ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
Food Science and Technology

DOI: https://doi.org/10.1590/fst.25820

Description of the antioxidant capacity of Calafate berries (*Berberis microphylla*) collected in southern Chile

Raúl SÁNCHEZ GUTIÉRREZ[1], Carla GUZMÁN PINCHEIRA[1,2*]

Abstract

The Berberidaceae family of shrubs has about 20 species present in Chile; however, the Calafate (*Berberis microphylla*) native to Chilean and Argentinean Patagonia, is the most widely distributed. The objective of this study was to compare the antioxidant capacity of Calafate collected at different locations in southern Chile, specifically Aysén and Magallanes. Methods: 2000 g of Calafate berries were harvested in both regions. The fruit was lyophilized for subsequent quantification of polyphenols, anthocyanins and antioxidant capacity. Results: All parameters evaluated were superior in the samples from Aysén. Conclusion: The results confirm the high content of polyphenolic compounds present in Calafate, with variations according to the geographical area where they grow. The higher antioxidant capacity of the fruit harvested in Aysén could be associated with the abiotic stress present in that location.

Keywords: phenolic compounds; anthocyanin; berberis; abiotic stress.

Practical Application: The results are a source of information to know the polyphenolic quality of Calafate according to the climatic conditions and in the future to improve its antioxidant capacity.

1 Introduction

Calafate (*Berberis microphylla*), native to South American Patagonia, is the most widely distributed member of the Berberidaceae family of shrubs in Chile (Bustamante et al., 2018). It has great antioxidant capacity, comparable to that contained in other native berries (Brito et al., 2014), which correlates with the high content of total polyphenols in the fruit and its concentration of anthocyanins (Speisky et al., 2012), mainly delphinidin 3-glycoside, cyanidin 3-glycoside and malvidin 3-glycoside (Chamorro et al., 2019), corresponding to a group of water-soluble pigments, which give the berries their blue, red, violet or purple color (Singla et al., 2019). The consumption of these compounds has been proposed as a method of protection against diseases (Yu et al., 2016), and may act as exogenous agents capable of protecting the cell from oxidative damage (Hostetler et al., 2017), However, their synthesis may be influenced by abiotic stress, corresponding to environmental factors such as temperature, humidity and/or ultraviolet radiation, to which the bush is subjected during its growth (Liu et al., 2017; Khoo et al., 2017). Studies on plant food sources have shown that exposure to different abiotic stressors increase anthocyanin concentrations (Li et al., 2018; Altangerel et al., 2017; Chavez-Barrantes & Gutiérrez-Soto, 2017), which may be due to the specific role that these compounds play in the plant during abiotic stress, such as ROS cooling, photoprotection, stress signaling and/or xenohormesis (Kovinich et al., 2015). The objective of this study was to compare the antioxidant capacity of Calafate collected at different locations in southern Chile, specifically Aysén and Magallanes.

2 Materials and methods

Sample Collection: Calafate (*Berberis microphylla*) were collected manually from the bush in the upper, middle and lower areas in a uniform manner in January 2017, obtaining approximately 2000 g of berries in each locality, specifically Aysén (46°18'32"S 73°30'55"O) and Magallanes (54°10'00"S 68°30'00"O) (Figure 1). The samples obtained showed no differences in their sensory attributes according to the geographical location, such as color intensity, brightness and firmness. Likewise, their appearance was globular or rounded up to 0.5 cm in diameter and purplish-black in color. After harvest, they were immediately refrigerated at 5 °C for transfer to the analysis site and then frozen at -20 °C until freeze-drying, which was carried out at a working temperature of -60 °C with a vacuum of 0.02 ATM (Christ Freeze-Dryer, Alpha 1-4 D'plus, Germany), a mechanism that reduces water activity, achieving a final humidity of less than 2% and reducing losses of the labile and photooxidative compounds contained in the fruit (Téllez-Pérez et al., 2020). Once this process was complete, the product was sprayed on ceramic mortar and stored in hermetically sealed high-barrier polyethylene containers at room temperature in a dark, dry place to maintain the stability of the bioactive components (Laleh et al., 2006).

