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Original article

Physiochemical and antibacterial characterization of fruits of *Citronella mucronata* (Cardiopteridaceae), *Pitavia punctata* (Rutaceae) and *Beilschmiedia berteroana* (Lauraceae), three endemic and threatened Chilean trees

F.A. Sáez[1], G.F. Narváez[2], M.F. Morales[3], H.M. Bello[3], C. Balbontín[4] and C.R. Figueroa[5,a]

[1] Master Program in Forest Sciences, Faculty of Forest Sciences, University of Concepción, Concepción, Chile
[2] Faculty of Forest Sciences, University of Concepción, Concepción, Chile
[3] Research Lab of Antibacterial Agents, Faculty of Biological Sciences, University of Concepción, Concepción, Chile
[4] Small Fruits and Berry Crops Research, Institute for Agricultural Research (INIA)-Quilamapu, Chillán, Chile
[5] Phytohormone Research Laboratory, Institute of Biological Sciences, University of Talca, Talca, Chile

Summary

Introduction – **Several native tree species are scarcely studied in relation to fruit properties. In order to bring about information of these plant resources, the characterization of ripening-associated properties of the fruit of three endemic and threatened Chilean trees (*Citronella mucronata*, *Pitavia punctata* and *Beilschmiedia berteroana*) was performed in the present study.** *Materials and methods* – **The physiochemical characterization of two developmental fruit stages in each species included the measurement of soluble solid content (SSC), titratable acidity (TA), pH, color and chlorophyll content; cell wall composition and lignin content; the total phenolic content and antioxidant capacity of the fruit extracts. Moreover, the minimal inhibitory concentration (MIC) was carried out in fruit extracts against 11 bacterial strains.** *Results and discussion* – ***Pitavia punctata* and *B. berteroana* fruits exhibited similar SSC and TA values showing a significant increase of the SSC:TA ratio during development. However, *C. mucronata* showed the highest SSC value at stage II with 8.1 °Brix, accompanied by a noticeable color change. The analysis of pectin fractions suggests that solubilisation of pectic polymers increase during fruit development in all species. The fruits of *C. mucronata* presented the highest level of total uronic acids (for stage I and II, a 63% and 71% of the cell wall material, respectively). The phenolic content, antioxidant capacity and MIC were particularly remarkable in *P. punctata* fruits at stage II, when fruit reaches 6.5 °Brix, with values of 924.4 mg GAE 100 g^{-1} FW, 2.51 mM $FeSO_4$ g^{-1} FW, and 6.25% v/v, respectively.** *Conclusion* – **These results relate interesting properties of *C. mucronata* and *P. punctata* fruits for food and pharmaceutical industries with physiological indexes at stage II, mainly soluble solids and color.**

Keywords
Chile, endemic species, non-timber forest products, underutilized species, fruit quality, cell wall composition, antioxidant compounds

[a] Corresponding author:
cfigueroa@utalca.cl or figlam@gmail.com.

Significance of this study

What is already known on this subject?

- *Citronella mucronata*, *Pitavia punctata* and *Beilschmiedia berteroana* are threatened endemic trees of central Chile whose fruit properties have been scarcely studied.

What are the new findings?

- *C. mucronata* and *P. punctata* fruits extracts showed a high amount of pectin and bacteriostatic effect, respectively. Harvest indexes were described for both fruits.

What is the expected impact on horticulture?

- Under a sustainable management, these fruits can be used for food and pharmaceutical industries.

Résumé

Caractéristiques physico-chimiques et anti-bactériennes des fruits de *Citronella mucronata* (Cardiopteridaceae), de *Pitavia punctata* (Rutaceae) et de *Beilschmiedia berteroana* (Lauraceae), trois arbres chiliens endémiques et menacés.

Introduction – **Nombreuses sont les espèces d'arbres indigènes dont les propriétés des fruits sont peu étudiées. Afin d'obtenir des informations sur ces ressources végétales, l'étude présente a caractérisé les propriétés associées au stade de maturité des fruits de trois arbres chiliens endémiques et menacés (*Citronella mucronata*, *Pitavia punctata* et *Beilschmiedia berteroana*).** *Matériel et méthodes* – **La caractérisation physicochimique de deux stades de développement de chaque espèce comprenait les mesures suivantes: la teneur en matières solubles (SSC), l'acidité titrable (TA), le pH, la couleur et la teneur en chlorophylle des extraits de ces fruits, ainsi que la composition des parois cellulaires, les teneurs en lignine, en composés phénoliques totaux et la capacité anti-oxydante. De plus, la concentration inhibitrice minimale (CMI) des extraits de ces fruits a été effectuée contre 11 souches**

bactériennes. *Résultats et discussion* **– Les fruits de *P. punctata* et de *B. berteroana* ont présenté des valeurs semblables de SSC et de TA, montrant une augmentation significative du rapport SSC: TA au cours de leur développement. Cependant, *C. mucronata* a montré la plus grande valeur de SSC au stade II avec 8,1 °Brix accompagné d'un changement notable de coloration. L'analyse des fractions de pectine suggère que la solubilisation des polymères pectiques augmente au cours du développement des fruits chez toutes les espèces étudiées. Les fruits de *C. mucronata* ont présenté le niveau le plus élevé d'acides uroniques totaux (pour les stades I et II, respectivement 63% et 71% de la paroi cellulaire). Le contenu phénolique, la capacité anti-oxydante et la CMI ont été particulièrement remarquables dans les fruits de *P. punctata* au stade II, quand les fruits atteignent 6,5 °Brix, avec des valeurs de 924,4 mg GAE 100 g^{-1} FW, 2,51 mM $FeSO_4$ g^{-1} FW et 6,25% v/v, respectivement.** *Conclusion* **– Ces résultats rapportent des propriétés particulières des fruits de *C. mucronata* et *P. punctata* peuvent intéresser l'industrie alimentaire ou pharmaceutique avec des valeurs de référence au stade II du développement des fruits, surtout en matières solubles et coloration.**

Mots-clés
Chili, espèces endémiques, produits forestiers non-ligneux, espèces sous-utilisées, qualité du fruit, composition des parois cellulaires, composés anti-oxydants

Introduction

Chile is considered a 'biogeographic island', with diverse climates, ecosystems, and an extraordinary biodiversity (Armesto *et al*., 1998). While the diversity of native plants may be low, considering with other South American countries, Chile has a high percentage of endemism what makes it a 'hotspot' of biodiversity (Myers *et al*., 2000). However, there is little information about the biology and usefulness of many of these endemic resources (Hechenleitner *et al*., 2005). This situation constitutes a major economic opportunity for the sustainable management of forests and their species with conservation problems. While forests have been studied for a long time under the approach of obtaining wood for industrial purposes, now its properties have gained renewed interest as a source of many non-timber forest products (NTFP), which add value to forests (Nepstad and Schwartzman, 1992). Some of the threatened endemic trees of native forests of central Chile whose fruits can be classified as fleshy drupe-type, are 'huillipatagua' (*Citronella mucronata* [Ruiz & Pav.] D. Don), 'pitao' (*Pitavia punctata* Molina) and 'belloto del sur' (*Beilschmiedia berteroana* [Gay] Kosterm.). These three species share a restricted distribution in wet ravines in south-central Chile and according to their conservation status they are threatened to extremely threatened (Hechenleitner *et al*., 2005). Although there are no reports that these fruits are edible, they could be interesting as raw materials or additives for food, pharmaceutical or natural products industries under a sustainable management.

