

# Phytochemical characterization and antioxidant properties of *Prumnopitys andina* fruits in different ripening stages in southern Chile

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## ABSTRACT

The native conifer lleuque (*Prumnopitys andina* (Poepp. ex Endl.) de Laub.) grows in southern Chile, and has an edible and fleshy ovoid fruit. Some species of the genus *Prumnopitys* are valuable for the medicinal value of their edible fruits. Thus, the aim of this research was to characterize the phytochemical and antioxidant compounds in four ripening stages of *P. andina* fruits from La Araucanía Region, Chile. Fruit quality related parameters, bromatological, antioxidant and phenolic compounds analyses were performed in order to highlight their potential for human consumption. Our study showed that fresh weight, equatorial diameter, and soluble solid content significantly increased ( $P < 0.05$ ) in *P. andina* fruits during ripening, reaching  $4.02 \pm 0.2$  g,  $17.9 \pm 0.6$  mm, and  $23.7 \pm 0.5$  °Brix, respectively, per fruit at stage IV. Our bromatological analyses showed that *P. andina* fruits had  $1.17 \pm 0.1$  g 100 g<sup>-1</sup> DW protein,  $1.55 \pm 0.2$  g 100 g<sup>-1</sup> DW crude fiber, and  $10.76 \pm 2.2$  g 100 g<sup>-1</sup> DW fruit of ash at fruit ripe. Likewise, we found  $2.6 \pm 0.2$  mg g<sup>-1</sup> FW total phenols,  $2.2 \pm 0.2$  mg trolox equivalent g<sup>-1</sup> FW of antioxidant activity, and  $6.4 \pm 0.2$  mg rutin equivalent g<sup>-1</sup> FW total flavonoid in *P. andina* fruits. Interestingly, ripening stages I and II showed higher antioxidant compound levels compared to stages III and IV, with the exception of total anthocyanins, which did not change throughout the ripening process. This study shows that *P. andina* has great potential as a fruit with significant functional properties, which could help promote the propagation, care, and use of this native conifer.

**Key words:** Conifer, flavonoids, native species, soluble solids.

## INTRODUCTION

*Prumnopitys andina* (Poepp. ex Endl.) de Laub, also known as “lleuque” in the Mapudungun native language of the “Mapuche” indigenous population, is an endemic conifer tree of Chile that belongs to the Pinales order and the family Podocarpaceae (Gardner, 2013). The *Prumnopitys* genus harbors at least 11 species, which are valuable from a commercial point of view for firewood and for their bark which has great medicinal value (Agrawal, 2018). *Prumnopitys andina* is distributed in the Andes Mountains from the Maule Region (Linares Province lat 35°52”S) to La Araucanía Region (Cautín Province lat 39°30”S), whereas in the coastal “Cordillera de la Costa” only one population is known, on the eastern slopes of the “Cordillera de Nahuelbuta”, close to Angol (La Araucanía Region; Clugston et al., 2017). According to Vargas-Gaete et al. (2020), 49.6% of *P. andina* trees are found in La Araucanía Region and 48.2% in the Biobío Region, covering around 9300 ha. Generally, *P. andina* grows on soils of volcanic, scoria, and lava origin in mountainous areas, and on shallow clay soils, tolerating different types of climate and low temperatures, and is frequently associated with *Nothofagus obliqua*, *N. dombeyi*, *Austrocedrus chilensis*, *Lomatia hirsuta*, and *Maytenus boaria* (Vargas-Gaete et

al., 2020). The International Union for Conservation of Nature and Natural Resources (IUCN) declared that *P. andina* is in a vulnerable state, proposing their conservation; indeed, they are protected in the Tolhuaca and Conguillío National Parks, and in the Ñuble National Reserve (IUCN, 2013).

