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Comportamiento de maqui silvestre (*Aristotelia chilensis* (Mol.) Stuntz; Elaeocarpaceae): manejo de canopia y compuestos bioactivos en frutos

Behavior wild maqui (*Aristotelia chilensis* (Mol.) Stuntz; Elaeocarpaceae): canopy management and bioactive compounds in fruits

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RESUMEN

Maqui (*Aristotelia chilensis* (Mol.) Stuntz; Elaeocarpaceae), especie endémica de Chile y Argentina. Se presenta en bosques denominados “macales” que corresponden a agrupaciones densas monoespecíficas. Sus frutos son considerados un producto forestal no maderero (PFNM) que ha experimentado un aumento sostenido en el consumo de éstos por parte de la sociedad. La especie presenta una reconocida plasticidad morfológica y fisiológica, que le permite adaptarse a diferentes condiciones edafoclimáticas. El maqui destaca por su alto contenido de polifenoles, antocianinas (delfinidinas y cianidinas), flavonoides, taninos y alcaloides, con potencial uso nutrifuncional, medicinal y aromático. Las concentraciones de compuestos bioactivos pueden variar entre plantas de la misma especie ubicadas en diferentes zonas geográficas, debido a que éstas los sintetizan para protegerse del estrés oxidativo producido a consecuencia de los factores ambientales, que, bajo ciertas condiciones, las afectan. Los macales silvestres son objeto de una fuerte presión antrópica siendo recolectados intensivamente, representando una amenaza a su producción. Usualmente, la cosecha de maqui se realiza mediante cortes o quiebres de ramas y vástagos, provocando que los árboles o arbustos no produzcan frutos en tres a cuatro temporadas. Para evitar que esto suceda se recomienda técnicas de manejo eficientes y eficaces, que garanticen su producción continua y, a su vez, cumplir con la ley de bosque nativo en vías de un desarrollo sustentable. El presente estudio plantea evaluar el comportamiento del maqui silvestre, su manejo y la influencia de las condiciones edafoclimáticas y estados de madurez sobre los compuestos bioactivos en frutos, en tres localidades del centro-sur de Chile. Para ello consta de los siguientes objetivos: Identificar los principales aspectos relacionados con maqui en condiciones silvestres para un manejo sustentable. En segundo lugar, analizar el contenido de compuestos bioactivos y capacidad antioxidante en frutos de maqui silvestre en diferentes estados de madurez y condiciones edafoclimáticas. En tercer lugar, determinar la variación en rendimiento de frutos en plantas de maqui silvestre sometidas a diferentes intensidades de poda

y/o raleo. En el capítulo I, se mencionan y abordan de manera general aspectos relacionados con maqui, manejo sustentable, manejo de canopia y fructificación y, finalmente compuestos bioactivos. El capítulo II, es una revisión con el objetivo de revisar y resumir los estudios publicados sobre las características generales, la distribución, la adaptación y el manejo de la producción del maqui, con énfasis en el manejo de la canopia para la producción sostenible de los frutos del maqui. En la revisión se resumen las investigaciones actualizadas sobre el manejo productivo del maqui, con énfasis en la sustentabilidad del recurso. La comunidad científica se ha centrado en el estudio de las características y propiedades químicas del maqui y su uso medicinal. Sin embargo, hay pocos estudios sobre la producción de maqui, la cosecha y la sustentabilidad de las poblaciones silvestres de maqui. Se han reportado efectos positivos de la poda en los árboles de maqui, ya sea de formación o de producción. Sin embargo, el momento y la intensidad de la poda no han sido definidos ni implementados en plantas silvestres. Un plan adecuado de manejo de canopia permitiría una óptima intercepción y distribución de la luz para aumentar la productividad y facilitar la cosecha en las poblaciones silvestres. El fruto de maqui se ha calificado como una "superfruta" debido a su composición fitoquímica y actividad antioxidante. El contenido de compuestos bioactivos en el maqui silvestre, específicamente la delfinidina, está influenciado por el tiempo de recolección, los rasgos genéticos y las condiciones ambientales (localidad). Considerando estos antecedentes en el capítulo III, se realizó un estudio de acumulación de derivados de delfinidina y otros compuestos bioactivos en maqui silvestre bajo diferentes condiciones ambientales y etapas de madurez del fruto. El objetivo del estudio fue evaluar el contenido de polifenoles totales, los niveles de antocianinas y flavonoles, y la capacidad antioxidante del maqui en tres estados de madurez del fruto (inmaduro, pre-maduro y maduro) en tres localidades diferentes de Chile Central (Coihueco, Cayumanque (Quillón) y Tregualemu (Pelluhue)) durante dos temporadas consecutivas (2019-2020 y 2020-2021). Hubo una interacción ($p \leq 0,05$) entre la localidad y el estado de madurez del fruto en todos los compuestos bioactivos evaluados. El contenido total de polifenoles varió en los diferentes estados de madurez, oscilando entre 2,290 (estado maduro) y 1,025 mg de equivalentes de ácido gálico (EAG) 100 g^{-1} de peso fresco (estado inmaduro). Se encontró una acumulación de derivados de delfinidina en la etapa de maduro, estos compuestos representaron una proporción significativa de alrededor del 80% de las antocianinas totales en ambas temporadas. La capacidad antioxidante varió en función de la temporada, la localidad y el estado de madurez del fruto. Los valores más altos se registraron en Cayumanque, con un valor medio de 8,926 µmol Trolox equivalente 100 g^{-1} de peso fresco, para ambas temporadas. Con el fin de suministrar materia prima de calidad similar, lo cual es una cuestión clave para las industrias alimentaria y farmacéutica, y permitir una producción sustentable, es necesario regular las variaciones de temporadas y ambientales de los compuestos bioactivos, así como las características químicas de los frutos silvestres de acuerdo con las condiciones edafoclimáticas.

ABSTRACT

Maqui (*Aristotelia chilensis* (Mol.) Stuntz; Elaeocarpaceae), a specie endemic to Chile and Argentina. It occurs in forests named "macales" that correspond to dense monospecific groupings. Its fruits are considered a non-timber forest product (NTFP) that has experienced a sustained increase in the consumption by society. The specie has a recognized morphological and physiological plasticity that allows it to adapt to different soil and climatic conditions. Maqui stands out for its high content of polyphenols, anthocyanins (delphinidins and cyanidins), flavonoids, tannins and alkaloids, with potential nutritional, medicinal and aromatic use. The concentrations of bioactive compounds may vary among plants of the same species located in different geographical areas, because they synthesize them to protect themselves from oxidative stress caused by environmental factors that, under certain conditions, affect them. The wild maqui trees are subject to anthropic pressure and are harvested intensively, representing a threat to their production. Maqui is usually harvested by cutting or breaking branches and stems, causing losses in production fruits in three to four seasons. To prevent this, efficient and effective management techniques are recommended to guarantee their continued production and, at the same time, fulfill with the native forest law for sustainable development. The present study aims to evaluate the behavior of wild maqui, its management and the influence of soil and climatic conditions and maturity stages on the bioactive compounds in fruits, in three locations at the Central-Southern in Chile. To identify the main aspects related to maqui in wild conditions under sustainable management. Second, to analyze the content of bioactive compounds and antioxidant capacity in wild maqui fruit at different stages of maturity and soil and climatic conditions. Third, to determine the variation in fruit yield in wild maqui plants subjected to different pruning and/or thinning intensities. In Chapter I, aspects of maqui related, sustainable management, canopy and fruiting management and, finally, bioactive compounds are addressed in a general manner. Chapter II, is a review made with the objective of reviewing and summarizing published studies related with general characteristics, distribution,