Citronella mucronata is a perennial tree belonging to the *Cardiopteridaceae* family whose species are used in some African countries as an effective molluscicide (Brackenbury, 1999). The fruits of this species are small drupes of about 1.2 cm diameter, dark purple at ripe stage (Hechenleitner *et al*., 2005). *Pitavia punctata* is a perennial tree belonging to the *Rutaceae* family, and its potential could be similar to other species of the family which have been characterized for antimicrobial properties (Esterhuizen *et al*., 2006). An early characterization of extracts from stems and leaves of *P. punctata* reported several flavonoid compounds (Silva *et al*., 1971). Fruits of *P. punctata* are ovoid drupes (1.8–2.5 cm diameter) green at the first developmental stages and yellowish green at ripe stage (Hechenleitner *et al*., 2005). *Beilschmiedia berteroana* is an evergreen tree that belongs to the *Lauraceae* family with globular and greyish-yellow drupes (2 × 2 cm) (Hechenleitner *et al*., 2005). The information about the fruit properties of this species is scarce, however the fruits could be interesting since they would present high amounts of mucilage (Rodríguez *et al*., 1983).

The characterization of fruit properties in this group of species has not been reported. Chilean native fruits have the potential to become in appreciated supplies for nutraceutical industry. In this sense, extracts from the Chilean native berries 'maqui' (*Aristotelia chilensis* [Molina] Stuntz), 'murtilla' (*Ugni molinae* Turcz.) and 'arrayan' (*Luma apiculata* [DC.] Burret) have shown interesting results for their antioxidant and antibacterial activities and potential health benefits, mainly due to the phenolic composition of their leaves and fruits (Genskowsky *et al*., 2016; Suwalsky *et al*., 2007; Fuentes *et al*., 2016). Nevertheless, other interesting metabolites and polymers could be found in endemic fruits. For instance, pectin, a main component of the cell wall found in a wide variety of fruits, is a valuable ingredient for food, cosmetic and pharmaceutical industries (Willats *et al*., 2006). In addition, other interesting and particular physiological processes can be described in indigenous or underutilized fruits that could help understand the ripening phenomena, as in the case of the Chilean strawberry (*Fragaria chiloensis* [L.] Mill.) (Cherian *et al*., 2014).

The objective of this work was to determine the potential of *Citronella mucronata*, *Pitavia punctata* and *Beilschmiedia berteroana* fruits as materials for the food and pharmaceutical industries. The characterization included the analysis of physiochemical and antimicrobial properties of the fruits. In a first step, fruit quality parameters such as color, solid soluble concentration (SSC) and titratable acidity (TA), and chemical attributes such as total phenolic content (TPC) and antioxidant capacity (AOX) were evaluated as a general characterization. In a second step, to deepen insight in certain fruit attributes, analyses of cell wall fractions and antibacterial assays were carried out on fruit extracts.

Materials and methods

Plant material

Fruits of the tree species were collected from three natural populations located in Biobío Region, Chile: 'Hualpén' (36°47'40''S, 73°09'42''W, 54 m a.s.l.) for *C. mucronata*, 'Nonguén' (36°49'55''S, 72°58'08'W, 250 m a.s.l.) for *P. punctata*, and 'Bulnes' (36°43'35''S, 72°15'26'W, 102 m a.s.l.) for *B. berteroana* (Figure 1). The harvested fruits were immediately transported to the laboratory under refrigerated conditions. The fruits were classified in two developmental stages according to the evolution of weight and/or color. The first stage (I) includes smaller fruits; and the second stage (II) includes bigger and pigmented fruits.

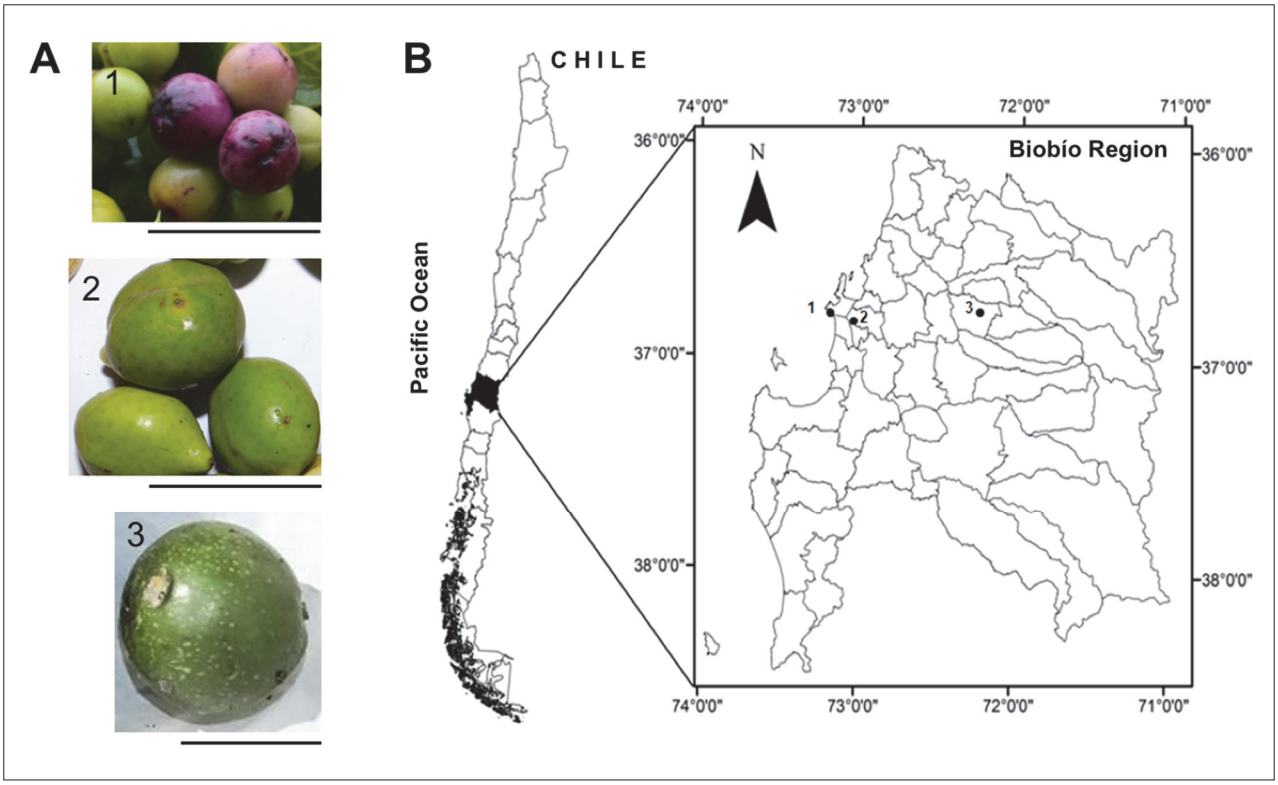

Figure 1. Fruits and locations of the three tree species studied in the present research. (A) Representative photographs of the tree fruits of: 1) *Citronella mucronata*, bar = 25 mm; 2) *Pitavia punctata*, bar = 25 mm and; 3) *Beilschmiedia berteroana*, bar = 20 mm. (B) Geographical locations of the natural populations in Biobío Region (Chile) for the three species: 'Hualpén' for *C. mucronata* (1), 'Nonguén' for *P. punctata* (2) and 'Bulnes' for *B. berteroana* (3). See text for geographic coordinates of each location.

