Bioagro 35(3): 227-236. 2023 doi: http://www.doi.org/10.51372/bioagro353.6

GEOGRAPHIC VARIATION IN TOTAL PHENOL CONTENT AND SPECIFIC LEAF AREA, AS ANTIOXIDANT INDICATORS OF MAQUI IN CENTRAL CHILE

Enrique Misle A.[1], Estrella Garrido G.[1], Hugo Contardo P.[2] and Besma Kahlaoui[3]

ABSTRACT

The high content of antioxidants of maqui (*Aristotelia chilensis* (Mol.) Stuntz) has recently motivated an increase in the research on this species. This study evaluates the variation in phenolic antioxidants of maqui leaves in the Chilean province of Curicó according to geographical location and sun exposure, using total phenol content (TP) and specific leaf area (SLA) as indicators. Leaves were collected from natural populations inside the province, sampling 15 leaves and three replicates per geographic location. The relationships between SLA and TP, SLA and geographical location, and TP content and geographical location, were assessed in 20 locations within the province. TP varied from 118.36 to 201.9 $mg \cdot g^{-1}$ (GAE) and SLA from 76.8 to 188.2 $cm^2 \cdot g^{-1}$. In general, north facing plants exhibited the highest TP and the lowest SLA. When grouping sampled populations according exposure (north facing, south facing and valley), only north facing points resulted in a significant negative correlation with longitude (r= -0.980, $P \leq 0.05$), implicating that moving westward less phenol content would be found. TP and SLA varied according to exposure and geographical location. Correlation analysis revealed a significant negative relationship between SLA and TP (r = -0.56, $P \leq 0.05$), suggesting the use of this parameter as practical indicator of phenolic antioxidants in maqui.
Additional keywords: *Aristotelia chilensis*, growth analysis, sun exposure

RESUMEN

Variación geográfica del contenido total de fenoles y área foliar específica como indicadores de antioxidantes del maqui en Chile central

El alto contenido de antioxidantes ha motivado en años recientes el aumento de la investigación en maqui (*Aristotelia chilensis* (Mol.) Stuntz). Este estudio evalúa la variación de antioxidantes fenólicos de hojas de maqui en la provincia chilena de Curicó de acuerdo con la ubicación geográfica y exposición solar, utilizando el contenido de fenoles totales (TP) y el área foliar específica (SLA) como indicadores. Se colectaron hojas de poblaciones naturales dentro de la provincia, tomando muestras de 15 hojas y tres repeticiones por ubicación geográfica. Las relaciones entre SLA y TP, SLA y ubicación geográfica, y contenido de TP y ubicación geográfica fueron evaluadas en 20 localidades dentro de la provincia. El contenido de fenoles totales varió de 118.36 a 201.9 $mg \cdot g^{-1}$ (GAE) y SLA de 76.8 to 188.2 $cm^2 \cdot g^{-1}$. En general, plantas en exposición norte exhibieron el mayor TP y el menor SLA. Al agrupar las poblaciones muestreadas de acuerdo con la exposición (norte, sur o valle) sólo los puntos en exposición norte resultaron tener una correlación negativa significativa con la longitud geográfica (r= -0.98, $P \leq 0.05$), implicando que hacia el oeste se encontraría menor contenido de fenoles. TP y SLA variaron de acuerdo con la exposición y ubicación geográfica. El análisis de correlación reveló una relación negativa significativa entre SLA y TP (r = -0.56, $P \leq 0.05$), lo que pudiera permitir el uso de este parámetro como un indicador práctico del contenido de antioxidantes fenólicos en maqui.
Palabras-clave adicionales Análisis de crecimiento, *Aristotelia chilensis*, exposición al sol

INTRODUCTION

The use of medicinal plants has been increasing along the last decades, since several studies have suggested positive correlations between the consumption of food rich in phenols and disease prevention (Hodzic et al., 2009; Guerrero et al., 2010; Quitete et al., 2021). These effects are mostly attributed to different phenolic antioxidants with bioactive properties coming from plants (Kim et al., 2005; Céspedes et al., 2008). As well known, total phenols as a parameter significantly correlates to antioxidant capacity (Hodzic et al., 2009; Olaya and Restrepo, 2012; Liu et al., 2018); a relationship also confirmed in wild and cultivated Chilean berries, including maqui berry (Guerrero et al., 2010). Maqui (*Aristotelia chilensis (*Mol.) Stuntz is a