Reagents: Folin-Ciocalteau, gallic acid, methanol, sodium carbonate, potassium chloride buffer pH 1, sodium acetate buffer pH 4.5, DPPH radical, Trolox (Merck, Germany), commercial standards of phenols and anthocyanins (Sigma Aldrich).

Received 27 May, 2020
Accepted 17 July, 2020
[1]*Programa Doctorado en Ciencias Médicas, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile*
[2]*Escuela de Nutrición, Facultad Ciencias de la Salud, Universidad San Sebastián, Concepción, Chile*
*Corresponding author: carla.guzman@uss.cl

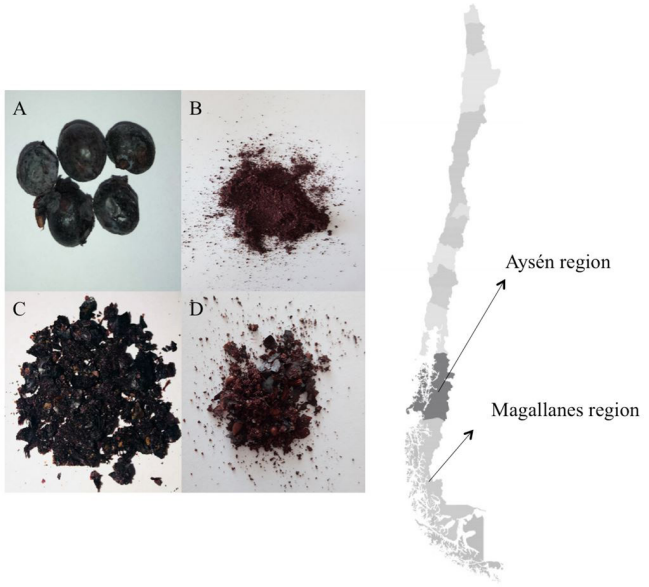

Figure 1. Regions of Chile. (A) and (B) images: Calafate fruit freeze-dried collected Aysén. (C) and (D) images: Calafate fruit freeze-dried collected Magallanes.

Sample preparation: 0.5 g of freeze-dried sample was mixed in 5 mL of methanol, formic acid and water at a ratio of 25:1:24 respectively, and then subjected to 60 minutes of ultrasound, 24 hours of incubation at 4 °C and centrifugation at 3500 rpm for 15 minutes to separate the supernatant, which was then filtered through a PVDF filter 22 µm (Millex HV13, Millipore, Bedford, MA, USA) and stored at 4 °C before analysis, following the protocol described (López et al., 2018).

Determination of total polyphenol content: This was performed using the Folin-Ciocalteu method (Singleton & Rossi, 1965), with a calibration curve of gallic acid as standard and absorbance measurement at a wavelength of 760 nm with a Thermo Scientific™ UV-vis spectrophotometer. The samples were prepared by adding 750 µL of Folin-Ciocalteu 1N reagent, 750 µL of 20% sodium carbonate and 500 µL of the previously extracted supernatant, after which it was incubated for 2 hours in the dark. The blank was prepared with distilled water to replace the sample supernatant. The polyphenol content was expressed as gallic acid equivalent/100 g dry weight. All results were done in quadriplicate.

Determination of total anthocyanin content: This was analyzed using the differential pH method (Lee et al., 2005). Two buffers were used, one of 0.025 M potassium chloride (KCl) at pH 1 and the other of 0.4 M sodium acetate (CH_3CO_2Na) at pH 4.5. From each sample 0.1 mL were extracted and added to two separate tubes. Then 2.9 mL KCl buffer was added to one tube and 2.9 mL CH_3CO_2Na buffer was added to tube 2. Subsequently the absorbance was measured with a Thermo Scientific™ UV-vis spectrophotometer at 510 and 700 nm from each of the tubes. Once the readings were complete, total absorbance was calculated: $A= (A_{510} - A_{700})\ pH_1 - (A_{510} - A_{700})\ pH_{4.5}$ where A = UV-vis absorbance at different wavelengths and according to pH. With the final absorbance value (A) the total anthocyanin content was calculated, taking into account the dilutions: AT = [(A x 1000 x (PM) 449.2)/(ε) 26900] x [3000/100] x [(5/(1000 x g sample)] x 100; where AT = total anthocyanins; PM = molecular weight of cyanidin-3-glucoside; ε = molar extinction coefficient. Data were expressed as mg cyanidin-3-glucoside/100 g dry weight. All results were done in quadruplicate.