Fruit quality assessments and chlorophyll determination

For each species and stage 30 fruits without external damage were analysed for weight, diameter and color. The fruits were weighed (g) and the equatorial diameter was measured (mm) using a digital caliper (Mitutoyo, USA). The skin color of the fruits was characterized using a colorimeter (model CR-400, Konica Minolta, Japan) and expressed as CIELAB scale (L^*, a^*, b^*) along with the dimensions of color chroma and hue angle (h°). Two measurements on each equatorial side were performed for each fruit. Firmness of stage II fruits was determined using a texture analyser (model CT3, Brookfield Engineering Labs., USA) expressing the results in Newton (N). The fruits were punctured at the equatorial region on opposite sides, using a 1-mm diameter cylinder probe. Thereafter, the fruits were cut into pieces and a bulk tissue sample was prepared in each species and stage and separated in three replicates, which were frozen in liquid nitrogen and stored at -20 °C until use for the rest of the analyses.

For the determination of solid soluble content (SSC), titratable acidity (TA) and pH, 2 g of each replicate were ground in liquid nitrogen, homogenized in 5 mL distilled water and filtered through miracloth obtaining three different juice extractions. The SSC was determined on the juice of each replicate at 20 °C using a hand-held temperature compensated refractometer (Atago, Japan) and expressed as °Brix. The pH was measured in the juice using a pH-meter (Model pH 20, HANNA Instruments, USA). TA was determined by diluting the remaining juice in distilled water (1/10, v/v), and then titrating an aliquot of 13 mL to pH 8.2 using 20 mM NaOH with a digital burette (Jencons, UK) and expressed as g citric acid 100 g^{-1} FW.

Chlorophyll quantification was performed according to the method described by Lichtenthaler and Wellburn (1983) with some modifications. Briefly, fruit skin (0.2 g) was ground with liquid nitrogen, homogenized in 2 mL acetone/H_2O (80/20, v/v) and centrifuged for 10 min at 12,000 rpm at 4 °C. The supernatant was then diluted (1/3, v/v) in acetone/H_2O (80/20, v/v) and then measured at 663–646 nm. The results of three replicates were expressed as µg chlorophyll g^{-1} fruit skin.

Cell wall analysis

Cell wall isolation and lignin determination

Cell wall material was extracted according to Figueroa *et al.* (2012), with some modifications. Five g of frozen fruit tissue was ground with liquid nitrogen, homogenized in 40 mL of 95% ethanol and boiled for 45 min. The insoluble material was filtered through miracloth and sequentially washed with 15 mL boiling ethanol, 15 mL chloroform/methanol (1/1, v/v) and 15 mL acetone. The alcohol insoluble residue (AIR) was dried overnight at 37 °C and weighed. The results of three replicates were expressed as mg AIR g^{-1} FW.

Lignin was extracted as described by Campbell and Ellis (1992). After cell wall preparation, the samples were diluted in 1 M NaOH (1/3, v/v) and hydrolysed. A colorimetric assay was performed using thioglycolic acid (Sigma-Aldrich, USA), and the absorbance was measured at 280 nm. The results of three replicates were expressed as µg lignin g^{-1} FW.

Cell wall fractionation

The fractionation of cell wall material was performed using a sequential chemical treatment of each AIR replicate as previously described (Figueroa *et al*., 2012). The water-soluble (WSF), the trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA)-soluble (CSF), and Na_2CO_3-soluble (NSF) fractions were obtained and considered mainly pectin-enriched fractions. Two independent extractions were obtained from each replicate. The uronic acid (UA) and neutral sugars (NS) concentrations in the different pectin fractions were determined colorimetrically as previously described (Blumenkrantz and Asboe-Hansen, 1973; Yemm and Willis, 1954). The results were calculated using standard curves of galacturonic acid and glucose for UA and NS, respectively. Measurements were performed in triplicate, and the results were expressed as µg galacturonic acid (UA) or glucose (NS) mg^{-1} AIR.

Total phenolic content (TPC) and antioxidant capacity (AOX) determination

Two grams of stage II fruits were ground with liquid nitrogen, homogenized in 10 mL of 100% methanol and centrifuged for 20 min at 5,000 rpm. The samples were filtered through miracloth to obtain a clear extract. Total phenolic content (TPC) were determined using the colorimetric method described by Singleton and Rossi (1965), using gallic acid as the standard. Briefly, 500 µL of methanol extract was mixed with 3.5 mL of distilled water and 250 µL Folin-Ciocalteu reagent, and incubated at room temperature for 5 min. Then, 500 µL of 10% (w/v) Na_2CO_3 was added and samples were incubated in darkness for 1 h at room temperature. Finally, the absorbance was read at 765 nm using an UV/Vis spectrophotometer (Spectronic Genesys, USA). The results of three replicates were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} FW.

Measurement of antioxidant capacity (AOX) of the fruit extracts was evaluated by the ferric reducing ability of plasma (FRAP) assay according to Benzie and Strain (1996). The FRAP reagent was prepared by mixing together 10 mM 2,4,6-tripyridyl triazine (TPTZ) and 20 mM ferric chloride in 0.25 M acetate buffer, pH 3.6. One hundred microliter of methanol extract was added to 300 µL distilled water followed by 3 mL FRAP reagent. The absorbance was read at 593 nm after 5 min of incubation at room temperature against a blank. The results of three replicates were expressed as mM $FeSO_4 \cdot 7H_2O$ equivalent g^{-1} FW.

Antibacterial assays

Fruit samples

Fruit samples of each species from stage II were oven-dried at 70 °C for 48 h. Dried samples were finely pulverized using a food processor. Seven grams of each dried and pulverized sample were mixed with 105 mL of 100% methanol in an orbital shaker at 100 rpm for 24 h at room temperature. After extraction, each sample was filtered and stored at 4 °C until use.

Bacterial strains

Each fruit sample was tested against Gram positive *Staphylococcus aureus* ATCC 29213 and ATCC 25923, *Enterococcus faecalis* ATCC 29212; Gram-negative *Acinetobacter baumannii* ATCC 19606, *Enterobacter cloacae* ATCC E705, *Escherichia coli* ATCC 25922 and ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC BAA-1706, ATCC BAA-1705 and ATCC 700603. All bacterial strains were obtained from the Research Lab of Antibacterial Agents, University of Concepción, Chile. The strains were maintained at -80 °C in a 2:1 ratio of 50% glycerol and broth. Cultures for antimicrobial activity tests were grown in 3 mL Tryptone Soy Broth at 37 °C overnight before the assay.