Received: September 1, 2022 Accepted: March 28, 2023
Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Curicó, Chile. e-mail: emisle@ucm.cl (corresponding author), luri_sma@yahoo.com.mx
[2] Servicio Agrícola y Ganadero, Curicó, Chile. e-mail: hugo_contardo@hotmail.com
[3]National Research Institute of Rural Engineering, Waters and Forestry (INRGREF). Ariana, Tunisia
e-mail: besma.kahlaoui@gmail.com

unique Chilean plant which has motivated research focused on revealing its properties (Araya et al., 2006; Céspedes et al., 2008; Misle et al., 2011). This plant is an evergreen shrub belonging to the family *Elaeocarpaceae* widely distributed in Chile (Donoso and Ramirez, 1994). Flowering normally occurs from September to December, while ripening of the fruit in summer. The plant got attention after the finding that it is one of the plants with the highest content of antioxidants (Araya et al., 2006; Brauch et al., 2016; González et al., 2020). In addition, Vogel et al. (2008) reported that leaves may have higher contents of tannins, alkaloids and flavonoids than fruits. A review on maqui highlighted weak points in the research of the plant and, among the main topics, on the need to study the variability of phenol content within natural populations (Misle et al., 2011). Concerning maqui there is an increasing interest for selecting plant material for growing the plant as a crop.

Along the consideration of the close relationship of total phenols to antioxidant capacity, some morphological traits can be also useful indicators. For long, Poorter (2002) and Poorter et al. (2009; 2012) have argued that specific leaf area (SLA) is a meaningful parameter in ecology, helping in the interpretation of plant responses to environmental changes as well as when comparing plant growth rates in different ecosystems. In addition, they explain that appreciable amounts of secondary compounds can be accumulated in leaves, like lignin and phenolics, increasing the dry weight of leaves in slow growing species or comparatively increasing under stressing environments which can be observed as changes in SLA.

The objective of this study was to evaluate the variation in phenolic antioxidants in leaves of natural populations of maqui widespread in the Chilean province of Curicó according to geographical location and sun exposure, using total phenol content and specific leaf area as indicators. This early assessment may contribute to the research on the species in general with particular novelty for the province.

MATERIALS AND METHODS

Site description. The research area is part of the province of Curicó (central Chile) and it is mainly composed of agricultural land where appreciable intensification is occurring during the last decades. The study was performed over an approximated area of 3300 km^2, covering 44% of the province, where the agricultural activity is mainly carried out, from 70°50’ to 72°04’ W and 34°45’ to 35°10’ S, approximately. Climate is temperate mesothermal Mediterranean semi-arid for most of the sampling area, excepting locations closer to Los Andes where it is Mediterranean sub-humid (Santibáñez et al., 2017). The main agroclimatic districts are described in Table 1.

Table 1. Relevant parameters of main agroclimatic districts in the Chilean province of Curicó, from West (coastal) to East (cordillera). Heat sum base = 10 °C. (adapted from Santibañez et al., 2017)

Sampling location	Tmax (Jan) (°C)	Tmin (Jul) (°C)	Heat sum (°C.days)	Precipit. (mm)	Water deficit (mm)	Dry season (months)
LL	23.7	6.5	1375	616	828	7
LO, LB, LH, LD, LG	26.7	5.4	1569	638	914	7
TO, PA, EC, PE, CO	29.7	5.2	1802	645	1027	7
SA, EP, LC	29.8	4.3	1706	656	1034	7
ZF, QA, ZA, SP, HE	29.6	3.8	1621	756	1025	7
PG	28.7	3.4	1433	1393	693	5

LL: Llico, LO: Lora, LB: Lautaro bridge, LH: Licanten hill, LD: Licanten Idahue, LG: La Higuera, TO: Tonlemo, PA: Palquibudi, EC: El Corazon, PE: Peteroa S. Familia, CO: Comalle, SA: Sarmiento, EP: Cuesta El Peral, LC: Los Cristales, ZF: Zapallar C. La Frutilla, QA: Quilvo Alto, ZA: Zapallar, SP: San Pedro de Teno, HE: Huemul El Escudo, PG: Potrero Grande hill.

Plant Material. Leaf samples were collected from naturally occurring individuals from populations of maqui widespread over the province, during the months of June and August, thus, winter season (Table 2). Sampling points located in hills facing to the North were grouped as "north facing" (NF); sampling points located in hills facing to the South were grouped as "south facing" (SF), and sampling points located in soils without a marked slope in the valleys were grouped as valley (V).

Table 2. Geographical location of 20 selected sampling points and slope exposure in the Chilean province of Curicó

Location	Latitude	Longitude	Slope exposure
Lautaro bridge	-35° 02' 33''	-72° 03' 34''	North facing
Llico	-34° 45' 36''	-72° 04' 56''	Valley(*)
Lora	-35° 01' 57''	-72° 07' 13''	South facing
Licantén hill	-34° 54' 01''	-71° 57' 03''	South facing
Licantén Idahue	-34° 59' 22''	-71° 54' 19''	North facing
La Higuera	-34° 58' 25''	-71° 50' 17''	Valley
Tonlemo	-35° 05' 12''	-71° 43' 48''	North facing
Palquibudi	-35° 02' 03''	-71° 33' 09''	Valley
El Corazón	-35° 00' 05''	-71° 30' 43''	South facing
Peteroa S. Familia	-35° 02' 10''	-71° 25' 08''	South facing
Comalle	-34° 51' 09''	-71° 20' 11''	South facing
Sarmiento	-34° 55' 07''	-71° 12' 12''	Valley
Cuesta El Peral	-34° 49' 19''	-71° 11' 32''	South facing
Los Cristales	-35° 03' 27''	-71° 07' 25''	South facing
Zapallar C. La Frutilla	-35° 02' 21''	-71° 09' 08''	South facing
Quilvo Alto	-34° 55' 41''	-71° 07' 46''	South facing
Zapallar	-34° 59' 45''	-71° 11' 29''	South facing
Potrero Grande hill	-35° 10' 52''	-71° 06' 35''	North facing
San Pedro de Teno	-34° 53' 38''	-71° 05' 53''	Valley
Huemúl El Escudo	-34° 52' 11''	-70° 58' 08''	Valley

