ GAE ($R^2 = 0.99$).

Experimental design and statistical analysis. Data obtained were analyzed on the basis of a random design with three replicates for each treatment and by ANOVA. Means were compared for the Tukey multiple comparison test ($p \leq 0.05$). A correlation analysis and linear regression were carried out to correlate AC with TP and TA. Data were processed by the StatsDirect v. 2.7.8 (StatsDirect, Cheshire, UK) statistical program.

RESULTS

Antioxidant capacity, total anthocyanins, and total phenol content (Table 2) were determined for 10 species of berries (four cultivated and six wild) separated into nine groups according to antioxidant potential (Figure 1). DPPH (antioxidant capacity) discoloration percentages of the berries studied ranged between 67.8% for red sarsaparilla and 95.3% for rosehip, and with a total mean of 86.3%. Antioxidant capacity of evaluated species was significantly correlated with TP (0.70), but was not significant with TA content (0.01).

Maqui berry showed a significantly higher TA content (2240.2 and 1445.3 mg L⁻¹) than the rest of the berries. Mean value of TA for all berries was 335.5 mg L⁻¹. The highest levels of TP content were obtained in the two saskatoon (773.9 and 1001.9) and two rosehip (1457.0 and 1140.4) cultivars for which values were significantly higher than for other berries.

The berries studied were grouped according to their antioxidant potential in nine main groups by means of cluster analysis of the three parameters (Figure 1).

The three blueberry cultivars were grouped in cluster 4 along with raspberry cv. Heritage, Chilean guava, and red sarsaparilla. It was observed when comparing means among groups that antioxidant capacity of group 4 was significantly different from elderberry, raspberry cv. Meeker, strawberry, saskatoon cv. Martin, and maqui berry. However, it did not show any significant difference with saskatoon cv. Honeywood, rosehip, and wild blackberry (Figure 2A). Total anthocyanin content was significantly higher in saskatoon cv. Honeywood and rosehip (Groups 2 and 7) (Figure 2B). Total phenol content was significantly higher in saskatoon cv. Martin (Figure 2C) than in any of the berries studied.

DISCUSSION

A significant difference was found in AC, TP, and TA between studied species and between cultivars of the same species. These results could be explained by the high variability of substances with antioxidant characteristics present in the berries. Consequently, Atala *et al.* (2009) cite that blueberry fruit is flavonoid-rich (quercetin)

Table 2. Antioxidant capacity (AC), percentage discoloration (2,2-diphenyl-1-picrylhydrazyl), total anthocyanin content (TA, referred to mg L⁻¹ cyanidin 3-glucoside), total phenol content (TP, as mg L⁻¹ gallic acid) of wild and cultivated berries in La Araucanía and Los Ríos Regions, Chile.

Species and cultivar	AC	TA	TP
Cultivated berries			
Blueberry cv. Elliot	82.5h	199.6de	429.6gh
Blueberry cv. Brigitta	76.0j	122.1efg	318.6jk
Blueberry cv. Brightwell	84.9f	149.6def	361.0hij
Raspberry cv. Meeker	91.8bc	52.4gh	521.4f
Raspberry cv. Heritage	82.6gh	65.6fgh	389.7ghij
Saskatoon cv. Honeywood	91.1c	196.7de	773.9d
Saskatoon cv. Martin	92.3b	213.6d	1001.9c
Strawberry	80.9i	43.8gh	538.3f
Wild berries			
Red Sarsaparilla	67.8k	11.9h	245.3k
Elderberry	91.4bc	416.3c	788.3d
Wild blackberry	87.5e	189.3de	644.7e
Chilean guava	90.0d	15.9h	420.9ghi
Rosehip	94.4a	3.8h	1457.0a
Rosehip	95.3a	3.2h	1140.4b
Maqui berry	83.6g	2240.2a	457.2fg
Maqui berry	89.5d	1445.3b	346.6ij
Mean	86.3	335.5	614.7
Correlation			
AC	---	0.01	0.70
TA	---	---	-0.22

Different letters among species according to treatment indicate a significant difference according to Tukey test ($P \leq 0.05$).