adaptation and management of maqui production, with emphasis on the management of the canopy for the sustainable production of maqui fruits. The review summarizes updated research on maqui production management, with emphasis on the sustainability of the resource. Most studies have focused on the characteristics and chemical properties of maqui and its medicinal use; however, there are few studies on maqui production, harvesting and sustainability protection of wild maqui populations. Positive effects of pruning on maqui trees, either by formation or production, have been reported. However, the timing and intensity of pruning have not been defined or implemented on wild plants. A proper canopy management plan may allow optimal light interception and light distribution to increase productivity and facilitate harvesting in wild maqui. The maqui fruit has been described as a "superfruit" due to its phytochemical composition and antioxidant activity. The content of bioactive compounds in wild maqui, specifically delphinidin, is influenced by the time of collection, genetic traits and environmental conditions (locality). Considering this background in Chapter III, a study of the accumulation of delphinidin derivatives and other bioactive compounds in wild maqui under different environmental conditions and stages of fruit maturity was carried out. The objective of the study was to evaluate the content of total polyphenols, the levels of anthocyanins and flavonols, and the antioxidant capacity of maqui at three stages of fruit maturity (immature, pre-ripe and ripe) in three different localities of Central Chile (Coihueco, Cayumanque (Quillón) and Tregualemu (Pelluhue)) during two consecutive seasons (2019-2020 and 2020-2021). There was an interaction ($p \leq 0.05$) between locality and fruit maturity stage for all bioactive compounds evaluated. Total polyphenol content varied at the different maturity stages, ranging from 2,290 (mature stage) to 1,025 mg gallic acid equivalent (GAE) 100 g^{-1} of fresh weight (immature stage). An accumulation of delphinidin derivatives was found at the mature stage, these compounds represented a significant proportion of about 80% of total anthocyanins in both seasons. Antioxidant capacity varied as a function of season, locality, and fruit maturity stage. The highest values were recorded in Cayumanque, with a mean value of 8,926 µmol Trolox equivalent 100 g^{-1} of fresh weight for both seasons. In order to supply raw material of similar quality, which is a key issue for the food and pharmaceutical industries, and to allow sustainable production, it is necessary to regulate seasonal and environmental variations of bioactive compounds, as well as the chemical characteristics of wild fruits according to soil and climatic conditions.

I. CAPÍTULO 1. INTRODUCCIÓN GENERAL

La demanda de productos derivados de maqui presenta un aumento sostenido en los últimos años, asociado a los múltiples beneficios que los compuestos bioactivos presentes mayoritariamente en sus frutos generan en la salud humana (Benedetti y Pavez, 2012). La recolección de frutos en maqui silvestre es la principal fuente de obtención de la materia prima a utilizar, ya sea por la industria farmacéutica o alimentaria (Salinas y Parra, 2012; Silva et al., 2017). La actual forma de recolección mediante quiebre de ramas y vástagos que prevalece entre los recolectores amenaza el desarrollo y producción de la especie (Chandía y Urra, 2017; FIA, 2017). Es necesario elaborar planes o protocolos de manejo de canopia que fomenten la producción de frutos de manera sustentable, de modo de favorecer la protección y producción de la planta.

1.1. Maqui

El maqui (*Aristotelia chilensis* (Mol.) Stuntz), es una especie endémica de los bosques subantárticos de Chile y Argentina (Rodríguez et al., 2017), cuyos frutos se consideran como uno de los berries más saludables en la naturaleza (Genskowsky et al., 2016).

Es un árbol o arbusto persistente (Rodríguez et al. 2017) o invierno-verde (Damascos y Prado, 2001), e incluso parcialmente caducifolio (Donoso y Ramírez, 1994; Lusk y Del Pozo, 2002). En lugares abiertos su crecimiento es arbóreo, mientras que en comunidades adquiere una forma arbustiva (Salinas y Parra, 2012). Se presenta en agrupaciones densas y monoespecíficas denominadas “macales”, de los cuales se extraen sus frutos mediante recolección silvestre (Fredes y Montenegro, 2011; Alonso, 2012; Benedetti y Pavez, 2012; Romo et al., 2018). En general, es de las primeras especies que invaden terrenos quemados o abandonados, siendo un agente importante para la conservación de suelo y generar condiciones que propician el establecimiento de otras especies más exigentes como roble (*Nothofagus obliqua* (Mirb.) Oerst.), coigüe (*Nothofagus dombeyi* (Mirb.) Oerst.), avellano (*Gevuina avellana* Mol.) y peumo (*Cryptocarya alba* (Mol.) Looser) (Donoso, 2006; Alonso, 2012; Silva et al., 2017).

Existen variados estudios en frutos silvestres, en particular las bayas (berries) con propiedades antioxidantes debido al impacto benéfico que estos han demostrado en la salud humana (Araya et al., 2006; Fredes, 2009; Ruíz et al., 2010; Lutz, 2012; Genskowsky et al., 2016; Céspedes et al., 2017; López et al., 2018; Fuentes et al., 2019; Chang et al., 2019). Estos adquieren la categoría de “superfrutas”, debido a sus altos contenidos de fitoquímicos saludables, principalmente por su acción antioxidante. A esta categoría pertenecen: maqui, calafate, granada, frutilla, mortiño, mora andina, goji y açaí, entre otros (Fredes, 2009; Lutz, 2012; Genskowsky et al., 2016; López et al., 2018; Chang et al., 2019).

El creciente interés de la comunidad científica por estudiar las propiedades y características químicas del maqui, con un claro enfoque en el área medicinal (Céspedes et al., 2008; Farías, 2009; Fuentealba et al., 2012; Gironés-Vilaplana et al., 2014; Genskowsky et al., 2016; Romanucci et al., 2016; Alvim et al., 2018; Romo et al., 2018), contrasta con los escasos estudios enfocados en manejo productivo de la especie en estado silvestre, que permitan favorecer la protección y producción de manera sustentable (Tacón, 2017; Romo et al., 2018). De esta manera, si se desea fomentar su consumo, se debe procurar, en paralelo, una mayor oferta, por lo que se deben desarrollar investigaciones que permitan mejorar su manejo productivo sustentable. Este debería dar énfasis en las características de fructificación y cosecha, en conjunto con generar manejos en las plantas silvestres y, a su vez, diferenciar la producción en términos de compuestos bioactivos, en las condiciones edafoclimáticas de donde proviene la materia prima (Romo et al., 2018).

1.2. Manejo sustentable

El fruto de maqui es considerado como producto forestal no maderero (PFNM), el cual, en los últimos 20 años, ha experimentado un aumento sostenido en el consumo de éstos por parte de la sociedad (Benedetti y Pavez, 2012).

El incipiente desarrollo económico del proceso exportador contrasta con el modelo productivo, lo que ha generado brechas, que pone en riesgo la sostenibilidad de la producción de maqui. En este sentido, se han generado problemas por carencia de información sobre la cuantificación de la producción y el consumo, y sobre métodos y técnicas sostenibles de recolección, por ausencia de planes de manejo, por insuficiente información de mercado y procesos de comercialización, y por el bajo nivel de desarrollo tecnológico asociado a la generación de valor agregado (Valdebenito, 2013).

En Chile, la mayor demanda de productos naturales y principios activos ha llevado a una transición hacia el cultivo de plantas nativas, incluyendo al maqui, incorporando técnicas y tecnología de manejo (Vogel et al., 2014; Vogel et al., 2016; Chandía y Urra, 2017; Doll et al., 2017; Muñoz-Espinoza et al., 2019). En general, muchas especies han mostrado una respuesta de crecimiento y producción favorables cuando se han cultivado en un entorno artificial; sin embargo, algunas disminuyen la concentración de ingredientes funcionales y otras han disminuido su producción (Vogel et al., 2005). Si bien, se dispone de algunos clones y plantaciones de maqui, desde el punto de vista de los compuestos bioactivos los productos silvestres siguen siendo preferidos en relación a los cultivados desde el punto de vista de los compuestos bioactivos (Salinas y Parra, 2012; Silva et al., 2017).

Los macales silvestres son recolectados intensivamente, representando una amenaza a su desarrollo y producción. Usualmente, la cosecha de maqui se realiza mediante cortes o quiebres de ramas y vástagos, provocando que los árboles o arbustos no produzcan frutos en tres a cuatro temporadas. Para evitar que esto suceda se recomienda técnicas de manejo sustentable, que garanticen su producción continua (Chandía y Urra, 2017; FIA, 2017), y así cumplir con la normativa actual que rige a esta especie, “Ley de bosque nativo Nº 20.283, 2008” (Benedetti y Pavez, 2012).