(*): plain surface without any marked slope

At each location three individuals were sampled, as replicates, where 15 mature leaves were taken from each tree, at medium height and around the perimeter. Samples were stored in a cooler in separate and labelled bags until processed in the lab.

Plant measures and analysis. Ten leaves from each sample were photographed on a flat surface of white paper with a graduated ruler as scaling reference. Later leaf area was determined through the software Compu Eye, Leaf & Symptom Area (Bakr, 2005). This method for estimating leaf area from digital photos has shown to be highly precise (Lopes et al., 2007). After taking pictures, leaves were oven dried at 70 °C until constant weight. Leaf dry mass was then related to leaf area to obtain the specific leaf area (SLA).

The remaining five leaves from each sample were used for phenols analysis. Leaves were dried and ground to a fine powder and 0.3 g was macerated in 10 mL methanol/water (80/20) at room temperature by a shaker for 24 h; the glass flasks were covered by aluminium film for securing dark conditions. After that, the filtered extract was used for total phenols determination (Waterman and Mole, 1994, Limam et al., 2020). Absorbance at 280 nm was measured on a Lambda 25 UV-Visible spectrophotometer (Perkin-Elmer, Waltham) with the software UV WinLab version 2.85.00. Obtained values were transformed by using a calibration curve of gallic acid and total phenolic content (TP) was expressed as gallic acid equivalents (GAE) ($mg \cdot g^{-1}$).

The results were statistically evaluated by ANOVA and Tukey test for phenol content, SLA, and locations. For evaluating the dataset of phenol content-slope exposure, and SLA-slope exposure, orthogonal contrasts were used. A correlation analysis was performed between SLA and TP. In addition, Pearson correlations were also run among these parameters and geographical position, latitude, and longitude. All the analysis were performed using the IBM SPSS Statistics 21 program.

RESULTS AND DISCUSSION

Phenol content was found to vary significantly (P=0.000) at some of the 20 locations defined within the area of study (Figure 1). Cerro Potrero Grande ranked the highest phenol content with 201.9 mg·g^{-1} as GAE with significant difference to nine of the locations sampled: Zapallar C. La Frutilla, Zapallar, Cuesta El Peral, Sarmiento, El Corazón, Licantén hill, Llico, Huemul El Escudo and Peteroa S. Familia, particularly the last, being highly significant (Tukey, $P\leq$0.01). Also, when comparing Peteroa S. Familia vs. Tonlemo significant differences were found. Cerro Potrero Grande is one of the eastern locations and located north facing which notably ranked the highest value of TP making a clear difference with the coastal locations with milder environmental conditions. So, in general, north exposed plants exhibited the higher phenol content. Notably, the lower content found at Peteroa S. Familia with 118.4 mg·g^{-1} (GAE) does not differ with any of the other locations probably due to the variability of the samples (CV= 0.12). TP found in leaves were in accordance to that reported by Vogel et al. (2008).

Significant differences (P=0.009) among some of the different locations studied were found in maqui leaves in terms of SLA (Figure 1). The lowest SLA values were found at Puente Lautaro, Tonlemo, Idahue, Peteroa S. Familia, Cerro Potrero Grande and La Higuera ranking 76.8 to 89,6 cm^2·g^{-1}, and significant differences were found between Cerro Potrero Grande and Llico, Sarmiento and Cerro Licantén; Tonlemo and Huemul el Escudo. Also significant differences were found between Llico and Zapallar, La Higuera and Palquibudi, Palquibudi and Cuesta El Peral and Peteroa Sgda. Familia; and Cuesta el Peral and Peteroa S. Familia (Tukey, $P\leq$0.01). Notably, the higher SLA was found in Llico (188.2 cm^2·g^{-1}), a location close to the sea shore with mild conditions. Häkkinen et al. (1999) also found significant variations in phenol content for Finnish berries at different localities. In a study on samples of maqui berries from four different regions, the southern location (Pucón) showed the lower TP (Fredes et al., 2014).