The fruits of *P. andina* are edible and contain a single seed with a hard and flattened testa. The fruits are ovoid, fleshy, and turn from a green to yellowish color when ripe; for this reason, they are commonly called mountain grapes. They ripen between January and April in the Maule and La Araucanía Regions (Figueroa and López, 2006). *Prumnopitys andina* fruits are eaten unprocessed or are cooked for the preparation of jam, as well as to elaborate fermented juice (“chicha”), and for feeding animals (Figueroa and López, 2006). This species is relatively easy to propagate vegetatively, whereas germination from seed is more variable, with germination times ranging from 20 d to 3 yr (Hechenleitner et al., 2005). In the context of the rising awareness concerning the origin, cultivation and health properties of fruits, consumers are increasingly interested in knowing the nutritional characteristics of a food, leading to higher demand for local products, grown with environmentally-friendly agricultural techniques (Mallor et al., 2018; Bhutia et al., 2021). Thus, Niedbała et al. (2020) argue that the culinary creations of rural areas are commercially attractive, and can constitute a viable alternative for improving the social and economic development of a community. Currently, there are very few studies - and thus a lack of knowledge and awareness - about the phytochemical and antioxidant characteristics of the different ripening stages of the fruits of *P. andina* species from the southern hemisphere. Indeed, Rosales (2004) determined that lleuque fruits are high in total protein and soluble sugars, but only evaluated fully-ripe fruits in the study. Thus, the aim of this research was to characterize the phytochemical and antioxidant compounds in four ripening stages of *P. andina* fruits from La Araucanía Region, Chile, in order to highlight their potential for human consumption.

## MATERIALS AND METHODS

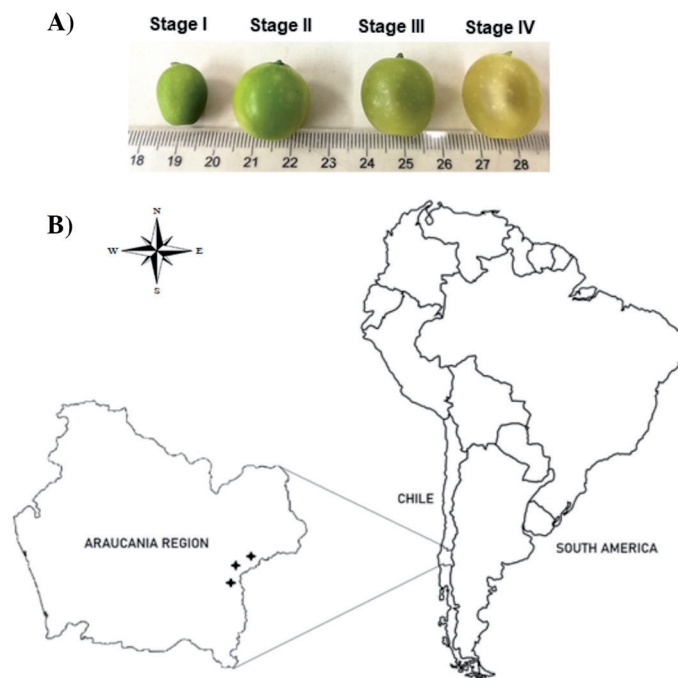
*Prumnopitys andina* (Poepp. ex Endl.) de Laub. fruits were collected in April 2019 in the Melipeuco commune (38°45'48" S, 71°35'05" W), La Araucanía Region, Chile. Fruits were collected on the same day and were evaluated in four ripening stages; initial (stage I), green (stage II), transition (stage III) and mature (stage IV), according to the classification of Hechenleitner et al. (2005), Donoso (2013) and Sáez (2016) (Figures 1A, 1B). Fruits collected from trees were immediately stored in a portable refrigerator and transferred to the Plant Physiology and Biotechnology Laboratory, Universidad Católica de Temuco. Fresh fruits were used for quality related parameter determinations, meanwhile, fruits for bromatological and antioxidant related parameter determinations were maintained at -80 °C until further analysis. Fruit fresh weight was determined using an analytical balance, where first the whole fruit was weighed and then only the seed. Equatorial and polar diameters were determined using a digital meter. Soluble solids were determined according to the methodology of Ribera et al. (2010). Five fruits were randomly selected from each ripening stage, individually ground, and then a drop was taken and placed on the refractometer plate to directly read the Brix degrees (°Brix) on a graduated scale.