DPPH antioxidant capacity (2.2-diphenyl-1-picrylhydrazil): Free radical scavenging activity was determined using the 2.2-diphenyl-1-picrylhydracil free radical method (Mena et al., 2011). 100 µL of the sample and 2.9 mL of the DPPH solution were added, stirred vigorously and incubated in the dark for 1 hour, and then absorbance was read at 515 nm with a Thermo Scientific™ UV-vis spectrophotometer. The blank contained 3 mL of methanol. The results were expressed as Trolox umol/100 g dry weight. All the results were done in quadruplicate.

Identification and quantification of phenolic compounds by HPLC-MS-DAD: This analysis was carried out on a Chromolith RP-18 reverse phase column equipped with a photodiode detector (DAD) (Merck Hitachi, Darmstadt, Germany) following the published protocol (Gironés-Vilaplana et al., 2012). The mobile phase consisted of two solvents: water (A) and formic acid (B) (99:1, v/v), with a flow rate of 1 mL/min. The gradient varied with 8% solvent B, reaching 15% at 25 min, 22% at 55 min and 40% at 60 min, and stayed constant at 70 min. Chromatograms were recorded at 254 nm, 280 nm, 320 nm, 360 nm and 520 nm. As standards pelargonidin-3-glucoside at 520 nm was used for the anthocyanins, quercetin at 360 nm and the ellagic acid derivatives as ellagic acid at 254 nm (Sigma Chemical Co. St. Louis, MO).

2.1 Statistical analysis

The data obtained were digitized in a Microsoft Office Excel 2011® database for later import and analysis in Graph Prism® version 8. The results are reported as mean ± standard deviation as a measure of dispersion. Given the normality of the data, the variables were analyzed by applying a t-test for independent samples, considering statistical significance with $p < 0.05$.

3 Results

3.1 Total polyphenol and anthocyanin content:

The berries harvested for this study showed higher phenol contents, expressed as mg gallic acid per 100 g of dry fruit, than reported for other berries (Grace et al., 2014). The sample from Aysén had a higher content of polyphenols than the sample from Magallanes with values of 1993 ± 75.7 and 1897 ± 134 mg of gallic acid per 100 g of dry fruit respectively ($p > 0.05$). Likewise, and associated with the high polyphenol content, it is observed higher concentration of anthocyanins in the sample of Aysén (1373 ± 50.2 cyanidin 3 glycoside/100 g of dry fruit), compared to Magallanes (1203 ± 47.7 cyanidin 3 glycoside/100 g of dry fruit) ($p > 0.05$). There were no significant differences between the two compounds.

3.2 Evaluation of the antioxidant capacity:

Both samples had DPPH inhibition capacity, expressed in Trolox umol per 100 g of fruit. The sample with the highest

Trolox equivalent antioxidant capacity (TEAC value) was from the Aysén region (8571 ± 358 umol) compared to Magallanes (7242 ± 123 umol), with no significant differences ($p > 0.05$). According to data from the Annual Environmental Report of the Chilean National Institute of Statistics 2017 (Instituto Nacional de Estadística, 2017) and 2018 (Instituto Nacional de Estadística, 2018), there were differences in both temperature and rainfall in both locations during the fruit ripening period, specifically October, November, December 2016 and January 2017 (months 1 - 2 - 3 - 4 respectively). On the one hand, the Aysén region recorded a lower average absolute minimum temperature than that recorded in Magallanes (0.2 ± 1.6 °C and 0.7 ± 0.8 °C respectively), with a marked but not statistically significant difference at the beginning of the period ($p > 0.05$) (Figure 2), while the absolute maximum temperatures were significantly higher in Aysén (25.3 ± 1.2 °C) than in Magallanes (20.1 ± 0.8 °C) ($p=0.014$) (Figure 3), which adds to the greater amount of precipitation in this area, especially in the second month analyzed ($p > 0.05$) (Figure 4). These variables can affect the ripening of the fruit and be partly responsible for the difference in antioxidant capacity between the two samples.