Grouping results according to slope exposure: north facing, south facing or valley revealed that north facing plants exhibited the highest phenol content and the lowest SLA (Figure 2). Orthogonal comparisons detected highly significant differences in phenol content between NF and SF ($P\leq$0.01; mean difference =39.44), and significant differences between NF and V ($P\leq$0.05; mean difference =36.02). Similarly, significant differences were detected for SLA comparing NF vs. V ($P\leq$0.05; mean difference =51.36), but no significant between NF and SF ($P>$0.05; mean difference =33.44), perhaps attributed to having much less NF sampling localities than SF. These results are not surprising since such variations in plant parameters by the environment have been well documented in the literature, so Poorter (2002) exposes as a characteristic of low-SLA species, leaves that accumulate more soluble phenolics, relating this to different competitive abilities among plant species and adaptation to the environment.

The correlation analysis for all the samples together revealed a significant negative relationship between SLA and TP in our study ($P\leq$0.05) (Figure 3). A negative correlation between TP and SLA was normally expected since samples of mature leaves were taken from locations with different soil and aerial environments and thinner leaves (higher SLA), and lower TP were found in less stressing environments; conversely, leaves with higher TP and lower SLA are formed in more stressing environments which has long been supported by Poorter (2002) and Poorter et al. (2009; 2012). Consequently, our confirmation of this relationship in maqui enables the use of SLA as indicator of TP in practical terms, e.g., the harvest of leaves for productive purpose, situation in which laboratory determinations of TP can be avoided, considering that in addition, TP may vary depending on plant age and growing conditions, as shown in *Vicia faba* L. (Fuentes et al., 2022).

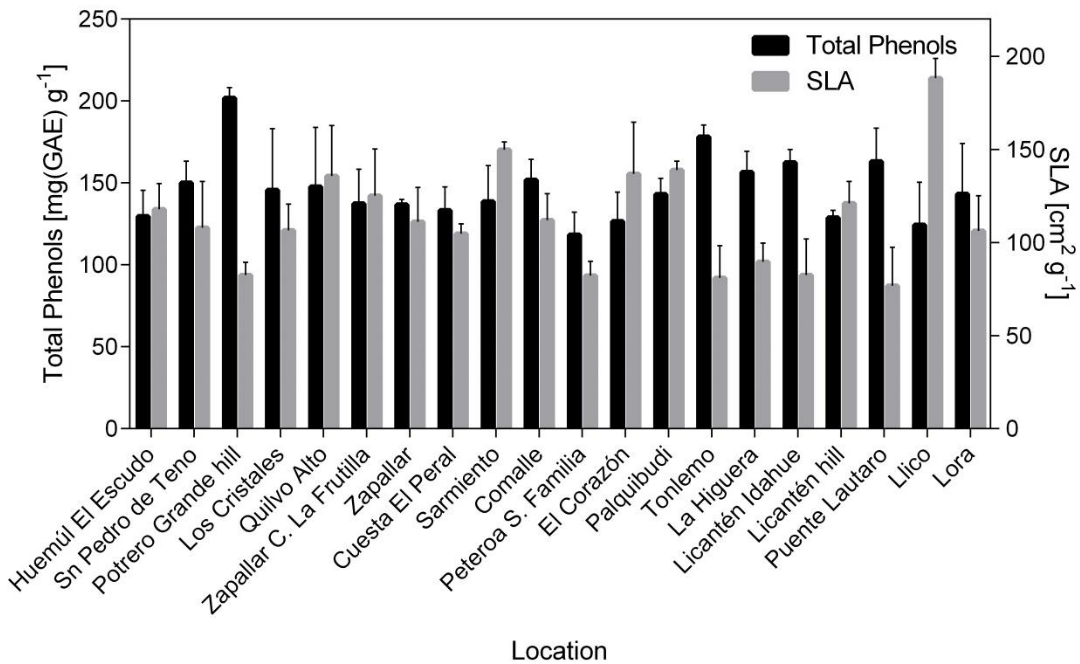

Figure 1. Total phenol content (GAE mg·g^{-1}) and specific leaf area (SLA) (cm^{-2}·g^{-1}) of maqui leaves sampled in natural populations at different locations in the Chilean province of Curicó. The locations are presented in increasing order of longitude. Vertical bars represent the standard deviation

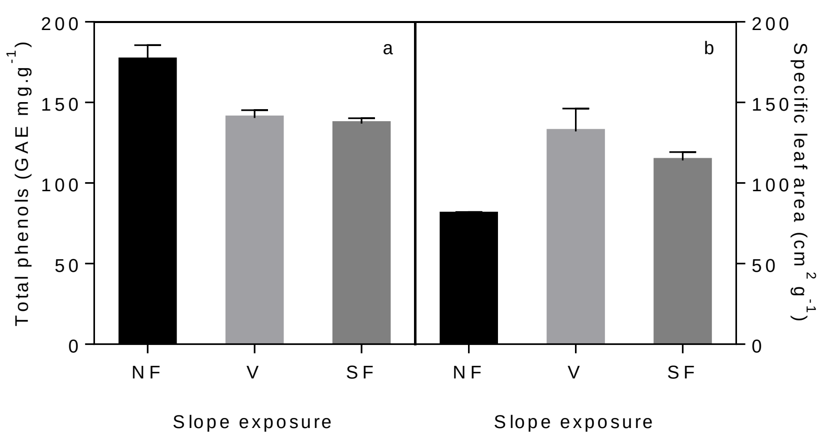

Figure 2. Total phenols (a) and specific leaf area (b) of maqui leaves coming from different locations grouped according to slope exposure: north facing (NF), valley (V) and south facing (SF) in the Chilean province of Curicó. Vertical bars indicate standard error. GAE: gallic acid equivalent