### Bromatological determinations

Dry matter was determined as described by AOAC (2000). Bags containing fresh fruits at each stage of maturation were weighed, then lyophilized for 12 d at -70 °C and kept in the oven at 105 °C for 5 d, obtaining a dried fruit which was re-weighed. Subsequently, with the help of a scalpel and tweezers, the pulp was separated from the seed, ground in a mortar, weighed in Gooch crucibles, then placed in the oven for 24 h at 105 °C. Finally, the crucibles were removed, allowed to cool for 20 min in the desiccator, and then re-weighed. Once the percentage of DM and the humidity factor had been obtained, the ground and dry pulp was kept in the desiccator for the subsequent determination of bromatological parameters.

The ethereal extract was determined using 3-micron fat filter bags (XT4, ANKOM Technology, Macedon, New York, USA). Approximately 1 g ground pulp sample was introduced into the bags, which were then sealed and labeled. Using an extractor (ANKOM<sup>XT10</sup>, ANKOM Technology, Macedon, New York, USA) and ether as solvent, samples were extracted for 2 h, and then dried in the oven at 105 °C for 24 h. Finally, samples were weighed, and the percentage of ethereal extract determined as described previously (AOAC, 2000).

**Figure 1. Fruits of *Prumnopitys andina* in different stages of ripening, initial (stage I), green (stage II), transition (stage III) and ripe (stage IV) (A), and map of natural populations of *P. andina*, indicating the location of sample collection in La Araucanía Region in southern Chile (B).**



For these analyses, samples subjected to ethereal extraction were used. Samples (0.5 g) were weighed, introduced into F57 ANKOM fiber filter bags (porosity of 25 microns), and placed in the ANKOM 200 fiber analyzer where acid hydrolysis was performed, applying sulfuric acid for 45 min at 95 °C. Then the samples were washed with distilled water and basic hydrolysis was performed by applying sodium hydroxide for 45 min at 95 °C. Once this process had finished, the samples were washed with acetone and left in the oven for 24 h at 105 °C, according to AOAC (2000). The samples were then removed from the oven, weighed, and placed in crucibles. After being kept for 3 h at 550 °C in a muffle, samples were weighed for calculating the crude fiber content.

Total ash was determined by placing crucibles with DM samples in the muffle at 550 °C for 4 h. After cooling in the desiccator, samples were weighed and the amount of ash determined according to AOAC (2000).

Determination of total proteins was carried out by Weende proximal analysis with the Kjeldahl method (AOAC, 2000). A digester was preheated to 400 °C (Labconco Corporation, Kansas City, Missouri, USA). Dry pulp samples (0.5 g) were weighed on tissue paper at 60 °C. The samples were put in digestion tubes with 2 g catalyst mixture and 10 mL sulfuric acid, then placed in the digester for 45 min, cooled and 50 mL distilled water was added. Furthermore, a flask containing 100 mL 2% boric acid plus indicator drops was placed in the nitrogen release tube. In the digestion tubes, 60 mL 40% NaOH was added, and then 100 mL distillate was collected, which was then titrated with standardized sulfuric acid. Finally, the protein content of samples was calculated.