Determination of minimal inhibitory concentration (MIC)

The MIC was determined by broth microdilution assay according to the Clinical and Laboratory Standards Institute (CLSI, 2012). Briefly, five microliter of inoculum adjusted to 0.5 McFarland of each of the strains were pipetted into 96-well microtiter plates with 100 µL Mueller-Hinton broth (Oxoid Ltd., UK) followed by a two-fold serial dilution of fruit extract samples (final concentration of 5×10^5 CFU mL^{-1}). A positive control (not including extracts) and a negative control (without inoculum) were also included. After incubation at 37 °C for 18–24 h, MIC was determined as the lowest concentration at which no growth occurred (devoid of turbidity).

Determination of minimal bactericide concentration (MBC)

The MBC was calculated by taking 100 µL of broth from each well without growth and transferred to 5 mL Trypticase soy broth. After incubation at 37 °C for 24–36 h, MBC were determined as the lowest concentration at which no growth occurred.

Statistical analysis

The entire experiment was conducted using a completely randomized design, with the main factors being the three species (*C. mucronata*, *P. punctata*, and *B. berteroana*) at one or two developmental stages, as the case. The data were analysed by ANOVA using INFOSTAT (version 2008) software, and differences were considered statistically significant at $P \leq 0.05$ (LSD test).

Results and discussion

Characterization of fruit quality attributes

The physical analysis of the fruit revealed that only *C. mucronata* fruit grows during development, since an increase in fresh weight and diameter was only observed in this species (Table 1). In addition, *C. mucronata* presented the smallest fruits among the three species (2.3 g FW and 13 mm diameter at stage II), and together with *B. berteroana* exhibited lower levels of fruit firmness than *P. punctata* at the stage II (data not shown). The biggest fruit corresponds to *B. berteroana* species, reaching 9.5 g FW and 23.1 mm diameter at stage II.

Regarding pH values, they remained constant during fruit development in *C. mucronata* and *P. punctata*, and increased in *B. berteroana* with the highest value compared with the other species (Table 1). The SSC values reported here for *C. mucronata* differ markedly when compared with the Chilean native *A. chilensis* berry, which showed a SSC close to 19 °Brix (Fredes *et al*., 2012). Nevertheless, other Chilean native berries show SSC values closer to those exhibited by *C. mucronata* fruits. In this sense, it has been reported for 'calafate' berry (*Berberis microphylla* G. Forst.) a wide range of SSC in ripe fruits from 9.3 to 22.9 °Brix, depending on location (Mariangel *et al*., 2013). In addition, it has recently been reported SSC values from 5.20 to 12.56 °Brix in green

Table 1. Quality assessments of *Citronella mucronata*, *Pitavia punctata* and *Beilschmiedia berteroana* fruits in two developmental stages. Data are means ± standard deviations ($n = 3$). SSC: soluble solid content, TA: titratable acidity.

Species	Stages	Fresh weight (g)	Diameter (mm)	pH	SSC (°Brix)	TA (g citric acid 100 g^{-1} FW)	SSC/TA
C. mucronata	I	1.0 ± 0.3 b[y]	11.8 ± 0.9 b	6.3 ± 0.2 a	8.0 ± 0.3 a	0.23 ± 0.03 b	34.4 ± 3.4 a
	II	2.3 ± 0.4 a	13.0 ± 1.1 a	6.1 ± 0.1 a	8.1 ± 0.9 a	0.33 ± 0.03 a	24.5 ± 1.1 b
P. punctata	I	5.0 ± 0.6 a	21.4 ± 1.5 a	6.6 ± 0.1 a	4.2 ± 0.4 b	0.07 ± 0.00 a	61.3 ± 6.6 b
	II	5.2 ± 0.9 a	20.9 ± 1.2 a	6.5 ± 0.1 a	6.5 ± 0.9 a	0.05 ± 0.01 b	108.4 ± 11.3 a
B. berteroana	I	8.7 ± 0.4 a	23.0 ± 1.3 a	7.0 ± 0.0 a	4.7 ± 0.1 a	0.04 ± 0.01 a	118.3 ± 13.9 b
	II	9.5 ± 0.5 a	23.1 ± 1.5 a	6.7 ± 0.1 b	6.3 ± 0.4 a	0.04 ± 0.01 a	164.8 ± 12.3 a

[y] Different letters indicate significant differences between developmental stages in each species ($P \leq 0.05$).

and ripe fruits of *L. apiculata*, respectively (Fuentes *et al.*, 2016). The SSC values for *C. mucronata* fruits are close to the lowest value for *B. microphylla* fruit (*i.e.*, 9.3 °Brix) and with those observed for cultivated berries such as cranberry (9.3 °Brix) (Celik *et al.*, 2008) and raspberry (10.9 °Brix) (Wang *et al.*, 2009). In the case of *P. punctata* and *B. berteroana*, their fruits exhibited lower SSC at stage II than those of *C. mucronata* (Table 1), similar to those values reported for ripe fruits of blackberry (Guedes *et al.*, 2013). Differences in SSC of fruit (juice) depend on the environment and the growing practices, among other factors. For instance, in *B. microphylla* high SSC values are in accordance with higher degrees of south latitude (Mariangel *et al.*, 2013), and in *L. apiculata* a variation between samples collected at different harvest seasons was reported (Fuentes *et al.*, 2016). In the present study, it was difficult to compare between different locations and growing seasons due to the scarcity of these threatened plant species.

On the other hand, *P. punctata* and *B. berteroana* fruits showed a significant increase of the SSC:TA ratio from stage I to II, suggesting that ripening process takes place between these two stages. Fruit at green stage has higher pectin content and starch, which decrease as the fruit ripens due to the action of enzymes that hydrolyse and produce an increase in the concentration of sugars (Osorio and Fernie, 2014). Moreover, the TA is considered as one of the physicochemical properties which affects the organoleptic properties, since the organic acid:sugar ratio defines quality parameters at harvest time in fruit (Osorio and Fernie, 2014).

Fruit pigmentation is a result of a coordinate and development-regulated process during ripening that involves the turnover of green chloroplasts into colored chromoplasts in yellow, orange and red colored fruits (Pech *et al.*, 2014), and the accumulation of anthocyanins that pigmented fruits of red, blue and black (Ageorges *et al.*, 2014). The CIELAB system represented in rectangular coordinates the lightness (L^*) and chromaticity (composed by a^* and b^*). Lightness goes from 0 to 100, where $L^* = 100$ represents a perfect reflecting diffuser, and $L^* = 0$ represents black. The components a^* and b^* have values from ($-a^*$) to ($+a^*$) and ($-b^*$) to ($+b^*$), where a^* goes from green to red, and b^* goes from blue to yellow (Yam and Papadakis, 2004). For L^* parameter, a significant decrease and increase were observed between stages in *C. mucronata* and *P. punctata* fruits, respectively (Table 2). This confirms a greater darkening and lightness observed in *C. mucronata* and *P. punctata* fruits, respectively. In the case of *B. berteroana* fruits, no changes in lightness were observed, with values close to the middle of the L^* scale (Table 2). Alongside this, the fruits of this species neither exhibited significant changes in the parameters a^* and b^*, suggesting that color change is not a good indicator for fruit ripening in *B. berteroana*. In contrast, the results of a^* and b^* for *C. mucronata* and *P. punctata* species showed significant differences between the developmental stages under study. The highest value in the a^* parameter and the lowest value in b^* were observed in *C. mucronata* fruit at stage II according to the purplish color that it reaches at maturity (Table 2; Figure 1A). *Pitavia punctata* fruit turns to yellow color in stage II according to the b^* value, which is the highest value registered (Table 2; Figure 1A).