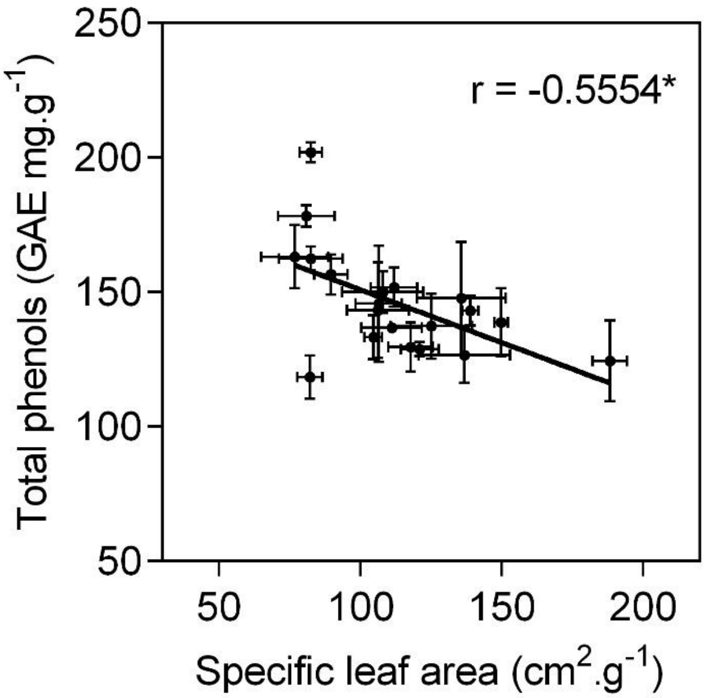

Figure 3. Correlation between total phenol content and specific leaf area in the Chilean province of Curicó. Vertical and horizontal bars indicate standard error

Geographical variation across the province was evaluated for latitude and longitude. The correlation analysis shows a significant positive relationship between total phenols and latitude, r = 0.6545 ($P \leq 0.05$) (Figure 4a). The implicit suggestion here is that moving south inside the ecosystems where maqui populations were sampled, the environment would be more stressing in resources, temperature or water availability which has not a simple interpretation. Across this narrow province, particularly along the valley of Mataquito river, the lowest latitudes correspond to locations south facing, which are typically more diverse ecosystems having more water availability and maintaining favourable conditions for longer in the season. Conversely, the greater latitudes in the province are typically north facing with the opposite situation. So, the relationship in Figure 4a is not necessarily a result of latitudinal movement southward. When grouping sampled populations according exposure, only north facing points result in a significant positive correlation, r = 0.994 ($P \leq 0.05$) (Figure 4b). In fact, Cerro Potrero Grande and Tonlemo possess the harder environments with poor soils, high summer temperatures and less water availability in the season (Santibañez et al., 2017).

On the other hand, there was found no significant correlation between total phenols and longitude ($P > 0.05$) (Figure 4c). However, when grouping sampled populations according exposure, only north facing points result in a significant negative correlation r = -0.980 ($P \leq 0.05$) with longitude, implicating that moving westward less phenol content will be found (Figure 4d). This can be explained by considering the higher thermal oscillation, seasonal and daily towards the eastern locations, while coastal areas possess mild thermal conditions and higher air humidity (note agroclimate 5-7-1 in contrast to 6-7-3 in Table 1)

SLA exhibits a significant negative correlation with latitude ($P \leq 0.05$) (Figure 5a). As known, variations in SLA are highly correlated with variations in the environmental conditions, and SLA differs among habitats, being an important indicator and parameter determining relative growth rates (Poorter et al., 2009). Locations as Potrero Grande hill and Puente Lautaro have soils with low fertility (CIREN, 1997), a condition added to water stress, resulting in lower SLA. Conversely a location like Llico (agroclimate 5-7-1 in Table 1) where the maqui was less exposed to direct sun radiation exhibits higher SLA accompanied with decreased phenols probably due to the comparative decreased carbon fixation per unit leaf area (decreased net assimilation rate) (Poorter, 2002). When grouping sampled populations according to latitude and slope exposure, no significant correlations were found in any of the three conditions ($P \leq 0.05$) (Figure 5b). On the other hand, there was found no significant correlation between SLA and longitude ($P \leq 0.05$) (Figure 5c) neither when grouping locations according to exposure (Figure 5d). This impossibility to find any significant correlation in this parameter on grouped results by exposure may be associated to the fact that it is not a single determination and requires two experimental measures (leaf area and mass) having one in the denominator of the fraction (mass), resulting in higher coefficient of variation for the calculated parameter.