### **Antioxidant related parameters**

Fruits (0.15 g) were macerated in a cold mortar with 1 mL ethanol (80% v/v), they were centrifuged at 13000 rpm at 4 °C for 5 min to finally extract the supernatant for antioxidant activity and total phenol determinations. The antioxidant activity of different ripening stages of fruit was determined using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described by Chinnici et al. (2004), with some modifications. The absorbance was measured at 515 nm in a spectrophotometer (BIOBASE model BK-UV1800PC UV-VIS, Jinan, Shandong, China) using Trolox as the standard (Sigma Aldrich, St. Louis, Missouri, USA). Antioxidant activity values were expressed as milligrams of Trolox equivalents (TE) per gram of fresh weight. Total phenols were determined using the Folin-Ciocalteu reagent according to the protocol described by Slinkard and Singleton (1977). Aliquots of 10 µL extract were mixed with 570 µL deionized water and

100  $\mu\text{L}$  Folin-Ciocalteu reagent. The tubes were shaken and incubated at room temperature for 5 min, then 300  $\mu\text{L}$   $\text{Na}_2\text{CO}_3$  (7% w/v) was added and incubated for 10 min at room temperature in the absence of light. The reaction mix was measured at 765 nm in a spectrophotometer (BK-UV1800PC UV-VIS, BIOBASE). Total phenols values were expressed as milligrams of caffeic acid per gram of fresh weight.

Flavonoids were determined using the aluminum chloride colorimetry method according to Meyers et al. (2003), with some modifications. Here, 150 mg fruits were homogenized with 1 mL acidified ethanol (pH 1), then centrifuged at 13000 rpm at 4 °C for 5 min to extract the supernatant. An aliquot of 150  $\mu\text{L}$  extract was diluted with 600  $\mu\text{L}$  deionized water, and the immediate addition of 100  $\mu\text{L}$   $\text{NaNO}_3$ , followed by incubation at room temperature for 10 min. Subsequently, 100  $\mu\text{L}$   $\text{AlCl}_3$  was added, stirred in a vortex and 100  $\mu\text{L}$  1 M  $\text{NaOH}$  was included. Finally, the absorbance at 510 nm was measured in the spectrophotometer. Total flavonoids were expressed as milligram of rutin per gram of fresh weight.

The extraction of anthocyanins in fruit was performed by the differential pH method proposed by Nyman and Kumpulainen (2001). Fruits (0.1 g) were macerated in a cold mortar with 1 mL acidified methanol (pH 1). The mix was incubated at 90 °C for 60 min, and subsequently placed in a shaker for 15 h at -20 °C. The samples were centrifuged at 13000 rpm for 10 min at 4 °C and the supernatant was removed. The absorbance at 530 and 657 nm was determined in the spectrophotometer. Anthocyanins were expressed as milligrams of cyanidin-3-*O*-glucoside (C3G) per gram of fresh weight.

### Statistical analysis

The experiment was carried out with five replicates for each treatment (fruit ripening stage). The data were subjected to normality and homoscedasticity tests. Then, a one-way ANOVA was performed using the IBM SPSS Statistics 25 program (IBM, Armonk, New York, USA). A Tukey's test was carried out for multiple comparisons ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

This is the first report that characterizes phytochemicals and phenolic compounds in different ripening stages and their quality as an edible fruit. In this study, fresh weight, pulp weight, equatorial diameter, polar diameter, and soluble solid content significantly increased ( $P < 0.05$ ) in *P. andina* fruits during ripening. However, the weight of the seed decreased marginally at stage IV (Table 1). The rising fresh weight of fruit and pulp reflected in the increases in polar and equatorial diameter from ripening stages I to IV, is probably associated with extensive cell division and growth, by which the fruit also undergoes subsequent sugar accumulation, softening, and changes in coloring during the ripening process. These results agree with those found by Hechenleitner et al. (2005), Donoso (2013) and Sáez (2016) who characterized *P. andina* fruits, indicating that in their initial stage they are small fruits with a green color, turning to a yellow-green color. Subsequently, fruits become sweeter, more yellow and even more mucilaginous, reaching a diameter of up to 19 mm and a weight of 3.4 g (Table 1). The seed weight increased significantly from stage I to stage III, decreasing at the end of the ripening process (stage IV). The soluble solids content also rose significantly during ripening, especially between stages III and IV (Table 1). This might be due to the hydrolysis of several structural polysaccharides found in fruits such as cell wall pectins and monomeric components, generating an accumulation of sugars such as glucose, fructose, and sucrose, which are the main constituents of soluble solids (Martínez-González et al., 2017). In our case, the soluble solids content of *P. andina* fluctuated from 14.7 to 23.7 °Brix, depending on the ripening stage (Table 1). Similar results were described by Rosales (2004) who reported about 20.8 °Brix in *P. andina* fruits. Other studies in berry fruits showed values of soluble solids around 21.5 °Brix in fruits of *Ugni molinae* (FIA, 2016), whereas in *Vaccinium corymbosum* only 15 °Brix is attained in fruits collected in La Araucanía Region (Ribera et al., 2010). On the other hand, the phytochemical analyses