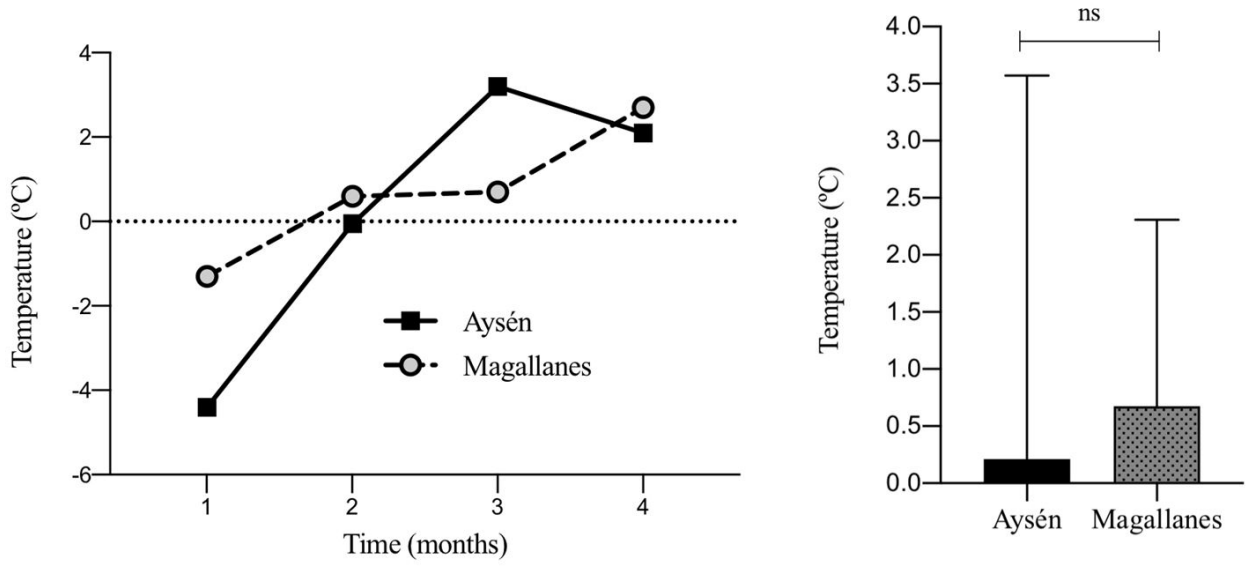

Figure 2. Average minimum temperature during the months of fruit ripening. The data are represented as mean ± standard deviation. (ns) non-significant ($p > 0.05$). T-test for independent samples.