.

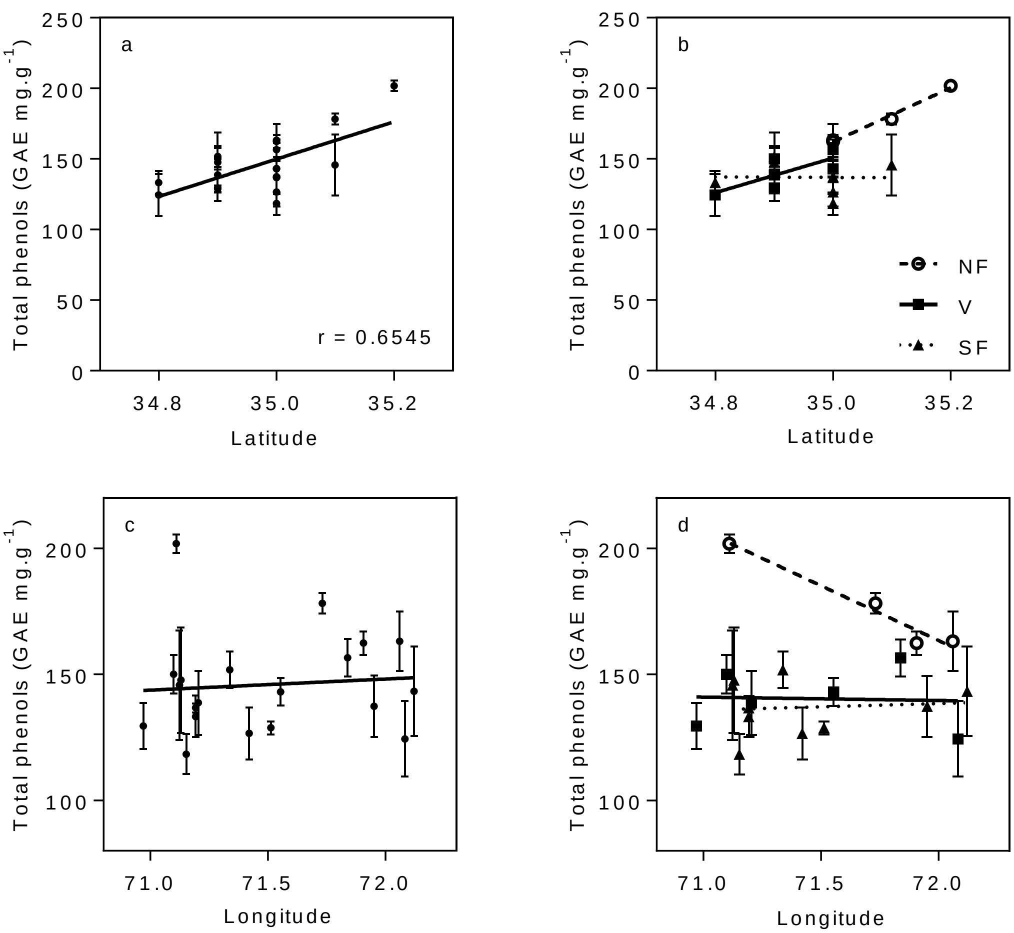

Figure 4. Geographical variation in total phenol content (TP) of maqui in the Chilean province of Curicó. a) TP variation in latitude of samples coming from 20 different locations, global plot; b) TP variation in latitude, grouped according to slope exposure: north facing (NF), valley (V) and south facing (SF); c) TP variation in longitude, global plot; d) TP variation in longitude, grouped. Vertical bars indicate standard error

From our results it is clear that TP significantly correlates to SLA (Figure 3), allowing the use of this parameter as indicator of higher phenolic antioxidants in maqui since in general, TP strongly correlates to antioxidant capacity (Guerrero et al., 2010; Hodzic et al., 2009; Rojas et al., 2021). SLA exhibits a notable plasticity to different abiotic factors. Poorter et al. (2012) calculated SLA plasticity index for different environmental abiotic factors, with larger changes for temperature and water, factors which characterize the comparative differences between some locations in our study (Table 1), particularly north facing ones, compared to south facing.