Los factores de sustentabilidad que se deben considerar en la recolección del maqui son: propagación, recolección y hábitat asociado a la especie. En propagación se debe facilitar la regeneración y generar nuevas plantas, de modo que el producto a cosechar sea abundante, cercano y más accesible para los recolectores (Tacón, 2017; Silva et al., 2017). En recolección, proteger la planta y el ambiente que la rodea, buscando técnicas o métodos que sean tanto ergonómicos como eficientes, asegurando la producción en las temporadas futuras. Logrando una producción constante, recolección de fruta rentable, y seguridad tanto para los recolectores como para los consumidores (Chandía y Urra, 2017; Tacón, 2017). Respecto al hábitat, se debe manejar el entorno natural en el que se desarrolla la especie, para lograr un manejo sustentable para el maqui. A su vez, es importante que la recolección se realice de manera de no perjudicar a la planta ni al ecosistema que la alberga y que favorezca la diversidad de la flora acompañante, atrayendo a insectos polinizadores nativos y aves que controlen plagas (Tacón, 2017).

1.3. Manejo de canopia y fructificación

El maqui puede crecer bajo las copas de árboles, en claros o áreas fragmentadas con luz (Doll et al., 1999; Rau, 2010; Arellano, 2012), debido a la plasticidad morfológica y fisiológica que presenta. Esto le permite mantener niveles adecuados de fotosíntesis (Repetto-Giavelli et al., 2007) y un desarrollo óptimo en renovales y matorrales (FIA, 2017). Aunque el maqui tolera condiciones de semisombra, el crecimiento de la planta y la producción de frutos dependen en gran medida de la disponibilidad de luz (Frank y Finckh, 1997; Fontúrbel et al., 2017).

El maqui presenta una respuesta favorable a la poda, ya sea de formación o de producción. Sin embargo, aún no se ha definido bien la intensidad de dicho manejo en plantas silvestres. Es una especie de rápido crecimiento, no obstante, no responde bien a la poda de ramas principales, requiriendo al menos dos años para volver a producir. Las ramas secundarias y terciarias se renuevan cada año, debido a la presencia de yemas en receso que se activan con la luz, por lo cual es recomendable realizar las labores de manejo de canopia en éstas (Valdebenito et al., 2003; Chandía y Urra, 2017). Durante el receso invernal de los “macales”, se pueden eliminar ramas bajas y sombrías. En contraste, también es posible realizar la poda en la época de cosecha, en verano, y así extraer de manera más sencilla los frutos de las ramas removidas. Esto permite aumentar la superficie expuesta a la luz induciendo a una mayor producción de yemas florales y finalmente de frutos por planta (Silva et al., 2017).

Esta especie rebrota vigorosamente después de la poda, sin embargo, si ésta se repite año a año, puede debilitar la planta hasta provocar su muerte (Tacón, 2017; FIA, 2017). Las labores silvícolas de poda y/o raleo, deben favorecer el ingreso de luz, para incrementar la producción de flores y frutos, y consecuentemente aumentar el rendimiento (Bonometti, 2000).

El manejo de canopia permite mejorar la disponibilidad de luz, influyendo en las interacciones planta-ambiente, alterando y/o estimulando los compuestos bioactivos (Fuentealba et al., 2021).

1.4. Compuestos bioactivos

El beneficio del consumo de frutas en la salud se ha relacionado con la presencia de un amplio número de compuestos que pertenecen al grupo de los denominados fitoquímicos o sustancias bioactivas (Martínez-Navarrete et al., 2008). Se considera componente bioactivo de un alimento, a aquel que aporta un beneficio a la salud más allá de los considerados como nutrición básica, cumpliendo una función en la prevención de enfermedades cancerígenas, cardiacas, entre otras. Estos componentes se encuentran en general en pequeñas cantidades en productos de origen vegetal y en alimentos ricos en lípidos (Herrera et al., 2014). En el reino vegetal se pueden distinguir cuatro grandes grupos de compuestos bioactivos, siendo sustancias de diversas familias químicas, como: sustancias nitrogenadas, azufradas, terpénicas y, las más ampliamente estudiadas, las fenólicas (Tomás-Barberán, 2003). En las frutas se presentan mayoritariamente terpénicas y fenólicas.

Los compuestos fenólicos son abundantes en las frutas rojas, moradas, cítricos y manzana. Estas se pueden clasificar en flavonoides (antocianinas, flavonoles y flavonas, flavanonas, chalconas y dihidrochalconas, isoflavonas y flavanoles), fenilpropanoides, estilbenoides y derivados del ácido benzoico. De todas estas sustancias bioactivas, el grupo más abundante es el de los flavonoides (Martínez-Navarrete et al., 2008; Peñarrieta et al., 2014).

Los compuestos fenólicos constituyen una familia de metabolitos secundarios con distintas características químicas y propiedades biológicas. Por esto, se ha estudiado el impacto del consumo de alimentos y suplementos que los contienen (Avello y Suwalsky, 2006; Avello et al., 2009; Aguilera-Ortíz et al., 2011), y por sus propiedades antioxidantes. Entre estos, los compuestos fenólicos presentes en el maqui, como flavonoides, ácidos fenólicos y antocianinas son valorados por destacarse en estas propiedades (Manach et al., 2004; Schreckinger et al., 2010; Fredes et al., 2014; Benahetrina et al., 2018).

El maqui sobresale de las demás especies nativas e introducidas por su alto contenido de polifenoles, antocianinas (delfinidinas y cianidinas), flavonoides, taninos y alcaloides, con potencial uso nutrifuncional, medicinal y aromático (Guerrero et al., 2010; Ruíz et al., 2010; Alonso, 2012; Echeverría y Niemeyer, 2012; Araneda et al., 2016). Comparado con otras frutas y vegetales, tiene una alta capacidad antioxidante con concentraciones de antocianinas de 9,3 g cianidina-3-glucósido kg^{-1} de peso fresco y compuestos fenólicos de 14,6 g de equivalentes de ácido gálico (EAG) por kg^{-1} de peso fresco (Fredes et al., 2014).

Los compuestos fenólicos son compuestos antioxidantes inocuos de las plantas, cuyas concentraciones pueden variar entre plantas de la misma especie cultivadas en diferentes zonas geográficas. Las plantas los sintetizan para protegerse del estrés oxidativo producido a consecuencia de los factores ambientales que, bajo ciertas condiciones, las afectan negativamente (Farías, 2009; Lattanzio, 2013;). Estos compuestos no tienen un rol reconocido sobre procesos vitales para la planta, como la fotosíntesis, asimilación de agua y nutrientes y la respiración celular. Sin embargo, tienen un rol importante en la defensa y adaptación de las plantas a su ambiente en condiciones desfavorables, evitando una mayor disminución de la fotosíntesis (Croteu et al., 2000; Lattanzio, 2013; Nogués et al., 2014). Se encuentran presentes, tanto en hojas, como frutos y son altamente valorados por la industria a nivel mundial, ya que constituyen la materia prima para la fabricación de suplementos dietéticos, aditivos alimentarios, cosméticos, alimentos, tés, tinturas, hierbas medicinales, fragancias y saborizantes (Vogel y Berti; 2003; Escribano-Bailón et al., 2006; Damascos et al., 2008; Alonso, 2012; Salinas y Parra, 2012; Tacón, 2017; Zuñiga et al., 2017; López et al., 2018; Fernández et al., 2019; Agulló et al., 2020).

Considerando los antecedentes anteriormente expuestos se propone la presente investigación, con el objetivo de evaluar el comportamiento del maqui silvestre, su manejo y la influencia de las condiciones edafoclimáticas y estados de madurez sobre los compuestos bioactivos en los frutos, en tres localidades del Centro-Sur de Chile.

1.5. Hipótesis

- Los compuestos bioactivos en frutos de maqui silvestre varían dependiendo de las condiciones edafoclimáticas y el estado de madurez.

- El manejo de canopia mediante poda y raleo en maqui silvestre favorece la sustentabilidad y productividad de la especie.

1.6. Objetivos

1.6.1. Objetivo general

Evaluar el comportamiento del maqui (*Aristotelia chilensis* (Mol.) Stuntz) silvestre, su manejo y la influencia de las condiciones edafoclimáticas sobre los compuestos bioactivos en los frutos en tres localidades del Centro-Sur de Chile.