**Table 1. Fruit quality and parameters in four stages of ripening of *Prumnopitys andina*.**

Ripening stage	Fruit fresh weight	Seed fresh weight	Pulp fresh weight	Soluble solids	Polar diameter	Equatorial diameter
	g			°Brix	mm	
Stage I	1.35 ± 0.54d	0.26 ± 0.18b	1.09 ± 0.05d	14.7 ± 0.44c	16.2 ± 0.23b	12.0 ± 0.49b
Stage II	3.37 ± 0.11bc	0.49 ± 0.06a	2.89 ± 0.09bc	15.8 ± 0.70bc	18.8 ± 0.64a	17.6 ± 0.11a
Stage III	3.90 ± 0.84ab	0.49 ± 0.06a	3.40 ± 0.07ab	21.1 ± 0.61ab	19.1 ± 0.19a	17.5 ± 0.40a
Stage IV	4.02 ± 0.29a	0.45 ± 0.08a	3.57 ± 0.26a	23.7 ± 0.59a	20.5 ± 0.62a	17.9 ± 0.60a

All values represent averages of five biological replicates ± SE and different letters indicate significant differences according to Tukey's test ( $P < 0.05$ ).

showed that the *P. andina* fruit significantly reduced ( $P < 0.05$ ) the contents of ethereal extract, protein and crude fiber as maturity proceeded, particularly after stage II of the ripening process. Meanwhile, total ash increased significantly in stages II and III (Table 2). In this sense, Martínez-González et al. (2017) reported that during fruit ripening, various physiological changes related to senescence occur that damage the cell membranes, stop carbohydrate synthesis, and degrade proteins, chlorophylls, nucleic acids and lipids. On the other hand, the decrease in protein content of fruit occurs in parallel to the ripening process, whereby proteins required for maturation in the early stages are subsequently degraded into amino acids, which are the precursors of volatile compounds in fruits (Evangelista-Lozano et al., 2019). In our case, total protein content of *P. andina* fell from 2.79 in stage I to 1.17 g 100 g<sup>-1</sup> DW in stage IV (Table 2). In this sense, Rosales (2004) reported similar protein values in fruits of the same species. Nonetheless, the protein content of this fruit is higher than that of other Chilean species such as *U. molinae* 0.3 g 100 g<sup>-1</sup> DW, and *Aristotelia chilensis* (0.8 g 100 g<sup>-1</sup> DW), but is lower than that of *Gevuina avellana* (12.4 g 100 g<sup>-1</sup> DW) (Tacón, 2004).

Crude fiber content decreased significantly with the advance of the maturation process and is higher in stages III and IV (Table 2). This reduction may be due to structural changes generated by enzymatic activity in the fruit, degrading pectic fractions, hemicellulose, and cellulose, which may contribute towards the decrease in firmness in the fruits when ripe (Ibarra et al., 2015). The results obtained in stage IV are similar to those reported by Rosales (2004).