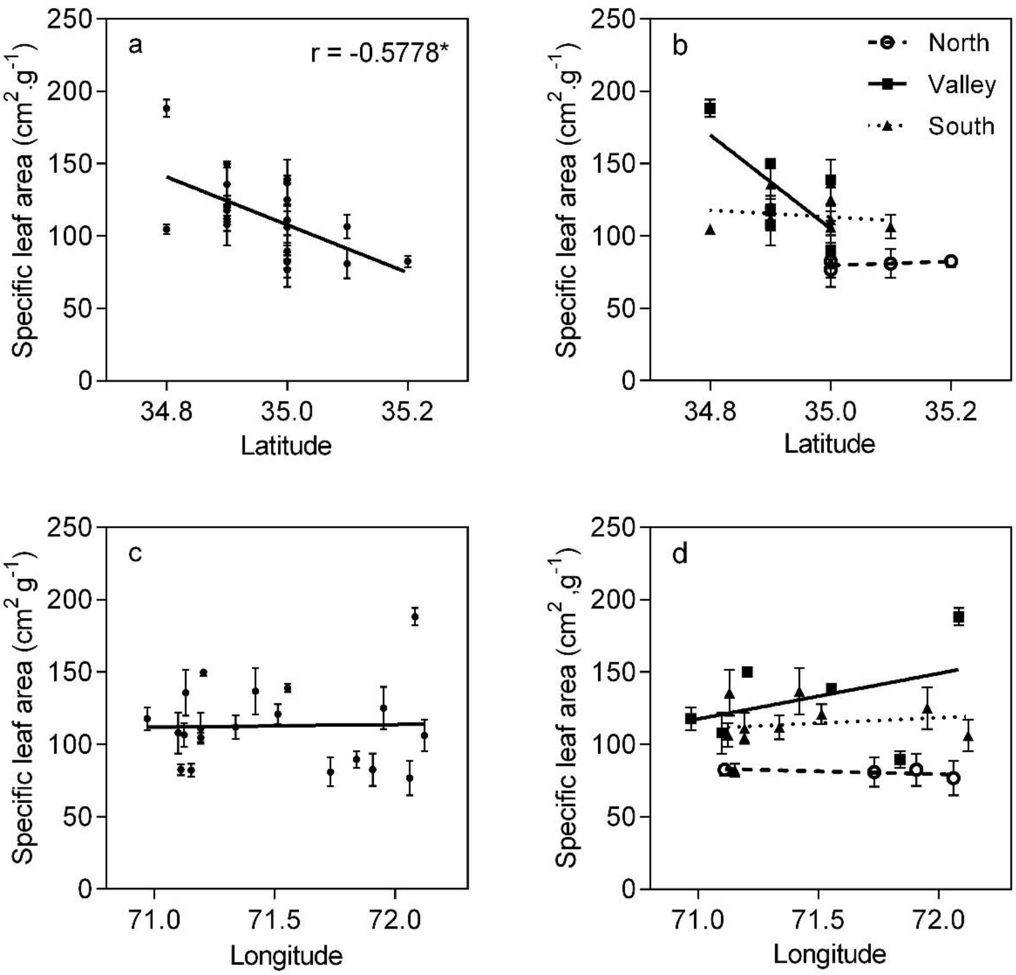

Figure 5. Geographical variation in specific leaf area (SLA) of maqui in the Chilean province of Curicó. a) SLA variation in latitude of samples coming from 20 different locations, global plot; b) SLA variation in latitude, grouped according to slope exposure: north facing (NF), valley (V) and south facing (SF); c) SLA variation in longitude, global plot; d) SLA variation in longitude, grouped. Vertical bars indicate standard error

CONCLUSIONS

Total phenol content (TP) in leaves of maqui varied within the province of Curicó from 118.36 to 201.9 mg·g^{-1} (GAE) and specific leaf area (SLA) from 76.8 to 188.2 cm^2·g^{-1}. In general, north facing plants exhibited the highest TP and the lowest SLA. North facing points resulted in a significant negative correlation with longitude, implicating that moving westward less phenol content would be found. Correlation analysis revealed a significant negative relationship between SLA and TP, allowing the use of this parameter as practical indicator of phenolic antioxidants in maqui.