1.6.2. Objetivos específicos

- Identificar los principales aspectos relacionados con maqui en condiciones silvestres para un manejo sustentable.
- Analizar el contenido de compuestos bioactivos y capacidad antioxidante en frutos de maqui silvestre en diferentes estados de madurez y condiciones edafoclimáticas.
- Determinar la variación en rendimiento de frutos en plantas de maqui silvestre sometidas a diferentes intensidades de poda y/o raleo.

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II. CAPÍTULO 2: MAQUI (*ARISTOTELIA CHILENSIS* (MOL.) STUNTZ), TOWARDS SUSTAINABLE CANOPY MANAGEMENT: A REVIEW.

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2.1. Abstract

The demand for bioactive compounds for the pharmaceutical industry have increased dramatically in the last decade. Phenolic compounds, such as flavonoids, phenolic acids and anthocyanins, are commonly present in many plants. These compounds, which act as antioxidants, have attracted much attention due to their beneficial health properties. Maqui (*Aristotelia chilensis* (Mol.) Stuntz) fruits have the highest content of total polyphenols in comparison with other berries with medicinal properties. This review summarizes up-to-date production management research on maqui, with emphasis on resource sustainability. Scientific community has focused on the study of chemical characteristics and properties of maqui and their medicinal use.

However, there are few studies on maqui production, harvest, and protection of maqui wild populations. Yield increases have been reported when pruning maqui trees, either by formation or production pruning. However, timing and intensity of pruning has neither been defined nor implemented in wild plants. An adequate canopy management plan would allow for optimal light interception and distribution to increase productivity and facilitate harvest in wild stands.

Keywords: Berries; Medicinal plants; Native; Polyphenols*;* Sustainability; Conservation.

2.2. Introduction

Nowadays, it seems clear that consumers consider health a priority, thus choosing products or supplements that prevent diseases or promote well-being (Sorenson and Bogue, 2005; Genskowsky et al., 2016). Today's consumers are increasingly driven to new flavors and demonstrating an increasing demand for products that are natural and without additives. This has resulted in the incorporation of products that have always been present in nature, but were not widely known (Martínez-Valverde et al., 2000; Verdi, 2004; Genskowsky et al., 2016).

There are several studies on wild fruits, particularly in wild berries with antioxidant properties due to their beneficial impact on human health (Araya et al., 2006; Fredes, 2009; Ruíz et al., 2010; Lutz, 2012; Genskowsky et al., 2016; Céspedes et al., 2017; López et al., 2018; Fuentes et al., 2019; Chang et al., 2019). Fruits, such as Maqui (*Aristotelia chilensis* (Mol.) Stuntz), calafate (*Berberis microphylla* G. Forst), pomegranate (*Punica granatum* L.), strawberry (*Fragaria x ananassa* Duch.), mortiño (*Vaccinium floribundum* Kunth), blackberry (*Rubus spp.* L.), goji (*Lycium barbarum* L.; *Lycium chinense* L.) and açaí (*Euterpe oleracea* Mart.), have been referred to as “superfruits” due to their phytochemical composition and antioxidant activity (Fredes, 2009; Lutz, 2012; Genskowsky et al., 2016; López et al., 2018; Chang et al., 2019).

Maqui is an endemic species of the Subantarctic forests of Chile and Argentina (Rodríguez et al., 2017). It produces small berries, also called maqui, which are considered as one of the berry species in nature with highest levels of polyphenols, tannins and anthocyanins in comparison with the other abovementioned "superfruits" (Escríbano-Bailón et al., 2006; Genskowsky et al., 2016; Lucas-Gonzalez et al., 2016). The importance of maqui berry lies in its benefits to human health, not only due to its extraordinary antioxidant capacity, but also due to its anti-inflammatory, antimicrobial, antiatherogenic, anticancer, astringent, analgesic and nutritional properties, among others (Table 1). Properties quantification vary according to the sample preparation, the extraction method, the quantification methodology and the standard used for the calibration of the curves. Therefore, comparison should consider the used method. In particular, the level of total phenolic content in maqui reach antioxidant level of 1,800 µg GAE/mL while blueberries 1,220 µg GAE/mL (Martín-Gómez et al., 2020; Dabulici et al., 2020). Anthocyanins level in maqui are reported to reach 11.6 g/L juice (Brauch et al., 2016) or 2.8 mg/g (Fredes et al., 2018) while in blueberries contents are between 0.7 and 2 mg/g (Fan-Chiang and Wrolstad, 2005).

Table 1. Research on medicinal and nutritional properties of maqui between 2002 and 2020.

Property	Dose/effectiveness	References
Antioxidant	1,800 µg GAE [a]/mL 88.1 TE[b], µmol/g of fresh weight 40 mmol TE[b]/ L juice 20,000 µmol TE[b]/100 g of fresh weight 194 - 241µmol TE[b]/g of dry weight 88.1 µmol TE[b]/g of fresh weight 28.18 g TE[b]/kg fruit 8.01 mg TE[b]/g pellet fraction 98 - 124 mg trolox/g fruit IC_{50}= 9.06 mg/mL methanol extract IC_{50}= 3.5 µg/mL and 45.3 µg/mL extract 27,600 ORAC[c] value/100 g 0.5% (w/w) maqui berry extract	(Dabulici et al., 2020) (Ruíz et al., 2010) (Miranda-Rottman et al., 2002) (Speisky et al., 2012) (González et al., 2015) (López et al., 2020) (Genskowsky et al., 2016) (Lucas-Gonzalez et al., 2016) (López de Dicastillo et al., 2016) (Gironés-Vilaplana et al., 2012) (Céspedes et al., 2017) (Zuñiga et al., 2017) (Tenci et al., 2019)
Analgesic	3.5% (w/w) hexane extract 4.5% (w/w) dichloromethane extract 2.7% (w/w) methanol extract	(Farías, 2009)
Anti-atherogenic	10 µM GAE[a] juice	(Miranda-Rottmann et al., 2002)
Anti-inflammatory	IC_{50}= 0.3 - 11.8 µg/mL 10.0 µg/mL against AChe[d] 20.0 µg/mL against BChe[e] 100 µM total polyphenols extract 0.5% (w/w) maqui berry extract	(Céspedes et al., 2010) (Céspedes et al., 2017) (Reyes-Farías et al., 2016) (Tenci et al., 2019)
Anti-diabetic	IC_{50}= 41.5 mg/L methanol extract 614.3 mg/g anthocyanin-rich fraction 180 mg of Delphinol® 100 µM total polyphenols extract	(Rubilar et al., 2011) (Rojo et al., 2012) (Alvarado et al., 2016) (Reyes-Farías et al., 2016)
Anti-depressive	1.9 – 4.74 mg/kg of anthocyanins	(Di Lorenzo et al., 2019)
Anti-cancer	50 ng/mL anthocyanins 1, 5, 10, 15, 30, and 50 µg/mL maqui berry extract 1,800 µg/mL GAE[a] 88.1 µmol TE[b]/g of fresh weight	(Ojeda et al., 2011) (Céspedes-Acuña et al., 2018) (Dabulici et al., 2020) (López et al., 2020)
Anti-obesity	IC_{50}= 0.67 mg/mL IC_{50}= 71.3 – 255.98 µg/mL 100 µM total polyphenols	(Gironés-Vilaplana et al., 2014) (Bastías-Montes et al., 2020) (Ovalle-Marín et al., 2020)
Anti-aging	1 sachet of Delphynol® diluted in 200 mL of water once a day for 12 weeks	(Addor et al., 2018)
Anti-microbial	4 mg/mL of hydroalcoholic extract 512 µg/mL ethanol and dichloromethane -	(Avello et al., 2009) (Mølgaard et al., 2011)

	metanol extract	
	2.81 mg TE[b]/g of dry weight	(Genskowsky et al., 2016)
	88.1 µmol TE[b]/g of fresh weight	(López et al., 2020)
	2, 4, 8, and 16% of methanolic extract maqui	(Arismendi et al., 2018)
Cardioprotective	10 mg/kg single-dose metanol extract	(Céspedes et al., 2008)
	88,1 µmol TE[b]/g of fresh weight	(López et al., 2020)
Neuroprotective	0.5 µM amyloid-β ± 1% maqui extract	(Fuentealba et al., 2012)
Ophtalmic	3 and 10 µg/mL of maqui berry extract	(Nakamura et al., 2014)
Nutritional	Lemon juice enriched with 5% maqui berry	(Gironés-Vilaplana et al., 2012)
	45.7 mg GAE[a]/g dry weight of fruit crude extract maqui	(Rubilar et al., 2011)
	11.6 g total anthocyanins/L juice	(Brauch et al., 2016)
	2.8 mg total anthocyanins/g	(Fredes et al., 2018)
	12.9 and 13.1 mg total anthocyanins/100 mL of the maqui-citrus juice	(Agulló et al., 2020)
	2,644.4 mg TE[b]/100 g maqui extract using inulin at 10%	(Romero-González et al., 2020)

Source: Own elaboration

[a] GAE - gallic acid equivalent.