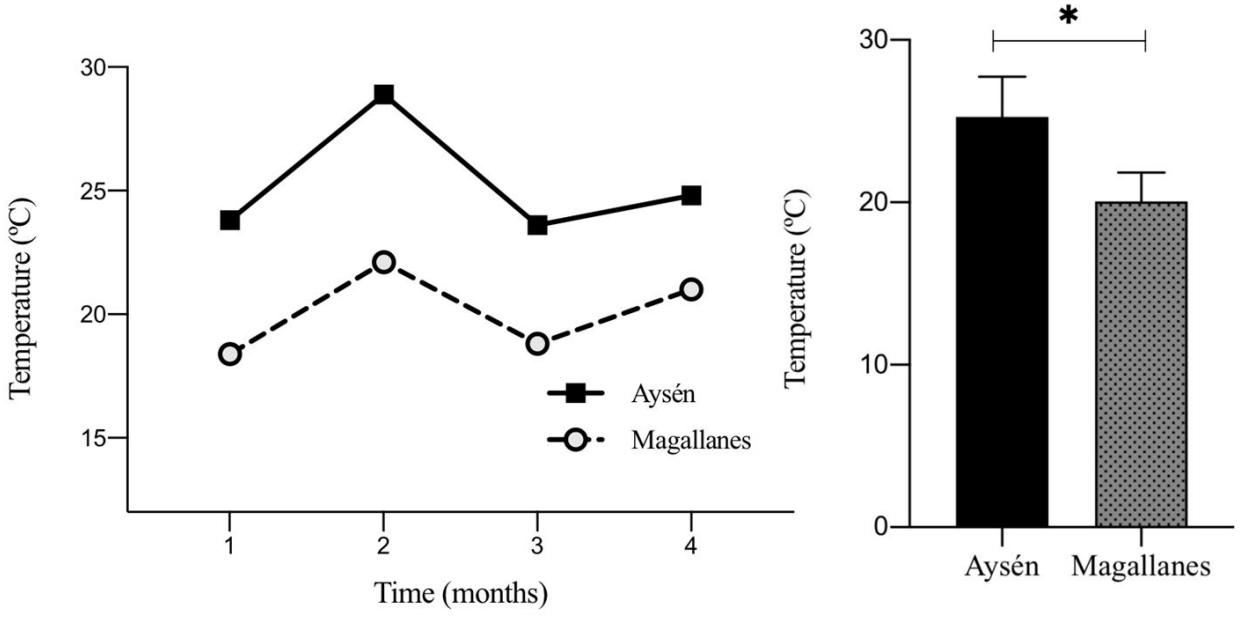

Figure 3. Average maximum temperature during the months of fruit ripening. The data are represented as mean ± standard deviation. (*) statistical significant ($p = 0.014$). T-test for independent samples.

3.3 Polyphenol profile

Regarding the levels of anthocyanins, the one observed in higher concentration corresponds to delphinidin 3 hexoside, followed by petunidin 3 hexoside (Table 1), while the highest flavonol is myricetin 3 glycoside in Aysén and quercetin hexoside derived in Magallanes (Table 2).

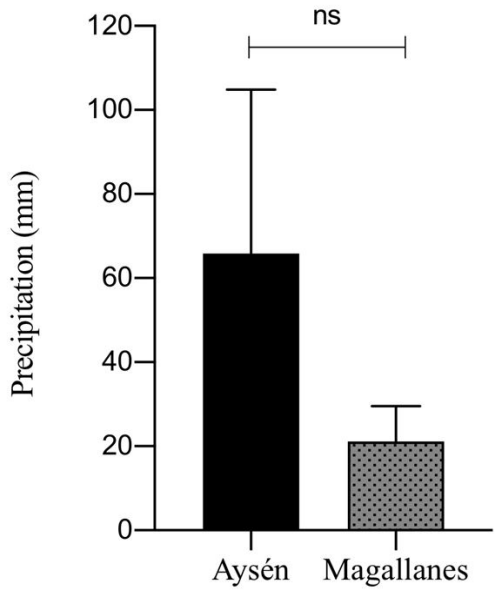

Figure 4. Average rainfall during the months of fruit ripening. The data are represented as mean ± standard deviation. (ns) non-significant ($p > 0.05$). T-test for independent samples

4 Discussion

Polyphenols represent a group of bioactive compounds present in fruits and vegetables, with implications in various physiological and pathological processes, such as their association with the favorable modification of the intestinal microbiota (García-Mazcorro et al., 2018) and their anti-inflammatory effects (Arulselvan et al., 2016; Dugo et al., 2017), and therefore the Chilean native berries can provide great antioxidant power after consumption. The fruit harvested for the purpose of this study showed a higher phenol content than reported for freeze-dried berries such as murtilla (*Ugni molinae Turcz*) and blueberry (*Vaccinium corymbosum*) (Brito et al., 2014). In addition, both samples showed the inhibition capacity of DPPH, expressed in Trolox umol/100 g of dry fruit, results consistent with previous research that identified several phenolic compounds for berries with similar phytochemical characteristics, such as maqui (*Aristotelia chilensis*), which contains antioxidants that can inhibit lipid peroxidation (Cespedes et al., 2010) and murtilla (*Ugni Molinae Turcz*), which even when subjected to extreme temperature changes has a high polyphenol content (Shene et al., 2009). The differences given between the samples collected at the two locations may be due to the content of polyphenols from various sources of plant origin varying according to genotype, climate and geographical location (Altangerel et al., 2017), which is supported by data obtained from the Annual Environmental Report by the INE, Chile in 2017 and 2018, where differences in the climatic conditions of both regions during the fruit ripening period were observed, and where more extreme temperature fluctuations and higher rainfall may favor the process. This may be due to abiotic stress, which includes the elevation of maximum environmental temperature,

Table 1. Anthocyanin content of different samples of Calafate (*Berberis microphylla*).