LITERATURE CITED

1. Araya, H., C. Clavijo. and C. Herrera. 2006. Capacidad antioxidante de frutas y verduras cultivadas en Chile. Archivos Latino-americanos de Nutrición 56: 361-365.
2. Bakr, E. M. 2005. A new software for measuring leaf area, and area damaged by *Tetranychus urticae* Koch. J. Appl. Entomol. 129: 173-175.
3. Brauch, J.E., M. Buchweitz, R.M Schweiggert. and R. Carle. 2016. Detailed analyses of fresh and dried maqui (*Aristotelia chilensis* (Mol.) Stuntz) berries and juice. Food Chemistry 190: 308-316.
4. Céspedes, C.L., M. El-Hafidi, N. Pavon and J. Alarcon. 2008. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis* (Elaeocarpaceae), Maqui. Food Chemistry 107: 820-829.
5. Donoso, C. and C. Ramírez. 1994. Native Shrubs of Chile, Recognition Guideline. Marisa Cuneo Eds., Valdivia, Chile.
6. Fredes, C, G.G. Yousef, P. Robert, M.H. Grace, M.A. Lila, M. Gómez et al. 2014. Anthocyanin profiling of wild maqui berries (*Aristotelia chilensis* [Mol.] Stuntz) from different geographical regions in Chile. J. Sci. Food Agric. 94: 2639-2648.
7. Fuentes-Herrera, P., A. Delgado-Alvarado, B. Herrera-Cabrera, M. Tornero-Campante, M. de L. Arévalo-Galarza, A.L. Martínez-Ayala and A. Barrera-Rodríguez. 2022. Effect of processing methods on the content of phenolic compounds in *Vicia faba* L. tissues grown in field and greenhouse. Bioagro 34(3):221-232.
8. González, J.R., K.S. Ah-Hen, R.L. Mondaca, and O.M. Fariña. 2020. Total phenolics, anthocyanin profile and antioxidant activity of maqui, *Aristotelia chilensis (*Mol.) Stuntz, berries extract in freeze-dried polysaccharides microcapsules. Food Chemistry 313: 1-25.
9. Guerrero, J., L. Ciampi, A. Castilla, F. Medel, H. Schalchli, E. Hormazabal et al. 2010. Antioxidant capacity, anthocyanins, and total phenols of wild and cultivated berries in Chile. Chilean Journal of Agricultural Research 70: 537-544.
10. Hodzic, Z., H. Pasalic, A. Memisevic, M. Srabovic, M. Saletovic and M. Poljakovic. 2009. The influence of total phenols content on antioxidant capacity in the whole grain extracts. European Journal of Scientific Research 28: 471-477.
11. Häkkinen, S.H., S.O. Kärenlampi, I.M. Heinonen, M. Mykkänen and A.R. Törrönen. 1999. Content of the Flavonols Quercetin, Myricetin, and Kaempferol in 25 Edible Berries. J. Agric. Food Chem. 47: 2274-2279.
12. Kim, H., F. Chen, X. Wang and N. Rajapkse. 2005. Effect of chitosan on the biological properties of sweet basil, J. Agric. Food Chem. 53: 3696-3701.
13. Limam, H., M.B. Jemaa, S. Tammar, N. Ksibi, S. Khammassi, S. Jallouli et al. 2020. Variation in chemical profile of leaves essential oils from thirteen Tunisian Eucalyptus species and evaluation of their antioxidant and antibacterial properties. Industrial Crops and Products 158: 112964.
14. Liu, F., M. Wang and M. Wang. 2018. Phenolic compounds and antioxidant activities of flowers, leaves and fruits of five crabapple cultivars (*Malus* Mill. species). Scientia Horticulturae 235: 460-467.
15. Lopes, S.J., B. Brum, V.J. dos Santos, E.B. Fagan, G.LD. Luz, and S.LP. Medeiros, 2007. Estimate of the leaf area of melon plant in growing stages by digital photos. Ciência Rural 37: 1153-115.
16. Misle, E., E. Garrido, H. Contardo, and W. González. 2011. Maqui [*Aristotelia chilensis* (Mol.) Stuntz] the Amazing Chilean Tree: A Review. Journal of Agricultural Science and Technology B1: 473-482.
17. Poorter, H. 2002. Plant Growth and Carbon Economy. Encyclopedia of Life Sciences. Nature Publishing Group, London. http://www.els.net (retrieved March 2023).

18. Poorter, H., Ü. Niinemets, L. Poorter, I.J. Wright and R. Villar. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist 182: 565–588.
19. Poorter, H., K.J. Niklas, P.B. Reich, J. Oleksyn, P. Poot, and L. Mommer. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. The New Phytologist 193: 30-50.
20. Quitete, F.T., G.M.A. Santos, L.O. Ribeiro, C.A. da Costa, S.P. Freitas, V.M. da Matta, and J.B Daleprane. 2021. Phenolic-rich smoothie consumption ameliorates non-alcoholic fatty liver disease in obesity mice by increasing antioxidant response. Chemico-Biological Interactions 336: 1-6.
21. Rojas-Ocampo, E., L. Torrejón-Valqui, L. D., Muñóz-Astecker, M. Medina-Mendoza, D. Mori-Mestanza, and E.M. Castro-Alayo. 2021. Antioxidant capacity, total phenolic content and phenolic compounds of pulp and bagasse of four Peruvian berries. Heliyon 7(8): e07787.
22. Santibáñez, F., P. Santibáñez, C. Caroca and P. González. 2017. Atlas agroclimático de Chile: Estado actual y tendencias del clima, Tomo III: Regiones de Valparaíso, Metropolitana, O´Higgins y Maule. Universidad de Chile, Primera edición. Santiago. 208 p.
23. Vogel, H., I. Rasmilic, J. San Martin, U. Doll and B. González. 2008. Medicinal plants of Chile, Editorial University of Talca, Talca, Chile.
24. Waterman, P. and S. Mole. 1994. Analysis of Phenolic Plant Metabolites. Blackwell Scientific Publication, Oxford.