[b] TE- trolox equivalent.

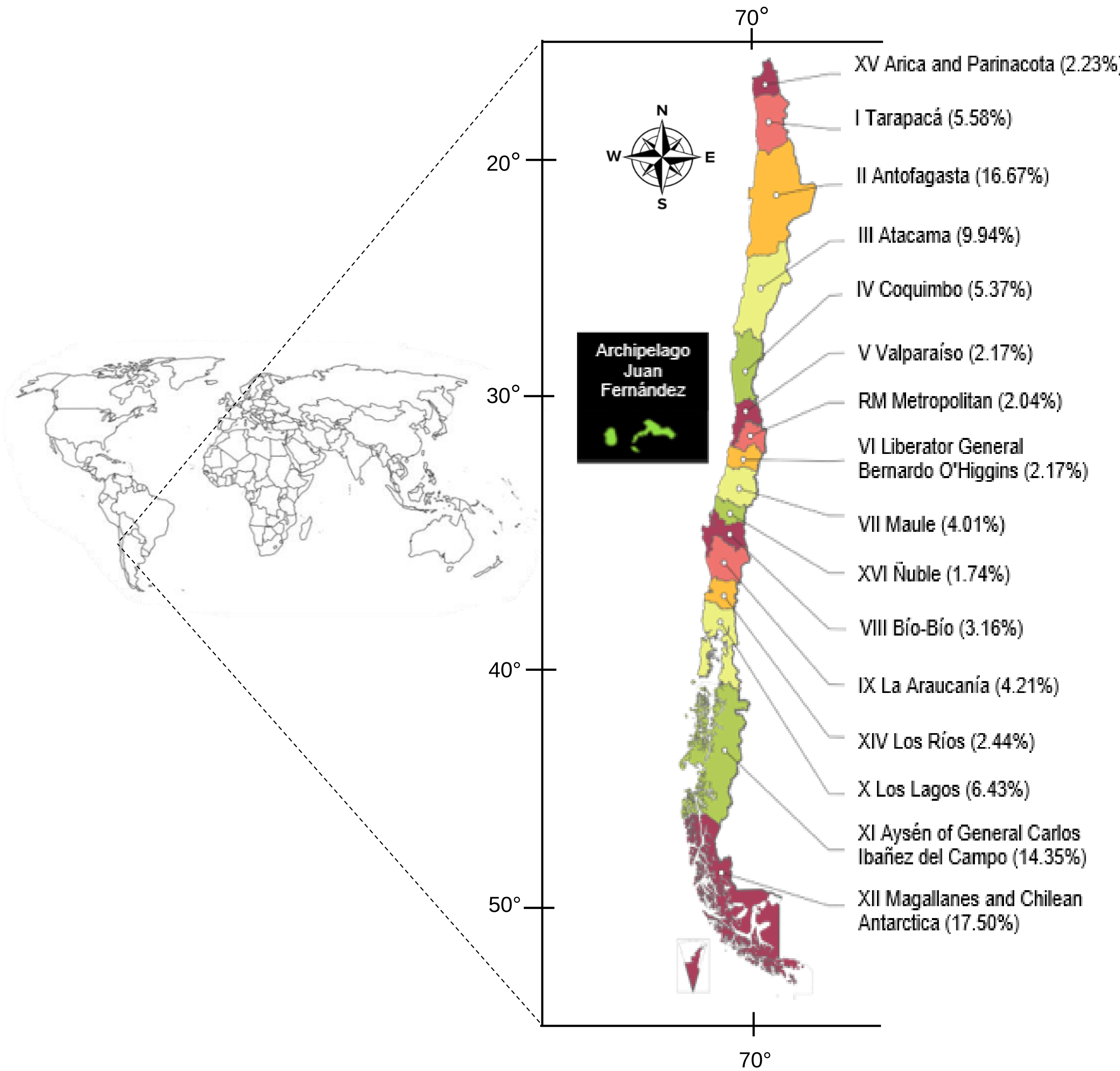
[c] ORAC- Oxygen radical absorbance capacity.

[d] BChe- butyryl cholinesterase.

[e] BChe- acetylcholinesterase.

Therefore, it is a great raw material for supplements and pharmaceuticals (Aguilera-Ortíz et al., 2011; Alonso, 2012; Brauch et al., 2015; Fredes et al., 2018; Agulló et al., 2020) and even the development of biopesticides to reduce the use of synthetic pesticides (Céspedes et al., 2020).

In Chile, it is distributed from the Coquimbo region (lat. 29°57'12''S; long. 71°20'22''W) in the North to the Aysén region (lat. 45°24'10''S; long. 72°41'30''W) in the South (Fig. 1) (Salinas and Parra, 2012; Silva et al., 2017; Tacón, 2017).



Source: Own elaboration

Figure 1. Regions distribution of and their percentage area in Chilean map.

Maqui fruit is not normally marketed as a fresh product, but dried or frozen for industrial uses. Given its beneficial properties, the maqui berry is highly demanded by the international market. However, as production and exports come mainly from wild-collected fruits, its supply is very limited. Currently, small volumes are being exported to Japan, South Korea, Italy, United States, and Germany among others, reaching an exported volume of 116,000 kg in 2019; as powder, dried, frozen, juice, and preserved maqui fruit (Table 2) (Chandía and Urra, 2017; ODEPA, 2019). Since maqui production comes mainly from harvesting fruits from wild stands, its availability is less than the growing demand. Unfortunately, most maqui production still is wild crafted, even many efforts for domestication, selection, and cultivation as an industrial crop have been attempted (Schreckinger et al., 2010; Alonso, 2012; Benedetti and Pavez, 2012; Vogel et al., 2014; Vogel et al., 2016; Chandía and Urra, 2017; FIA, 2017).

Table 2. Percentages of participation of the destinations of the exports of maqui, reported to 2019, based on 116,000 kg of exports. Prepared by the authors based on data from ODEPA 2019.

Country	Participation (%)
Japan	25
South Korea	24
Italy	18
United States	16
Germany	9
Australia	3
Denmark	2
Others	3

Source: Own elaboration

In Chile, a higher demand for natural products and active principles has led to a transition towards the cultivation of native plants, including maqui, incorporating management techniques and technology (Vogel et al., 2014; Vogel et al., 2016 Chandía and Urra, 2017; Doll et al., 2017; Muñoz-Espinoza et al., 2019). In general, many species have shown favorable growth response and production when grown in an artificial environment, but others have decreased their production (Vogel et al., 2005). The raw material for maqui comes mainly from wild plant collection (Salinas and Parra, 2012; Silva et al., 2017).

Maqui is a small tree, which usually is the first species to colonize burned forest or abandoned soil (Alonso, 2012). It is considered an important agent for soil conservation and to generate conditions that favor the establishment of more demanding species such as Chilean oak ‘roble’ (*Nothofagus obliqua* (Mirb.) Oerst.), coigüe (*Nothofagus dombeyi* (Mirb.) Oerst.), Chilean hazelnut (*Gevuina avellana* Mol.) and peumo (*Cryptocarya alba* (Mol.) Looser) (Donoso, 2006; Silva et al., 2017). Maqui populations are called “macal”, which are dense, monospecific populations of *A. chilensis*, and are characterized by fast growth (Fredes and Montenegro, 2011; Alonso, 2012; Benedetti and Pavez, 2012; Romo et al., 2018).