Crude fiber analysis determines the cellulose and lignin content in the cell walls of plants or fruits and is carried out since fiber represents the organic portion of food that is most difficult to digest. From the point of view of human nutrition, it should be noted that improved dietary fiber intake contributes to reducing idiopathic chronic constipation in adults (Liu et al., 2020). In this context, *P. andina* fruits have higher crude fiber ranges compared to *Aristotelia chilensis* with 0.8 g 100 g<sup>-1</sup> DW of fruit (Tacón, 2004). Moreover, in our study total ash content increases as the fruit matures, with a significant rise between stage I (6.81 g 100 g<sup>-1</sup> DW fruit) and stage IV (10.76 g 100 g<sup>-1</sup> DW fruit), and even higher ash levels at stage III (Table 2). This analysis determines the total content of minerals in food which are found as inorganic salts or organic acid salts (Liu, 2019). In this sense, it is necessary to take into account that *P. andina* fruit has high levels of total ash compared to several Chilean fruits such as *U. molinae*, *Gaultheria mucronata*, *Berberis microphylla*, *Aristotelia chilensis*, *Berberis darwinii* and *G. avellana* (Velásquez et al., 2017).

In this work, we determined the antioxidant activity in *P. andina* fruit, observing that antioxidant activity was maintained in the first three ripening stages (stage I, II and III), decreasing significantly ( $P < 0.05$ ) only at full ripening (stage IV) (Figure 2A). The highest antioxidant activity level was reached in stage I (immature fruit; 2.2 mg TE g<sup>-1</sup> FW), decreasing to values around 1.0 mg TE g<sup>-1</sup> FW at full ripening (stage IV). The antioxidant activity of *P. andina* is similar to that of some frequently consumed fruits, such as *Rubus fruticosus* (1.2-1.4 mg TE g<sup>-1</sup> FW), *Malus domestica* (1.5-2.4 mg TE g<sup>-1</sup> FW), and *Aristotelia deliciosa* (2.3 mg TE g<sup>-1</sup> FW), and is lower than in *Vitis vinifera* (3.8 mg TE g<sup>-1</sup> FW), *Pyrus* (4.7 mg TE g<sup>-1</sup> FW), *Prunus persica* (4.6 mg TE g<sup>-1</sup> FW) and *Citrus ×sinensis* (4.5 mg TE g<sup>-1</sup> FW) (Palomo et al., 2010; Armijos, 2018). Furthermore, in our study we observed that total phenol content was highest in immature and green fruits (stage I and II) and decreased significantly ( $P < 0.05$ ) during ripening (Figure 2B). Total phenols fluctuated around 2.5 mg caffeic acid g<sup>-1</sup> FW in stage I and II, whereas in the stage of full ripening, levels decreased to around 1.2 mg caffeic acid g<sup>-1</sup> FW. This could be due to the higher activity of enzymes such as polyphenol oxidases involved in the ripening process in fruits, that are known to cause the degradation of phenols in species such as *Prunus domestica*, *P. avium*, *V. vinifera*, *Rubus idaeus*, *V. corymbosum*, and *Fragaria ×ananassa* (López-Palestina et al., 2019). The total phenols content of *P. andina* fruits is similar to that of several species that are commonly consumed in Chile, such as