	Anthocyanin (mg/g dry fruit)			
	Aysén		Magallanes	
	Mean	SD	Mean	SD
Delphinidin-3-hexoside	12.19	0.274	8.81	0.043
Cyanidin-3-hexoside	0.47	0.052	0.55	0.014
Petunidin-3-hexoside	5.09	0.079	4.09	0.066
Petunidin-3-coumaroyl-hexoside	0.19	0.016	0.07	0.025
Peonidine-3-hexoside	2.60	0.118	2.41	0.029
Malvidin-3-hexoside	0.12	0.033	0.02	0.006
Malvidin-3- coumaroyl-hexoside	0.01	0.006	<0.01	0.003

The data are represented as mean ± standard deviation (SD). (ns) non-significant ($p > 0.05$). T-test for independent samples.

Table 2. Flavonol content of different samples of Calafate (*Berberis microphylla*).

	Flavonol (mg/g dry fruit)			
	Aysén		Magallanes	
	Mean	SD	Mean	SD
Myricetin-3-glucoside	0.33	0.002	0.08	0.039
Quercetin-3-hexoside	0.02	0.002	0.17	0.022
Quercetin-3-hexoside derivative	0.03	0.010	0.19	0.092
Isorhamnetin-3-hexoside	0.01	0.003	<0.01	0.001
Isorhamnetin-3-hexoside derivative	0.04	0.001	0.04	0.001
Isorhamnetin-3-rutinoside-7-glucoside	0.11	0.003	0.05	0.030

The data are represented as mean ± standard deviation (SD). (ns) non-significant ($p > 0.05$). T-test for independent samples.

as is the case in the Aysén region, and induces synthesis and accumulation of secondary metabolites in plants, including flavonoids and anthocyanins (Liu et al., 2017). The former plays a variety of roles, such as defending against pathogens, attracting pollinators and reducing the growth of nearby competing plants, while anthocyanins increase their production under adverse temperature conditions, nutrient deficiency and salinity (Wahid, 2007). In several plants an increase has been noted in the concentration of antioxidants when they are subjected to relatively high temperatures compared to those plants that grow in lower temperatures (Wang & Zheng, 2001), and this tolerance to heat stress can be generated as a result of a reprogramming of the transcriptome, mediated by the levels of cellular ATP/AMP in plants and regulated by energy sensors, represented biochemically by calcium and ATP-dependent enzymes, whose activity is stimulated by heat, also promoting the survival of the plant or shrub (Chavez-Barrantes & Gutiérrez-Soto, 2017).

5 Conclusion

The Calafate has a wide geographical distribution in the extreme south of Patagonia; therefore, the berries of the bush may have different concentrations of phenolic compounds according to the climatic characteristics present during the ripening of the fruit. In this study, Aysén berries had higher content of polyphenols and anthocyanins than the Magallanes berries, confirming that variations in climatic conditions affect the antioxidant capacity of the fruit.

Acknowledgements

To the Department of Plant Production of the University of Concepción, Chillán campus, for their support in the experimental analyses.

References

Altangerel, N., Ariunbold, G. O., Gorman, C., Alkahtani, M. H., Borrego, E. J., Bohlmeyer, D., Hemmer, P., Kolomiets, M. V., Yuan, J. S., & Scully, M. O. (2017). In vivo diagnostics of early abiotic plant stress response via Raman spectroscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 114(13), 3393-3396. http://dx.doi.org/10.1073/pnas.1701328114. PMid:28289201.

Arulselvan, P., Tangestani-Fard, M., Sean-Tan, W., Gothai, S., Fakurazi, S., Esa-Norhaizan, M., & Kumar, S. (2016). Role of antioxidants and natural products in inflammation. *Oxidative Medicine and Cellular Longevity*, 2016, 1-15. http://dx.doi.org/10.1155/2016/5276130. PMid:27803762.