Maqui harvest is an important summer source of income for many people in rural areas who collect non-wood forest products (NWFP) in Chile. The current harvest practice in for collection of maqui is by cutting the whole fruit–bearing branch, which suppresses the fruit growth and yield in the following seasons. This causes the tree to concentrate fruit production near the top of the plant over the years. Therefore, one important part of picking wild maqui is harvesting the fruit in a way that does not harm the plant, the ecosystem surrounding it, and other macro and microfauna that depend on maqui to survive (Chandía and Urra, 2017; Tacón, 2017). For the Mapuche native culture, maqui is one of the three sacred plant species, a symbol of good intention, very much used in popular medicine. They are careful with the harvest to protect the tree (Misle et al., 2011).

There is very limited information on the management of wild maqui for sustainable production (Tacón, 2017; Romo et al., 2018). Sustainable management of species and forests is a key issue for endemic plants (Villagrán and Armesto, 2005). Therefore, knowledge of the production management of maqui, mainly of the characteristics of fruiting and harvesting, is crucial to develop sustainable management programs (Romo et al., 2018). The objective of this work is to review and summarize published studies on the general characteristics, distribution, adaptation, and production management of maqui, with emphasis on canopy management for sustainable production of maqui fruits. The value of our review is that most of the sources of information are not available to public and most are in Spanish and by including them in this review you are making the information available.

2.3. General information

2.3.1. Geographical distribution and soil and climate conditions

In Chile, maqui trees are a component of sclerophyllous scrub forest (Doll et al., 1999; Crisóstomo et al., 2019), deciduous forests, as well as perennial forests (San Martín et al., 1984; Donoso, 2006). It can also be found as a pioneer species under the canopies of adult *Pinus radiata* D. Don. plantations. It is estimated that there are approximately 170,000 ha of maqui, predominantly wild stands (Benedetti and Pavez, 2012; Chandía and Urra, 2017; FIA, 2017; López et al., 2018). These formations extend for more than 2,100 km, distributed between latitudes 30° and 46° S, from the Limarí Valley (IV Coquimbo region) in the North to General Carrera Lake (XI Aysén region) in the South (Fig. 2) (Donoso, 1994; Donoso, 2006; Fredes and Montenegro, 2011; Benedetti and Pavez, 2012). Maqui is also present in the Archipelago Juan Fernández (lat. 33°38'19''S; long. 78°51'29''W) (Fig. 2), where it is considered an invasive species, that establishes in clear areas generated when forests have lost canopy density or have suffered fragmentation of their stands or in forest edges (Alonso, 2012; FIA, 2017; Rojas-Badilla et al., 2017; Vargas-Gaete et al., 2018). It grows both in the central valley (intermediate depression) and mountainous areas up to 2,500 m.a.s.l. (Coastal and Andes Mountain Ranges), but preferably in the wet soils of the valley (Fig. 2) (Cuevas et al., 2004; Arellano, 2012).

In the semi-arid climate zone, in the northern part of Chile near to the Coquimbo region (Fig. 2), maqui is present in humid environments such as ravines, shaded slopes, areas exposed to fog, riverbanks, and small lakes. However, maqui trees are more commonly found in the Coastal Mountain Range since moisture conditions in coastal areas facilitate the development of species with higher humidity requirements (Donoso, 2006). Further, in the South of the Bío-Bío region (lat. 36°46'22''S; long. 73°03'22'47'W), temperate-rainy climate allows for the development of hygrophilous vegetation, typical of the evergreen forests where “macal” (Fig. 3A) are located contiguously, maintaining humidity. Towards the South near to Aysén region, within the temperate climate zone, dependence on water courses, ravines and other humid zones, is not crucial, allowing maqui to grow in wide range of places, such as edges of forest patches, river beds, streams, abandoned meadows and other places with low canopy cover (Fredes et al., 2012; Salinas and Parra, 2012).

The spread and regeneration of a “macal” depends on the degree of forest fragmentation and the presence of birds, since these eat the berries and spread the seeds (Donoso, 2006; Fernández et al., 2019). The plant has morphological and physiological plasticity that allows adaptation to different climates, from semi-arid to sub-humid and humid (Fig. 2) (Fredes and Montenegro, 2011; Lusk and Del Pozo, 2002).

Regarding edaphic terms, maqui prefers soils with abundant organic matter and moisture. In Central Chile, it grows in Ultisols of the Coastal Mountain Range from 300 to 1,100 m.a.s.l. In the Andes Mountains, maqui develops more frequently in Andosols and other soil types with high humidity and organic matter content (Casanova et al., 2007; Silva et al., 2017; Tacón, 2017). Although the species preferably grows in wet soils, it has also been found in degraded and dry soils, where it can tolerate short drought periods of less than one month (Donoso, 2006; Crisóstomo et al., 2019).

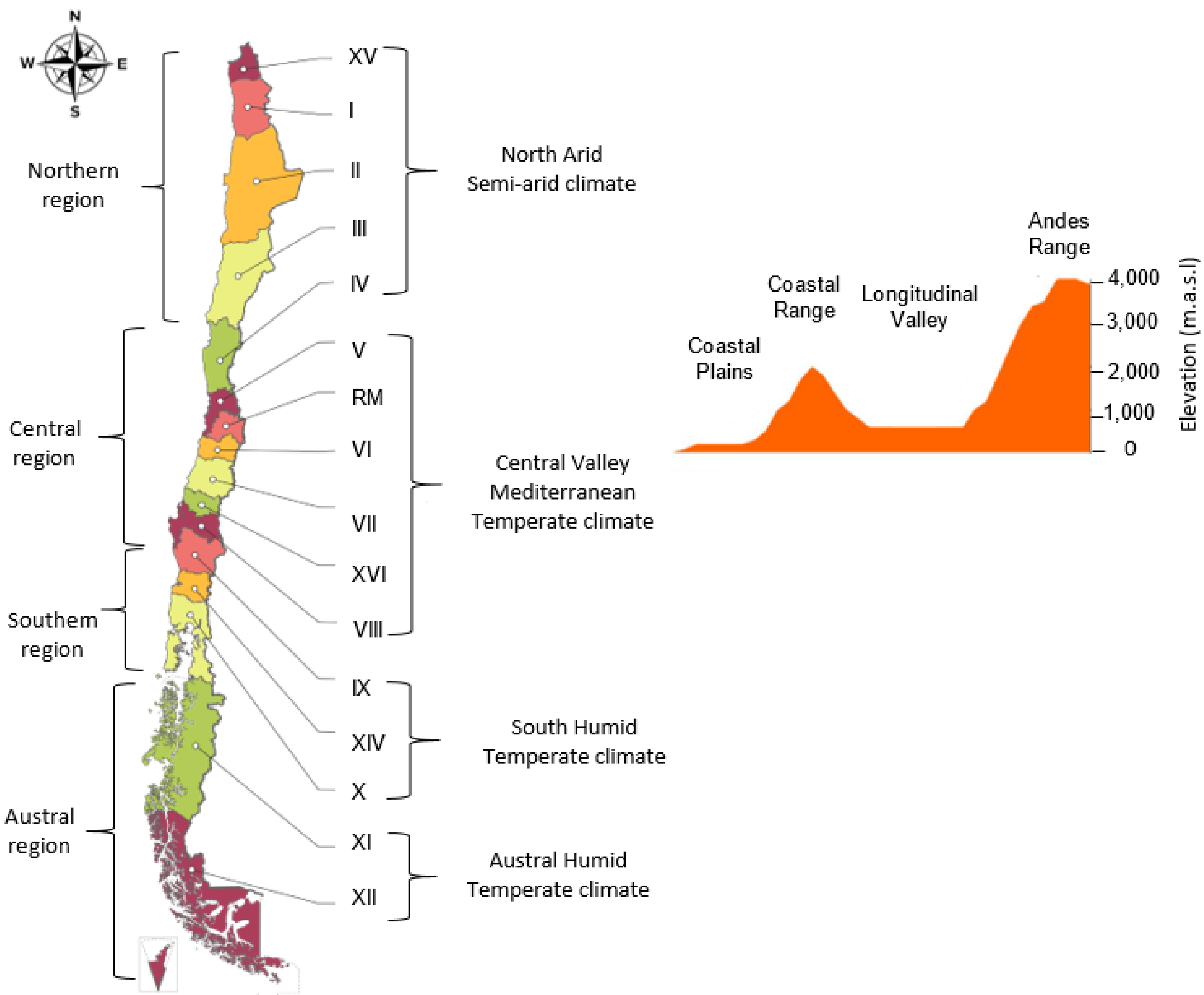


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Figure 2. Map showing the main macro-regions and climatic zones of Chile and Central region topographic profile.