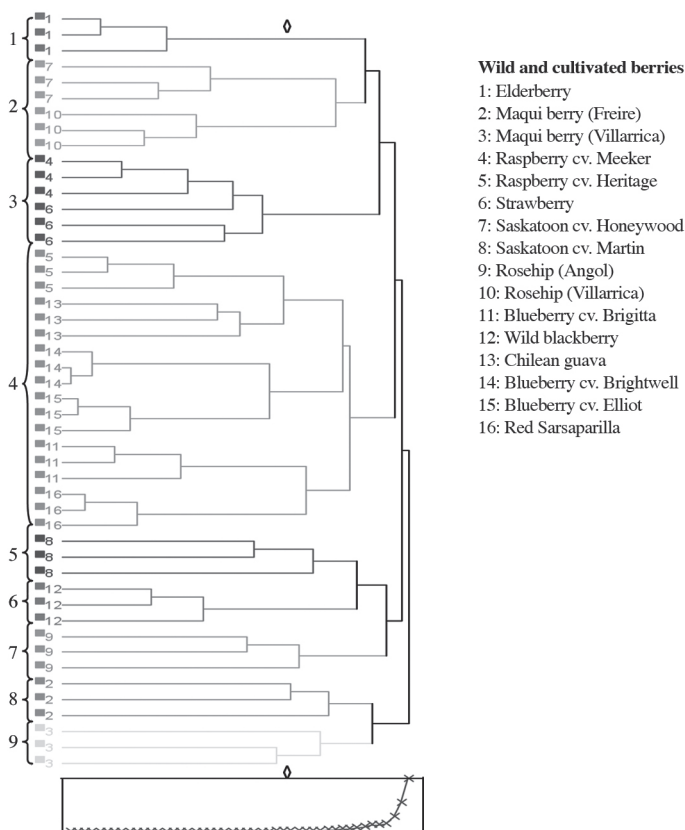


Figure 1. Similarity dendrogram of 16 wild and cultivated berries grown in Chile in relation to their antioxidant potential.

while strawberry and raspberry have higher ellagic acid content. In addition, blueberries are especially rich in hydroxycinnamic acid derivatives which have been shown to possess high antioxidant activity (Chen and Ho, 1997; Meyer *et al.*, 1998; Slimestad and Solheim, 2002; Cheel *et al.*, 2005; Parry *et al.*, 2005; Araya *et al.*, 2006; Rubilar *et al.*, 2006).

The wild rosehip species was identified as having high AC in fruit picked in both Villarrica and Angol. In the cultivated species, the two saskatoon (Martin and Honeywood) and raspberry cv. Meeker cultivars were underscored. On the subject, Tsao *et al.* (2003) indicate that there is a big difference in phenol content among wild and cultivated varieties and is much greater in wild raspberries than in cultivated raspberries. Halvorsen *et al.* (2002) also found that wild blueberry has higher AC than cultivated species.

Ding *et al.* (2006) and Pantelidis *et al.* (2007) cite that TA in cultivated strawberry species, unlike wild species, can contribute more to AC than TP. They add that AC was positively correlated with TP and TA in cultivated berries but not in wild berries. Antioxidant capacity was significantly correlated with TP in this study but not with TA. Furthermore, the significant correlation

between AC and TP recorded in this study coincides with values reported by Connor *et al.* (2002b) who suggest that phenols could be used as a measure for determining specific antioxidant molecules.

When comparing cultivars in the same locality, for example raspberry, it was determined that cv. Meeker showed significantly higher AC, TP, and TA than cv. Heritage. The latter showed differences attributable to genotype. A similar tendency was observed when comparing antioxidant capacity of two blueberry cultivars in the same locality. Likewise, differences were significant when comparing two blueberry species (highbush and rabbiteye) in distinct localities. Along this line, studies carried out by Hakkinen *et al.* (1999) on strawberry and blueberry reported significant differences in phenolic compound content, differences that were positively correlated with the cultivar and growing region. The same authors add that TP content varied a great deal. The more specific phenolic compound profile also recorded significant variations among harvested cultivars in distinct localities and among growing seasons, thus making it very difficult to clearly distinguish the factors causing these differences.