The chroma and hue angle (h°) are combinations of a^* and b^* parameters and its behavior can be easily differentiate the color vividness and color appearance of each fruits in the developmental stages. As well as a^* and b^* parameters, only *C. mucronata* and *P. punctata* showed changes in chroma and h° between developmental stages. The fruits of all species at stage I have similar h° and chroma values (green tones, 117-120°, with middle chroma values 36 to 39). *Pitavia punctata* and *B. berteroana* fruits remained in this range at the stage II, although *P. punctata* exhibited a more vivid color in stage II (chroma 43.9). On the contrary, *C. mucronata* fruits showed a severe reduction in chroma and h° values (Table 2), indicating a change of color and saturation in the fruit skin (Figure 1A).

The analysis of chlorophylls of *C. mucronata, P. punctata* and *B. berteroana* fruits revealed a significant decrease in the first two species during development, except in the case of chlorophyll *a* for *B. berteroana* which remained without significant differences (Table 2). Remarkably, only *C. mucronata* fruits showed a dramatic decrease of both chlorophylls during development (almost 95% reduction for chlorophyll *a* and a 85% reduction for chlorophyll *b*). At the stage II, *P. punctata* and *B. berteroana* fruits still have high levels of chlorophyll in relation to *C. mucronata* fruits. However, the total content of chlorophyll becomes lower during fruit ripening in all the studied fruits. This loss of chlorophyll is related with the decrease in the hue value, from green to yellow-orange colors tones, as occurred with *C. mucronata* fruits (Table 2).

Analysis of cell wall material, lignin and cell wall composition

The alcohol insoluble residue (AIR) content decreased during the development only in *P. punctata* and *B. berteroana* fruits, without significant differences between *C. mucronata* fruits (Table 3). This suggests a degradation of the cell wall occurring during the fruit development of both *P. punctata* and *B. berteroana* species. Regarding the lignin content, a significant decrease was recorded in *C. mucronata* and *P. punctata* fruits during development. In contrast, *B. ber-*

Table 2. Characterization of color and composition of chlorophylls of *Citronella mucronata*, *Pitavia punctata* and *Beilschmiedia berteroana* fruits in two developmental stages. Data are means ± standard deviations ($n = 3$).

Species	Stages	*L**	*a**	*b**	Chroma	Hue angle (h°)	Chlorophyll *a* (µg g^{-1} FW)	Chlorophyll *b* (µg g^{-1} FW)
C. mucronata	I	52.5 ± 3.0 a[y]	-17.9 ± 1.2 b	33.8 ± 2.4 a	38.3 ± 2.3 a	117.7 ± 0.8 a	468.5 ± 36.1 a	253.2 ±15.1 a
	II	22.2 ± 1.6 b	1.7 ± 0.3 a	1.8 ± 0.3 b	2.4 ± 0.8 b	47.1 ± 3.9 b	25.2 ± 14.3 b	38.8 ± 22.2 b
P. punctata	I	52.4 ± 2.5 b	-18.2 ± 0.4 b	31.6 ± 0.9 b	36.7 ± 2.4 b	119.9 ± 0.2 a	170.9 ± 10.1 a	278.6 ± 14.3 a
	II	62.7 ± 0.9 a	-9.4 ± 1.1 a	43.3 ± 0.5 a	43.9 ± 0.9 a	101.2 ± 2.5 b	144.3 ± 5.7 b	246.1 ± 2.4 b
B. berteroana	I	49.8 ± 2.8 a	-18.2 ± 1.6 a	34.6 ± 3.7 a	39.1 ± 3.1 a	118.3 ± 1.0 a	170.5 ± 10.5 a	277.0 ± 5.9 a
	II	45.1 ± 2.1 a	-16.0 ± 2.6 a	35.4 ± 5.0 a	38.4 ± 1.4 a	114.8 ± 3.6 a	141.8 ± 17.8 a	245.5 ± 11.6 b

[y] Different letters indicate significant differences between developmental stages in each species ($P \leq 0.05$).

Table 3. Cell wall material (AIR), lignin content, and content of uronic acids (UA) and neutral sugars (NS) in water-soluble (WSF), CDTA-soluble (CSF) and Na_2CO_3-soluble (NSF) fractions of *C. mucronata, P. punctata* and *B. berteroana* fruits in two developmental stages. Data are means ± standard deviations ($n = 3$).

Species	Stages	Cell wall material (AIR) (mg g^{-1} FW)	Lignin (µg g^{-1} FW)	Cell wall fractions (µg mg^{-1} AIR)					
				WSF		CSF		NSF	
				UA	NS	UA	NS	UA	NS
C. mucronata	I	151.5 ± 9.5 a[y]	45.0 ± 0.3 a	383.3 ± 20.2 a	3.4 ± 0.7 b	124.9 ± 7.2 b	32.2 ± 3.1 a	116.6 ± 9.8 b	3.6 ± 0.8 a
	II	171.6 ± 3.7 a	36.0 ± 0.5 b	154.6 ± 11.6 b	27.6 ± 1.8 a	319.1 ± 25.9 a	8.9 ±1.1 b	237.0 ± 15.0 a	2.7 ± 1.0 a
P. punctata	I	270.7 ± 7.7 a	53.0 ± 0.2 a	62.4 ± 3.7 a	3.9 ± 0.5 a	30.3 ± 2.9 b	1.4 ± 0.1 b	64.2 ± 0.8 b	2.0 ± 0.4 b
	II	167.8 ± 0.9 b	21.0 ± 0.4 b	71.8 ± 4.7 a	4.8 ± 0.9 a	81.9 ± 5.41 a	3.2 ± 0.4 a	157.1 ± 14.1 a	4.8 ± 0.7 a
B. berteroana	I	247.1 ± 3.5 a	24.0 ± 0.3 b	7.2 ± 2.6 b	0.5 ± 0.3 b	8.8 ± 3.2 b	3.1 ± 1.0 b	16.3 ± 9.0 a	11.8 ± 1.9 a
	II	207.9 ± 3.3 b	31.0 ± 0.1 a	66.2 ± 2.9 a	23.8 ± 1.7 a	19.7 ± 2.1 a	7.3 ± 0.4 a	15.5 ± 2.9 a	17.0 ± 2.0 a

[y] Different letters indicate significant differences between developmental stages in each species ($P \leq 0.05$).

teroana fruits exhibited an increase in lignin during fruit development (Table 3). Generally, the unripe or green-stage fruits have higher fiber content, because the fruit has many polymeric carbohydrates (cellulose, hemicellulose, lignin and pectin substances), which are enzymatically degraded during the ripening process (Waldron *et al.*, 2003). In the three species, some extent of degradation of these polymers seems to occur during fruit development, either of cell wall in *P. punctata* and *B. berteroana*, or of lignin in the case of *C. mucronata* (Table 3).