As a pioneer species, maqui can develop in places with edaphic problems associated to erosion, due to human-induced disturbances (affected by fire or exploited) or surface runoff in landslide during the rainy season. In this sense, it is considered an important agent for soil erosion control, with a great capacity for regeneration so other species that require better conditions can establish thereafter (Silva et al., 2017; Donoso, 2006). Maqui's wide ecological range of distribution has been increasing due to the abovementioned alterations, forest fragmentation and changes in land use (Benedetti and Pavez, 2012). Maqui can grow in any exposure and various slope ranges. The collection of fruits in the wild is mainly conducted in areas with slopes less than 30% due to easier accessibility (Salinas and Parra, 2012).

2.3.2. Taxonomy

Maqui, *Aristotelia chilensis* (Mol.) Stuntz (= *A. glandulosa; A. glabra; A. maqui* is an endemic specie of the Subantarctic forests of Chile and Argentina, belonging to the Elaeocarpaceae family (Alonso, 2012; Matte and Wilckens, 2019). In Chile, there are two genera of this family; *Crinodendron* (*Crinodendron hookerianum* Gay and *Crinodendron patagua* Mol.) and *Aristotelia* (Mösbach, 1992; Salinas and Parra, 2012) with three species, maqui being the most abundant. The genus *Aristotelia* includes five species: *A. australasica* F. Muell., *A. chilensis*, *A. fruticosa* Hook. f, *A. peduncularis* (Labill.) Hook. f., and *A. serrata* (J.R.Forst. & G.Forst.) Oliv. (Coode, 1985), distributed in the temperate zones of the South Pacific, including Chile, Argentina, New Zealand, Australia, and Tasmania Island (Silva et al., 1997).

2.3.3. Botanical description

It is an evergreen small tree (multi-stem tree) (Fig. 3A) (Ramírez et al., 1984; Damascos and Prado, 2001). However, Donoso and Ramirez (1994), and Lusk and Del Pozo (2002) described it as partially deciduous, behaving like a facultative trophite, adding organic matter to the soil. The species presents a primary growth in spring with senescence in autumn, and a secondary growth (less intense than the primary growth) in summer with senescence in mid-spring in lesser quantity (Bonometti, 2000; Damascos and Prado, 2001). It has a divided or branched trunk, with a variable height of up to 5 m in the southern zone and 8 m in the southernmost zone of Chile (Misle et al., 2011; Alonso, 2012).

Maqui leaves are simple, opposite, and decussated (in a characteristic cross arrangement), pendulous, oval-lanceolate in shape, with blades 2 to 5 cm in width, 3 to 8 cm in length and serrated edges (Fig. 3B). Leaves have drooping stipules, petioles 1.5 to 2 cm in length and reddish, leathery texture, bright-green color, glabrous, and with a marked reticular rib (Zevallos and Matthei, 1992; Verdi, 2004; Bonometti, 2000; Salinas and Parra, 2012). Even though maqui keeps its leaves in winter, they are still photosynthetically active under moderate temperatures of autumn and winter days as well as early in the spring (Damascos and Prado, 2001).

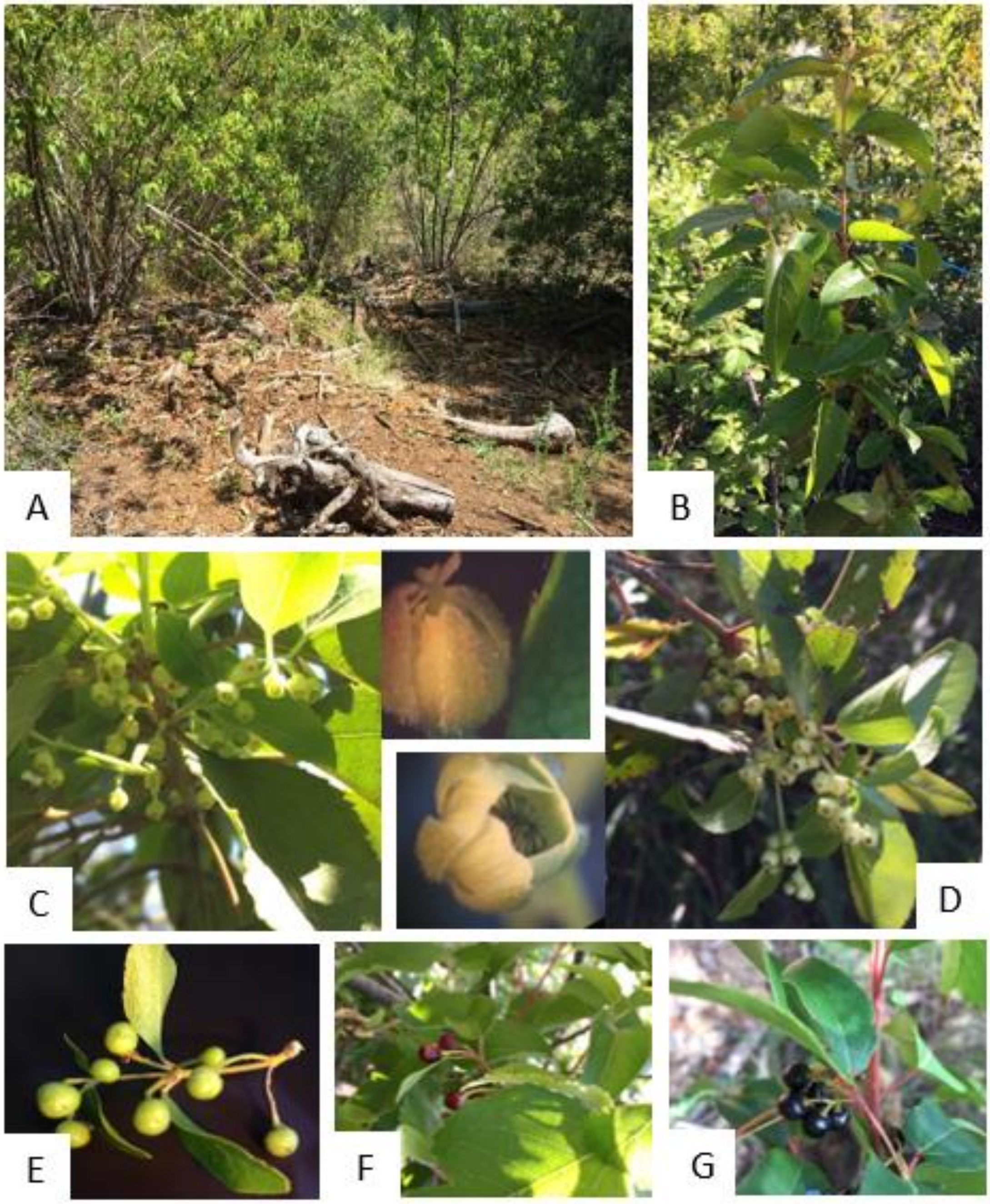
Depending on its distribution, maqui blooms from September to December (southern Hemisphere spring season) (Cárdenas, 1998). Blooming occurs from late September to early October in the central zone of Chile and from November to the end December in colder climates in the South of Chile (Riveros and Smith-Ramírez, 1995).