Howard *et al.* (2003) established a positive correlation

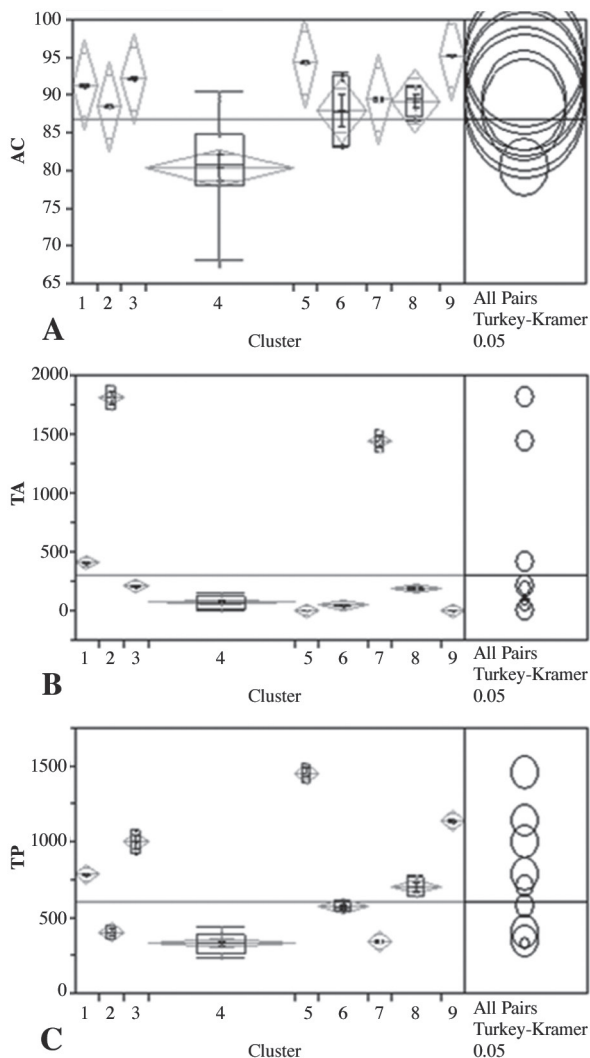


Figure 2. Multidimensional scale and statistical distance for: A) antioxidant capacity (AC); B) total anthocyanins (TA), and C) total phenolic content (TP).

between AC and TP, TA, tartaric ester, and flavonoids in two growth periods for various highbush blueberry cultivars. The evaluated germplasm was more affected by cultivar and cultivar x growing season interaction than by the growing season itself. The content of TA between the two growing seasons indicates that TA synthesis was affected more by differences in environmental growth conditions. It can be inferred from this that cultivars having a higher TP content could be selected to increase AC and that it would be convenient to evaluate the cultivars in various growing seasons.

Regarding the influence of environmental conditions in the crop site, diverse authors report a variation in AC of berries among localities and years for a single crop site (Connor *et al.*, 2002a; Howard *et al.*, 2003). The effect of

this crop practice is an aspect which also influences AC in blueberry. Accordingly, Asami *et al.* (2003) found that AC was lower for blueberries cultivated in a traditional way rather than organically. The authors attribute this response to the fact that plants in organic production are in an increased environmental stress condition.

CONCLUSIONS

There are significant differences in antioxidant capacity in wild and cultivated fruits in Chile, and a strong correlation between antioxidant capacity and total phenol content was observed. Of all the berries evaluated in this study, rosehip picked in the localities of Villarica and Angol was underscored for its antioxidant capacity. The cultivated berries with the greatest antioxidant capacity were the two saskatoon (Martin and Honeywood) and the raspberry cv. Meeker cultivars. From these study results, future studies are directed to evaluating the effect of climatic and edaphic conditions of different locations in Southern Chile on the antioxidant potential of cultivated and wild berries with greater antioxidant capacity, as well as the effect of agronomic management on the antioxidant capacity of different cultivars of the same species.

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RESUMEN

Capacidad antioxidante, antocianinas y fenoles totales de berries silvestres y cultivados en Chile.

Por medio del consumo de berries es posible incorporar al organismo una gran cantidad de antioxidantes capaces de prevenir múltiples enfermedades generadas por la acción de los radicales libres. Los antioxidantes actúan neutralizando los radicales libres y de esta forma protegen al organismo del daño oxidativo de lípidos, proteínas y ácidos nucleicos. Entre variadas frutas y hortalizas, se destacan los berries como una de las fuentes más ricas en fitonutrientes antioxidantes. El objetivo de esta investigación fue determinar la capacidad antioxidante (AC), antocianinas totales (TA) y el contenido de fenoles totales (TP), de berries silvestres y cultivados en diferentes localidades de la Región de La Araucanía y Región de Los Ríos (Chile). Para evaluar estos parámetros se utilizó el método de radicales libres 2,2-difenil-2-picrilhidrazilo (DPPH), pH diferencial y Folin-Ciocalteu, respectivamente. Los porcentajes de decoloración del DPPH estuvieron

comprendidos entre 67,8% para zarzaparrilla roja y 95,3% para rosa mosqueta. El maqui presentó un contenido de AT significativamente mayor (2240,2 y 1445,3 mg L⁻¹ cianidina 3-glucósido) que el resto de los berries, el promedio de AT fue de 335,5 mg L⁻¹. En cuanto al contenido FT los mayores niveles se obtuvieron en los dos cultivares de saskatoon (773,9 y 1001,9 mg L⁻¹ ácido gálico) y en rosa mosqueta silvestre (1457,0 y 1140,4 mg L⁻¹ de ácido gálico). En este estudio se concluye que existen diferencias significativas en la capacidad antioxidante de frutos silvestres y cultivados en Chile, observándose una fuerte correlación entre el CA y el contenido de FT.

Palabras clave: *Vaccinium corymbosum*, 2,2-diphenyl-1-picrylhydrazyl, potencial antioxidante.

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