Brito, A., Areche, C., Sepúlveda, B., Kennelly, E., & Simirgiotis, M. (2014). Anthocyanin characterization, total phenolic quantification and antioxidant features of some chilean edible berry extracts. *Molecules*, 19(8), 10936-10955. http://dx.doi.org/10.3390/molecules190810936. PMid:25072199.

Bustamante, L., Pastene, E., Durán-Sandoval, D., Vergara, C., Von-Baer, D., & Mardones, C. (2018). Pharmacokinetics of low molecular weight phenolic compounds in gerbil plasma after the consumption of calafate berry (Berberis microphylla) extract. *Food Chemistry*, 268, 347-354. http://dx.doi.org/10.1016/j.foodchem.2018.06.048. PMid:30064768.

Cespedes, C., Valdez-Morales, M., Avila, J. G., El-Hafidi, M., Alarcón, J., & Paredes-López, O. (2010). Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae). *Food Chemistry*, 119(3), 886-895. http://dx.doi.org/10.1016/j.foodchem.2009.07.045.

Chamorro, M. F., Reiner, G., Theoduloz, C., Ladio, A., Schmeda-Hirschmann, G., Gómez-Alonso, S., & Jiménez-Aspee, F. (2019). Berberis species and wild strawberry from the Argentinean Patagonia. *Molecules*, 24(18), 1-24. http://dx.doi.org/10.3390/molecules24183331. PMid:31547424.

Chavez-Barrantes, N., & Gutiérrez-Soto, M. (2017). Crop physiological responses to high temperature stress. I. Molecular, biochemical and physiological aspects. *Agronomía Mesoamericana*, 28(1), 237-253. http://dx.doi.org/10.15517/am.v28i1.21903.

Dugo, L., Belluomo, M. G., Fanali, C., Russo, M., Cacciola, F., Maccarrone, M., & Sardanelli, A. M. (2017). Effect of Cocoa Polyphenolic Extract on Macrophage Polarization from Proinflammatory M1 to Anti-Inflammatory M2 State. *Oxidative Medicine and Cellular Longevity*, 2017, 1-11. http://dx.doi.org/10.1155/2017/6293740. PMid:28744339.

García-Mazcorro, J., Pedreschi, R., Chew, B., Dowd, S., Kawas, J., & Noratto, G. (2018). Dietary supplementation with raspberry extracts modifies the fecal microbiota in obese Diabetic db/db mice. *Journal of Microbiology and Biotechnology*, 28(8), 1247-1259. http://dx.doi.org/10.4014/jmb.1803.03020. PMid:29943551.

Gironés-Vilaplana, A., Valentão, P., Andrade, P. B., Ferreres, F., Moreno, D. A., & García-viguera, C. (2012). Phytochemical profile of a blend of black chokeberry and lemon juice with cholinesterase inhibitory effect and antioxidant potential. *Food Chemistry*, 134(4), 2090-2096. http://dx.doi.org/10.1016/j.foodchem.2012.04.010. PMid:23442660.

Grace, M., Esposito, D., Dunlap, K., & Lila, M. A. (2014). Comparative analysis of phenolic content and profile, antioxidant capacity and anti-inflammatory bioactivity in wild Alaskan and commercial vaccinium berries. *Journal of Agricultural and Food Chemistry*, 62(18), 4007-4017. http://dx.doi.org/10.1021/jf403810y. PMid:24219831.

Hostetler, G., Ralston, R., & Schwartz, S. (2017). Flavones: food sources, bioavailability, metabolism and bioactivity. *Advances in Nutrition*, 8(3), 423-435. http://dx.doi.org/10.3945/an.116.012948. PMid:28507008.

Instituto Nacional de Estadística – INE. (2017). *Annual environment report*. Chile. Retrieved from https://www.ine.cl/

Instituto Nacional de Estadística – INE. (2018). *Annual environment report*. Chile. Retrieved from https://www.ine.cl/

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, 61(1), 1361779. http://dx.doi.org/10.1080/16546628.2017.1361779. PMid:28970777.