In order to determine the proportion of the different pectic substances in the fruits, the AIR was sequentially extracted with water, CDTA and Na_2CO_3 to obtain the WSF, CSF and NSF fractions, respectively (Table 3). Cell wall fractionation and quantification of uronic acids (UA) and neutral sugars (NS) allowed the identification of pectic composition associated to WSF, CSF and NSF fractions. It is well known that high methoxylated pectins can be easily extracted with water, although it can only partially solubilize some of the pectic substances of the primary cell walls and middle lamellae (Selvendran and O'Neill, 1987). On the other hand, chelating agents, such as CDTA ions solubilises low methoxylated pectin fractions, and this procedure also preferentially solubilises pectic substances from the middle lamellae. WSF fraction usually has a relatively higher degree of neutral sugar content compared with the CSF fraction. Disruptive treatment with Na_2CO_3 captured pectin fractions with lower molecular weight due to cleavage of glycosidic bonds mainly from primary cell walls (Selvendran and O'Neill, 1987).

In terms of UA content, higher values were observed at the stage II of *C. mucronata* and *P. punctata* in CSF and NSF fractions. In the case of *B. berteroana*, higher UA values were observed at the stage II in WSF and CSF fractions. Only *C. mucronata* fruit showed a reduction in UA content in the WSF fraction during development. Interestingly, the greater proportion of pectins is in different fractions depending on the species. Thus, WSF, CSF and NSF are the majority fraction in *B. berteroana*, *C. mucronata* and *P. punctata*, respectively (Table 3). In terms of NS content, values are markedly lower in all species and stages with respect to UA values. Among different fractions, WSF exhibited the greater NS values: it represents 70% and 50% for *B. berteroana* and *C. mucronata* at the stage II, respectively. During fruit development, an increase in NS content can be observed in *P. punctata* and *B. berteroana* in the CSF fraction, and in *C. mucronata* and *B. berteroana* in the WSF fraction (Table 3). These data suggested that the solubilisation of pectic polymers increases during fruit development and differences in the pectin metabolism could be specific to each species, both events widely reported for many fruits (Ruiz-May and Rose, 2013). In this sense, *C. mucronata* presented the highest level of total UA in relation to AIR in the two developmental stages (for I and II, 63% and 71%, respectively). In contrast to the observed in this species, *P. punctata* and *B. berteroana* showed lower UA levels in stage I and II: 16% and 31%; 3% and 10%, respectively. Thus, differences in pectin contents were observed between species, suggesting that *P. punctata* and *B. berteroana* might have a higher proportion of hemicellulosic polymers in comparison to *C. mucronata*.

Analysis of total phenolic content (TPC) and antioxidant capacity (AOX)

The analysis of TPC, AOX and antimicrobial assays was performed in fruits at stage II, since these fruits, when ripen, could accumulate bioactive compounds as most studied (Kanellis and Manganaris, 2014). The results of TPC and AOX showed significant differences between species. The greatest values of TPC and AOX were detected in *P. punctata* fruits whereas that the lowest values of AOX were observed in *C. mucronata* fruits (Table 4). This result suggests that other phytochemicals rather than anthocyanins can be related with the higher TPC and AOX founded in *P. punctata* fruit since this fruit has a light yellow color (chroma 43.9 and h° 101.2) at stage II (Table 2). It has been reported that in Citrus crops, which belong to the same botanical family as *P. punctata*, several biocompounds such as flavanones and triterpenoid acids can contribute to a significant antioxidant capacity (Yu *et al.*, 2005).

Table 4. Total phenolic content (TPC) and antioxidant capacity (AOX) by FRAP method of *C. mucronata*, *P. punctata* and *B. berteroana* fruits at developmental stage II. Data are means ± standard deviations ($n = 3$).

Species	TPC (mg GAE 100 g^{-1} FW)	FRAP (mM $FeSO_4$ g^{-1} FW)
C. mucronata	742.7 ± 56.0 b[y]	0.710 ± 0.001 c
P. punctata	942.4 ± 64.2 a	2.510 ± 0.100 a
B. berteroana	724.5 ± 60.7 b	1.660 ± 0.124 b

[y] Different letters indicate significant differences between species ($P \leq 0.05$).

The TPC values exhibited by *P. punctata* fruits are comparatively higher than those found in the worldwide known berries grown in Chile such as raspberry 'Heritage' (300 mg GAE 100 g^{-1} FW), blueberry 'Brigitta' (610 mg GAE 100 g^{-1} FW) and strawberry 'Camarosa' (630 mg GAE 100 g^{-1} FW) (Fredes *et al.*, 2014), and in the native 'calafate' berry (870 mg GAE g^{-1} FW) (Ruiz *et al.*, 2010), being overcome only by the 'maqui' berry (1,460 mg GAE g^{-1} FW) (Ruiz *et al.*, 2010). On the other hand, the antioxidant capacity observed for *C. mucronata* fruit is equivalent to those reported for the 'maqui' berry (0.152 mmol Fe^{2+} g^{-1} FW) also measured by means of FRAP assay (Ruiz *et al.*, 2010).

Determination of antibacterial activity of fruit extracts

The results of antibacterial activity on various bacterial species are shown in Table 5. Additionally, the minimal inhibitory concentration (MIC) for methanol was evaluated being this 50% v/v. The results indicated that most of methanol extracts obtained from fruits samples showed a MIC > 12.5% (v/v). The only extract that presented the lowest MIC was from *P. punctata* against *P. aeruginosa* (6,25% [v/v]). When minimal bactericide concentration (MBC) was determined for this extract, a value > 12.5% (v/v) was obtained, indicating that the extract from *P. punctata* against *Pseudomonas aeruginosa* has a bacteriostatic behavior.

The high resistance presented by *Pseudomonas aeruginosa* to antibiotics and compounds from plants or fungal producing antibiotics (Adwan *et al.*, 2010; Sharifi-Rad *et al.*, 2015; Mota *et al.*, 2015) is apparently resulting from a particular waterproofing membrane and the presence of efflux mechanisms (Mayaud *et al.*, 2008; Tyagi and Malik, 2011). Furthermore, it has been reported that the low activity of lipophilic compounds from plants may be due to multidrug pumps expulsion, including ATP-binding cassette (ABC) transporters type (active transport) that remove antibiotics and other harmful lipophilic substances (Mulyaningsih *et al.*, 2011). In this sense, it could be interesting to deepen into the

Table 5. Minimum inhibitory concentration (MIC, % v/v) of extracts of *P. punctata*, *C. mucronata* and *B. berteroana* fruits at developmental stage II against American Type Culture Collection (ATCC) bacterial strain.