**Table 2. Bromatological composition of *Prumnopitys andina* fruits.**

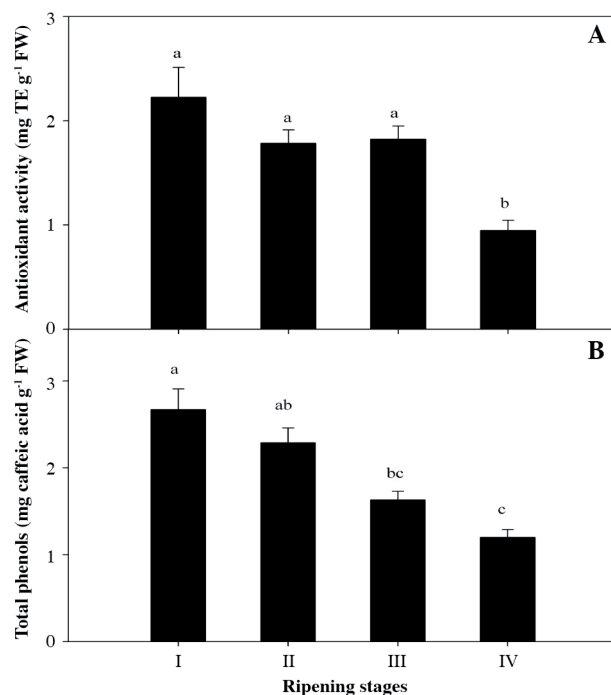
Ripening stage	Ethereal extract	Protein	Crude fiber	Ash
	g 100 g <sup>-1</sup> DW			
Stage I	2.39 ± 0.16a	2.79 ± 0.26a	4.17 ± 0.43a	6.81 ± 0.31cd
Stage II	2.59 ± 0.19a	2.64 ± 0.28a	2.40 ± 0.35b	3.70 ± 0.42d
Stage III	1.48 ± 0.12b	2.33 ± 0.14a	1.51 ± 0.21c	16.33 ± 0.09a
Stage IV	1.42 ± 0.22b	1.17 ± 0.17b	1.55 ± 0.21c	10.76 ± 2.28bc

All values represent averages of five biological replicates ± SE and different letters indicate significant differences according to Tukey's test ( $P < 0.05$ ).

*Smilax aspera* (1.8 mg g<sup>-1</sup> FW), *F. xananassa* with (1.9 mg g<sup>-1</sup> FW), *R. idaeus* (2.4 mg g<sup>-1</sup> FW), and *M. domestica* (1.5-2.9 mg g<sup>-1</sup> FW) (Palomo et al., 2010; FIA, 2016). Furthermore, in our study total flavonoids decreased significantly ( $P < 0.05$ ) from the immature stage to the transition stage (stage I to III), before increasing slightly at full ripening (stage IV) of *P. andina* (Figure 3A). The highest concentration (about 6.5 mg rutin g<sup>-1</sup> FW) was found in the immature fruit (stage I), falling to about 1.0 mg rutin g<sup>-1</sup> FW at the transition state (stage III) (Figure 3A). Similar contents have been reported in *F. xananassa* (5 mg g<sup>-1</sup> DW), whereas in *V. corymbosum* and *Vitis idaea*, higher contents of flavonoids were recorded (up to 11 mg g<sup>-1</sup> DW; Liu et al., 2020). On the other hand, species like *R. idaeus* has low flavonoid concentrations in fruit (0.36 to 0.93 mg g<sup>-1</sup> DW) compared to *P. andina* fruit (Armijos, 2018; Ochoa et al., 2019). Interestingly, Jiménez-Aspee et al. (2019) reported that rutin, caffeic acid  $\beta$ -glucoside were the major components in *P. andina* fruits, which are recognized phenolic compounds with antioxidant activity.