Kovinich, N., Kayanja, G., Chanoca, A., Otegui, M. S., & Grotewold, E. (2015). Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. *Plant Signaling & Behavior*, 10(7), e1027850. http://dx.doi.org/10.1080/15592324.2015.1027850. PMid:26179363.

Laleh, G., Frydoonfar, H., Heidary, R., Jameei, R., & Zare, S. (2006). The effect of light, temperature, pH and species on stability of anthocyanin pigments in four berberis species. *Pakistan Journal of Nutrition*, 5(1), 90-92. http://dx.doi.org/10.3923/pjn.2006.90.92.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the ph differential method: collaborative study. *Journal of AOAC International*, 88(5), 1269-1278. http://dx.doi.org/10.1093/jaoac/88.5.1269. PMid:16385975.

Li, X., Lv, X., Wang, X., Wang, L., Zhang, M., & Ren, M. (2018). Effects of abiotic stress on anthocyanin accumulation and grain weight in purple wheat. *Crop & Pasture Science*, 69(12), 1208-1214. http://dx.doi.org/10.1071/CP18341.

Liu, Y., Liu, Y., Tao, C., Liu, M., Pan, Y., & Lv, Z. (2017). Effect of temperature and pH on stability of anthocyanin obtained from blueberry. *Journal of Food Measurement and Characterization*, 12(3), 1744-1753. http://dx.doi.org/10.1007/s11694-018-9789-1.

López, M., Baenas, N., Retamal-Salgado, J., Zapata, N., & Moreno, D. (2018). Underutilized native biobío berries: opportunities for foods and trade. *NPC*, 13(12), 1681-1684. http://dx.doi.org/10.1177/1934578X1801301226.

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D., & Martí, N. (2011). Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L) cultivars grown in Spain. *Journal of the Science of Food and Agriculture*, 91(10), 1893-1906. http://dx.doi.org/10.1002/jsfa.4411. PMid:21480278.

Shene, C., Reyes, A., Villarroel, M., Sineiro, J., Pinelo, M., & Rubilar, M. (2009). Plant location and extraction procedure strongly alter the antimicrobial activity of murta extracts Plant location and extraction procedure strongly alter the antimicrobial activity of murta extracts. *European Food Research and Technology*, 228(3), 467-475. http://dx.doi.org/10.1007/s00217-008-0954-3.

Singla, R. K., Dubey, A. K., Garg, A., Sharma, R. K., Fiorino, M., Ameen, S. M., Haddad, M. A., & Al-Hiary, M. (2019). Natural polyphenols: chemical classification, definition of classes, subcategories, and Structures. *Journal of AOAC International*, 102(5), 1397-1400. http://dx.doi.org/10.1093/jaoac/102.5.1397. PMid:31200785.

Singleton, V., & Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(1), 144-159.

Speisky, H., López-Alarcón, C., Gómez, M., Fuentes, J., & Sandoval-Acuña, C. (2012). First web-based database on total phenolics and oxygen radical absorbance capacity (ORAC) of fruits produced and consumed within the south andes region of South America. *Journal of Agricultural and Food Chemistry*, 60(36), 8851-8859. http://dx.doi.org/10.1021/jf205167k. PMid:22512599.

Téllez-Pérez, C., Cardador-Martínez, A., Tejada-Ortigoza, V., Soria-Mejía, M. C., Balderas-León, I., & Alonzo-Macías, M. (2020). Antioxidant content of frozen, convective air-dried, freezedried, and swell-dried chokecherries (*Prunus virginiana* L.). *Molecules*, 25(5), 2-15. PMid:32155801.

Wahid, A. (2007). Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *Journal of Plant Research*, 120(2), 219-228. http://dx.doi.org/10.1007/s10265-006-0040-5. PMid:17024517.

Wang, S. Y., & Zheng, W. (2001). Effect of plant growth temperature on antioxidant capacity in strawberry. *Journal of Agricultural and Food Chemistry*, 49(10), 4977-4982. http://dx.doi.org/10.1021/jf0106244. PMid:11600054.

Yu, J., Bi, X., Yu, B., & Chen, D. (2016). Isoflavones: anti-inflammatory benefit and possible caveats. *Nutrients*, 8(6), 1-16. http://dx.doi.org/10.3390/nu8060361. PMid:27294954.