Bacterial strains		MICs (% v/v)		
		P. punctata	*C. mucronata*	*B. berteroana*
Gram negative				
Acinetobacter baumannii	ATCC 19606	> 12.5	> 12.5	> 12.5
Enterobacter cloacae	ATCC E705	> 12.5	> 12.5	> 12.5
Escherichia coli	ATCC 25922	> 12.5	> 12.5	> 12.5
Escherichia coli	ATCC 35218	> 12.5	> 12.5	> 12.5
Klebsiella pneumoniae	ATCC BAA-1706	> 12.5	> 12.5	> 12.5
Klebsiella pneumoniae	ATCC BAA-1705	> 12.5	> 12.5	> 12.5
Klebsiella pneumoniae	ATCC 700603	> 12.5	> 12.5	> 12.5
Pseudomonas aeruginosa	ATCC 27853	6.25	> 12.5	> 12.5
Gram positive				
Staphylococcus aureus	ATCC 29213	> 12.5	> 12.5	> 12.5
Staphylococcus aureus	ATCC 25923	> 12.5	> 12.5	> 12.5
Enterococcus faecalis	ATCC 29212	> 12.5	> 12.5	> 12.5

mechanisms of the bacteriostatic effect of *P. punctata* fruit extract, since our results are hopeful when compared with those of other plant extracts.

Conclusion

The present research is a first approach to the characterization of the physiochemical and antibacterial properties of the fruits of *Citronella mucronata*, *Pitavia punctata* and *Beilschmiedia berteroana*, three endemic and threatened trees of south-central Chile. Interestingly, this study provides indications as harvest indexes for possible use of these fruits associating physiological properties to fruit developmental stages. Specifically, it was possible to associate the highest pectin content observed at stage II of *C. mucronata* fruit with purplish color (*i.e.*, color parameters of L^* = 22.2; a^* = 1.7; b^* = 1.8) and SSC value (8.1 °Brix). These indexes are good indicators to select fruits at that stage. Likewise, *P. punctata* fruits at stage II, with a yellowish color (*i.e.*, L^* = 62.7; a^* = -9.4; b^* = 43.3) and 6.5 °Brix, present a high phenolic content and antioxidant capacity, that can be related with interesting antimicrobial properties of the fruit extract against the opportunistic human pathogen *Pseudomonas aeruginosa*.

The importance of this study lies on the identification of harvest indexes for two types of fruits with promising features of interest to the food and pharmaceutical industries. Further studies should address the physiological factors that determine fruit ripening (*e.g.*, phytohormones), the analysis of the genetic variability of different populations for potential genetic improvement and the challenge of producing these interesting non-timber forest products under proper sustainable management.

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References

Adwan, G., Abu-Shanab, B., and Adwan, K. (2010). Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug resistant *Pseudomonas aeruginosa* strains. Asian Pac. J. Trop. Med. *3*, 266–269. https://doi.org/10.1016/S1995-7645(10)60064-8.

Ageorges, A., Cheynier, V., and Terrier, N. (2014). Polyphenols. In Fruit Ripening: Physiology, Signalling and Genomics, P. Nath, M. Bouzayen, A.K. Mattoo, and J.-C. Pech, eds. (Oxfordshire, UK: CAB International). https://doi.org/10.1079/9781845939625.0151.

Armesto, J.J., Rozzi, R., Smith-Ramírez, C., and Arroyo, M.T.K. (1998). Conservation targets in South American temperate forests. Science *282*, 1271–1272. https://doi.org/10.1126/science.282.5392.1271.

Benzie, I.F., and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. Anal. Biochem. *239*, 70–76. https://doi.org/10.1006/abio.1996.0292.

Blumenkrantz, N., and Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. Anal. Biochem. *54*, 484–489. https://doi.org/10.1016/0003-2697(73)90377-1.

Brackenbury, T. (1999). The molluscicidal properties of *Apodytes dimidiata* (Icacinaceae): geographical variation in molluscicidal potency. Ann. Trop. Med. Parasit. *93*, 511–518. https://doi.org/10.1080/00034983.1999.11813451.

Campbell, M., and Ellis, B. (1992). Fungal elicitor-mediated responses in pine cell cultures. Planta *186*, 409–417. https://doi.org/10.1007/BF00195322.

Celik, H., Özgen, M., Serce, S., and Kaya, C. (2008). Phytochemical accumulation and antioxidant capacity at four maturity stages of cranberry fruit. Sci. Hortic. *117*, 345–348. https://doi.org/10.1016/j.scienta.2008.05.005.

Cherian, S., Figueroa, C.R., and Nair, H. (2014). 'Movers and shakers' in the regulation of fruit ripening: a cross-dissection of climacteric versus non-climacteric fruit. J. Exp. Bot. *65*, 4705–4722. https://doi.org/10.1093/jxb/eru280.

CLSI, Clinical and Laboratory Standards Institute (2012). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (CLSI document M07-A9) (Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute).

Esterhuizen, L.L., Meyer, R., and Dubery, I.A. (2006). Antimicrobial compounds from *Coleonema album* (Rutaceae). Z. Naturforsch. C *61*, 489–498. https://doi.org/10.1515/znc-2006-7-805.

Figueroa, C.R., Opazo, M., Vera, P., Arriagada, O., Díaz, M., and Moya-León, M. (2012). Effect of postharvest treatment of calcium and auxin on cell wall composition and expression of cell wall-modifying genes in the Chilean strawberry (*Fragaria chiloensis*) fruit. Food Chem. *132*, 2014–2022. https://doi.org/10.1016/j.foodchem.2011.12.041

Fredes, C., Montenegro, G., Zoffoli, J.P., Gómez, M., and Robert, P. (2012). Polyphenol content and antioxidant activity of maqui (*Aristotelia chilensis* Molina Stuntz) during fruit development and maturation in Central Chile. Chil. J. Agr. Res. *72*, 582–589. https://doi.org/10.4067/S0718-58392012000400019.

Fredes, C., Montenegro, G., Zoffoli, J.P., Santander, F., and Robert, P. (2014). Comparison of the total phenolic content, total anthocyanin content and antioxidant activity of polyphenol-rich fruits grown in Chile. Cienc. Investig. Agrar. *41*, 49–60. https://doi.org/10.4067/s0718-16202014000100005.

Fuentes, L., Valdenegro, M., Gómez, M.G., Ayala-Raso, A., Quiroga, E., Martínez, J.P., Vinet, R., Caballero, E., and Figueroa, C.R. (2016). Characterization of fruit development and potential health benefits of arrayan (*Luma apiculata*), a native berry of South America. Food Chem. *196*, 1239–1247. https://doi.org/10.1016/j.foodchem.2015.10.003.

Genskowsky, E., Puente, L.A., Pérez-Álvarez, J.A., Fernández-López, J., Muñoz, L.A., and Viuda-Martos, M. (2016). Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristotelia chilensis* (Molina) Stuntz] a Chilean blackberry. J. Sci. Food Agric. *96*, 4235–4242. https://doi.org/10.1002/jsfa.7628.

Guedes, M.N.S., Abreu, C.M.P., Maro, L.A.C., Pio, R., Abreu, J.R., and Oliveira, J.O. (2013). Chemical characterization and mineral levels in the fruits of blackberry cultivars grown in a tropical climate at an elevation. Acta Sci.-Agron. *35*, 191–196. https://doi.org/10.4025/actasciagron.v35i2.16630.