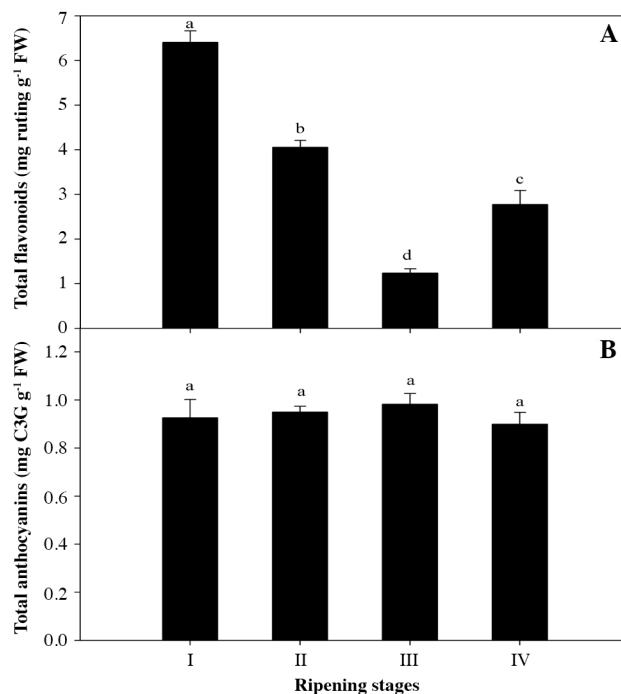
In addition, the anthocyanin concentration did not differ significantly ( $P > 0.05$ ) between the different ripening stages of *P. andina* fruits (Figure 3B), fluctuating around 1.0 mg C3G g<sup>-1</sup> FW at all four stages (Figure 3B). Dalgo et al. (2014) indicated that in the initial stages of maturation, fruits are green with higher contents of chlorophylls, which are subsequently degraded. In our case, as the fruit of *P. andina* ripens, its coloration varies from green to yellowish tones (Donoso, 2013). This would suggest that during lleuque ripening, the chlorophyll concentration is more uniform than in other fruits that change to red or blue colors, and in which anthocyanin production is triggered. In addition, it has been shown that the enzyme phenylalanine ammonium lyase, responsible for the synthesis of secondary metabolites, decreases in green fruits (Ochoa et al., 2019), which could be associated with the similar concentrations of anthocyanins found during the four ripening stages in *P. andina* fruits. In Chile, fruits with low anthocyanin concentration are often consumed, such as *Smilax ornata*, which contains 0.5 mg 100 g<sup>-1</sup> FW, and *B. microphylla* with 0.24 mg 100 g<sup>-1</sup> FW. Nonetheless, *P. andina* showed higher levels of anthocyanin (around 1.0 mg g<sup>-1</sup> FW) in their fruits, values that are lower than other more-commonly ingested fruits such as *U. molinae* (6.4 mg g<sup>-1</sup> FW), *V. corymbosum* (2.8 mg g<sup>-1</sup> FW), *R. idaeus* (7.1 mg g<sup>-1</sup> FW) and *A. chilensis* (21.7 mg g<sup>-1</sup> FW) (Ribera et al., 2010; FIA, 2016). The anthocyanin concentration did not differ significantly ( $P > 0.05$ ) between the different ripening stages of *P. andina* fruits (Figure 3B), fluctuating around 1.0 mg C3G g<sup>-1</sup> FW at all four stages (Figure 3B).

**Figure 2. Antioxidant activity (A) and total phenol (B) of *Prumnopitys andina* fruits at different ripening stages, initial (stage I), green (stage II), transition (stage III) and mature (stage IV).**



All values represent averages of five biological replicates  $\pm$  SE. Different letters indicate significant differences in different ripening stages, according to the Tukey's test ( $P < 0.05$ ). TE: Trolox equivalent.

**Figure 3. Total flavonoid (A) and anthocyanin concentrations (B) in *Prumnopitys andina* fruits at different stages of ripening, initial (stage I), green (stage II), transition (stage III) and ripe (stage IV).**



All values represent averages of five biological replicates  $\pm$  SE. Different letters indicate significant differences in different ripening stages, according to the Tukey's test ( $P < 0.05$ ).  
C3G: Cyanidin 3-*O*-glucoside.

## CONCLUSIONS

In conclusion, the findings of our study on the phytochemical and antioxidant parameters of lleuque fruits, show that they undergo quantitative changes in nutritional and functional compounds during ripening, which allows us to suggest different strategies for their use according to their specific ripening stage. From these results, further studies could be proposed, such as determining the influence of the climate on the fruit in different areas, since this fruit could be used to develop new functional and nutraceutical food products, encouraging the propagation, care, and sustainable use of this native conifer.

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