Hechenleitner, P., Gardner, M.F., Thomas, P.I., Echeverría, C., Escobar, B., Brownless, P., and Martínez, C. (2005). Plantas Amenazadas del Centro-Sur de Chile. Distribución, Conservación y Propagación (Valdivia, Chile : Universidad Austral de Chile y Real Jardín Botánico de Edimburgo).

Kanellis, A.K., and Manganaris, G.A. (2014). Antioxidants and bioactive compounds in fruits. In Fruit Ripening: Physiology, Signalling and Genomics, P. Nath, M. Bouzayen, A.K. Mattoo, and J.-C. Pech, eds. (Oxfordshire, UK: CAB International). https://doi.org/10.1079/9781845939625.0099.

Lichtenthaler, H.K., and Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvent. Biochem. Soc. T. *11*, 591–593. https://doi.org/10.1042/bst0110591.

Mariangel, E., Reyes-Diaz, M., Lobos, W., Bensch, E., Schalchli, H., and Ibarra, P. (2013). The antioxidant properties of calafate (*Berberis microphylla*) fruits from four different locations in southern Chile. Cienc. Investig. Agrar. *40*, 161–170. https://doi.org/10.4067/S0718-16202013000100014.

Mayaud, L., Carricajo, A., Zhiri, A., and Aubert, G. (2008). Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. Lett. Appl. Microbiol. *47*, 167–173. https://doi.org/10.1111/j.1472-765X.2008.02406.x.

Mota, A.S., Martins, M.R., Arantes, S., Lopes, V.R., Bettencourt, E., Pombal, S., Gomes, A.C., and Silva, L.A. (2015). Antimicrobial activity and chemical composition of the essential oils of Portuguese *Foeniculum vulgare* fruits. Nat. Prod. Commun. *10*, 673–676.

Mulyaningsih, S., Sporer, F., Reichling, J., and Wink, M. (2011). Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. Pharm. Biol. *49*, 893–899. https://doi.org/10.3109/13880209.2011.553625.

Myers, N., Mittermeier, R., Mittermeier, C., Da Fonseca, G., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature *483*, 853–858. https://doi.org/10.1038/35002501.

Nepstad, D.C., and Schwartzman, S. (1992). Non-timber products from tropical forests: Evaluation of a conservation and development strategy. Advances in Economic Botany, Vol. 9. (Bronx, New York: The New York Botanical Garden).

Osorio, S., and Fernie, A.R. (2014). Fruit ripening: primary metabolism. In Fruit Ripening: Physiology, Signalling and Genomics, P. Nath, M. Bouzayen, A.K. Mattoo, and J.-C. Pech, eds. (Oxfordshire, UK: CAB International). https://doi.org/10.1079/9781845939625.0015.

Pech, J.-C., Bouzayen, M., and Latché, A. (2014). Cellular, metabolic and molecular aspects of chromoplast differentiation in ripening fruit. In Fruit Ripening: Physiology, Signalling and Genomics, P. Nath, M. Bouzayen, A.K. Mattoo, and J.-C. Pech, eds. (Oxfordshire, UK: CAB International). https://doi.org/10.1079/9781845939625.0028.

Rodríguez, R., Mattei, O., and Quezada, M. (1983). Flora Arbórea de Chile (Concepción, Chile: Editorial Universidad de Concepción).

Ruiz, A., Hermosín-Gutiérrez, I., Mardones, C., Vergara, C., Herlitz, E., Vega, M., Dorau, C., Winterhalter, P., and von Baer, D. (2010). Polyphenols and antioxidant activity of calafate (*Berberis microphylla*) fruits and other native berries from Southern Chile. J. Agric. Food Chem. *58*, 6081–6089. https://doi.org/10.1021/jf100173x.

Ruiz-May, E., and Rose, J.K.C. (2013). Cell wall architecture and metabolism in ripening fruit and the complex relationship with softening. In The Molecular Biology and Biochemistry of Fruit Ripening, G.B. Seymour, M. Poole, J.J. Giovannoni, and G.A. Tucker, eds. (Oxford, UK: Wiley-Blackwell). https://doi.org/10.1002/9781118593714.ch7.

Selvendran, R.R., and O'Neill, M.A. (1987). Isolation and analysis of cell walls from plant material. In Methods of Biochemical Analysis, Vol. 32, D. Glick, ed. (Hoboken, NJ, USA: John Wiley & Sons, Inc.). https://doi.org/10.1002/9780470110539.ch2.

Sharifi-Rad, J., Hoseini-Alfatemi, S.M., Sharifi-Rad, M., Sharifi-Rad, M., Iriti, M., Sharifi-Rad, M., Sharifi-Rad, R., and Raeisi, S. (2015). Phytochemical compositions and biological activities of essential oil from *Xanthium strumarium* L. Molecules *20*, 7034–7047. https://doi.org/10.3390/molecules20047034.

Silva, M., Cruz, M.A., and Sammes, P.G. (1971). Some constituents of *Pitavia punctata*. Phytochemistry *10*, 3255–3258. https://doi.org/10.1016/S0031-9422(00)97383-7.

Singleton, V.L., and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. *16*, 144–158.

Suwalsky, M., Orellana, P., Avello, M., and Villena, F. (2007). Protective effect of *Ugni molinae* Turcz against oxidative damage of human erythrocytes. Food Chem. Toxicol. *45*, 130–135. https://doi.org/10.1016/j.fct.2006.08.010.

Tyagi, A., and Malik, A. (2011). Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapour phase against food spoilage microorganisms. Food Chem. *126*, 228–235. https://doi.org/10.1016/j.foodchem.2010.11.002.

Waldron, K.W., Parker, M.L., and Smith, A.C. (2003). Plant cell walls and food quality. Compr. Rev. Food Sci. F. *2*, 101–119. https://doi.org/10.1111/j.1541-4337.2003.tb00019.x.

Wang, S.Y., Chen, C.-T., and Wang, C.Y. (2009). The influence of light and maturity on fruit quality and flavonoid content of red raspberries. Food Chem. *112*, 676–684. https://doi.org/10.1016/j.foodchem.2008.06.032.

Willats, W.G.T., Knox, J.P., and Mikkelsen, J.D. (2006). Pectin: new insights into an old polymer are starting to gel. Trends Food Sci. Tech. *17*, 97–104. https://doi.org/10.1016/j.tifs.2005.10.008.

Yam, K.L., and Papadakis, S.E. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. J. Food Eng. *61*, 137–142. https://doi.org/10.1016/S0260-8774(03)00195-X.

Yemm, E.W., and Willis, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. *57*, 508–514. https://doi.org/10.1042/bj0570508.

Yu, J., Wang, L., Walzem, R.L., Miller, E.G., Pike, L.M., and Patil, B.S. (2005). Antioxidant activity of citrus limonoids, flavonoids, and coumarins. J. Agric. Food Chem. *53*, 2009–2014. https://doi.org/10.1021/jf0484632.

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