It is a dioecious plant, with female (Fig. 3C) and male flowers (Fig. 3D) in different specimens. The pale-yellow to whitish maqui flowers are either female or male but a few flowers can be hermaphrodite. Flowers are grouped in clustered inflorescences in the axils of the leaves, either in umbels of 2 to 3 units or pauciflore corymbs with 2 to 4 flowers (Coode, 1985; Cárdenas, 1998; Benedetti and Pavez, 2012; Silva et al., 2017; Fernández et al., 2019). It blooms for a short period of time, approximately 45 to 60 days and female flowers are receptive during 5 to 6 days (Cárdenas, 1998). Maqui flowers are hardly visible and, therefore, it is predominantly wind pollinated (Donoso, 2006). However, it can also be pollinated by insects. The plants do not have a clear nectar-producing structure. But nectar has been found in both female and male flowers, with a higher amount in female flowers (Cárdenas, 1998; Mora, 1999). Flowering occurs from tertiary branches, and flower clusters spring from their buds (Tacón, 2017). The floral induction transition of the vegetative meristem to reproductive meristem occurs in spring, during fruit development. The axillary buds develop a flowering shoot system that is protected by bud scales during dormancy. These buds will be activated in spring, flowering in corymbs (Vogel et al., 2014).

The fruit is a small berry of 4 to 5 mm in diameter, round, fleshy and with a sweet pulp, dark violet to shiny black, rarely white, with three locules inside, with 2 to 4 and even 8 angular seeds of 3 mm in length and 2 mm in width (Oyanadel, 2002; Misle et al., 2011; Mendoza, 2012; Alonso; 2012; Chandía and Urra, 2017). Most of the fruit volume is filled in by the seeds (Romo et al., 2018). The type of fruit gives the name to the plant, since maqui is the Mapuche indigenous name for berry (Cárdenas, 1998; Schreckinger et al., 2010; Benedetti y Pavez, 2012; Romo et al., 2018).

The fruit is green when unripe (Fig. 3E) and it tends to turn reddish in the pre-ripening stage (Fig. 3F). When ripe, it acquires a dark-violet to deep-purple (almost black) color (Fig. 3G) due to its high concentration of anthocyanin pigments, reaching a soluble solids concentration of 18 to 19°Brix (mostly glucose and fructose) (Forcone and Kutschker, 2006; Fredes and Montenegro, 2011; Fredes et al., 2012; Gonzalez et al., 2015; Brauch et al., 2016). Maturation occurs in the first months of summer, with 1,100 °C growing degree-days accumulated (base temperature of 5 °C), around 90 days after fruit set (Fredes and Montenegro, 2011; Fredes et al., 2012). There is no parameter of maturity index to estimate the optimal harvest time, but the fruit is harvested when it is dark purple (Fredes and Montenegro, 2011).

In Chile, maqui ripens in the summer, between December and March (Benedetti and Pavez, 2012; Salinas and Parra, 2012; Chandía and Urra, 2017), more precisely in December in northern and central-southern in Chile, and by the end of summer (March) in the southern area. Then, these berries become overripe and dehydrated (Chandía and Urra, 2001). Blooming length and days to ripening depend on environmental factors associated with changes in elevation and latitude, consequently varying according to the geographical area (Donoso, 2006; Chandía and Urra, 2017).



Source: Own elaboration

Figure 3. Details of maqui plants (A) type of growth (macal); (B) leaves; (C) female flowers; (D) male flowers; (E) fruit green; (F) fruit reddish; (G) mature fruit.

2.4. Wild and cultivated maqui

Maqui is a non-wood forest product (NWFP) that in the last decades has slowly turning into a cultivated crop. Even though some maqui clones were developed and selected for commercial cultivation, with a total of only 27.8 ha planted, most production is coming from wild stands (CIREN, 2019). In the wild, maqui fruits harvest practices are unsustainable since whole branches are cut to easily remove the fruits. This affects the fruit production potential of next season (Doll et al., 2017).

Maqui fruits demand has experienced a steady increase, associated with their use as medicinal plant or in supplements manufacturing (Benedetti and Pavez, 2012). The incipient production and export process of maqui contrasts with existing productive models of the fruit industry and economic development, generating gaps that have put maqui wild production sustainability at risk. In fact, encountered problems include insufficient market information, such as volumes produced, deficient marketing processes, added-value, and low level of technological development and absence of management plans of wild stands (Valdebenito et al., 2012; Romo et al., 2018). To prevent this, sustainable management techniques should be encouraged to ensure continuous production of wild stands (Chandía and Urra, 2017; FIA, 2017) and in turn to comply with the current regulation, Native Forest Law No. 20,283 of 2008 (Benedetti and Pavez, 2012).

Tacón (2017) and Silva et al. (2017) suggest that to facilitate regeneration and sustainability, new plants should be incorporated in wild formations to ensure an abundant production. The plant selection is focused on yield from wild stands (Poblete, 2014; Doll et al., 1999; Molina, 2001; Valdebenito et al., 2003; Rodríguez et al., 2017). However, other parameters related to medicinal properties and tolerance to abiotic stress, should be considered.

To propagate maqui plants cuttings from branches or seeds from selected plants are used (Molina, 2001). Using seeds for propagation; however, produces highly variable plant material and a mix of female and male plants.

2.4.1. Harvest

The current harvest practice in wild stands is done by cutting the whole fruit–bearing branch and shoots, which does not take into account future flowering and fruiting (Doll et al., 2017). From these practices the induced floral bud that develops in the new shoots are also removed with ensuing effects on inhibition of fruit production for the following seasons. In addition, cutting branches or shoots weakens the plant, making it susceptible to diseases. In this sense, harvest should consider plants protection to ensure production in future seasons and their environment. In addition, residues such as twigs broken during harvest can help spread fires, resulting in serious environmental damage (Silva et al., 2017).

Harvesting methods are crucial to maintain productivity of the plant. It has been reported that maqui is harvested at about 4 kg of fruits per day per person. Such low harvest certainly requires innovations in tree management fruit extraction and postharvest (Valdebenito et al., 2013) to achieving a steady supply, profitable fruit picking, and safety for both local collectors and consumers (Chandía and Urra, 2017; Tacón, 2017).

2.4.2. Ecosystem aspects

The sustainable production and management of wild maqui should be oriented to conserve the ecosystem, reducing erosion, water and CO2 balance and energy flow, in which the species develops, maintaining the accompanying floral diversity, attracting native pollinating insects, and birds that control pests and allows natural regeneration by seed dissemination (Silva et al., 2017; Tacón, 2017). Tacón (2017) suggest keeping at least 30% of the branches with fruit to ensure food availability for birds. It is also necessary to keep male plants for pollination, and establish trails for harvest.

In the ecosystem, interaction plant-environment can significantly affect some processes associated with growth and development of the plants, even their ability to synthesize secondary metabolites. In fact, phytochemical components such as polyphenols, tannins and anthocyanins, are modified by abiotic factors such as light, temperature, water, and soil fertility (Lattanzio, 2013; Yang et al., 2018) generating differences in the raw material obtained. Therefore, the cultivated maqui could have a lower amount of functional elements due the optimization of culture condition and consequently less abiotic stress.

2.5. Canopy management

2.5.1. Importance of light interception and distribution

Light interception and distribution within tree canopies play key roles in physiological plant process affecting fruit production and quality, such as photosynthesis, carbohydrate partitioning, fruit growth, flower induction and fruit pigment synthesis (Corelli-Grappadelli, 2003; Buler and Mika, 2009; Raffo, 2014). A basic factor in all fruit trees is fruit buds formation, flower bearing buds through the transformation of the vegetative to floral meristem, is commonly divided in two stages: induction phase and differentiation phase. Considering the heterogeneous light conditions present in the forest, both spatially and temporally, with an adequate management of canopy a better interception and distribution of light is achieved (Quevedo et al., 2016).

Although maqui tolerates semi-shade conditions, plant growth and fruit production are highly dependent on light availability (Frank and Finckh, 1997, Fontúrbel et al., 2017). Maqui plants adequately develops beneath tree canopies with sunlight regimes characterized by continuous sunflecks (Doll et al., 1999; Repetto-Giavelli et al., 2007; Rau, 2010; Arellano, 2012). Moya et al. (2019), evaluated different light conditions in maqui clones and observed that the quantity and quality of light affect the growth and fruit production in maqui, with differences being genotype dependent.

Maqui tree develops a dense canopy with poor light transmission to inner parts of the plant due to the annual vigorous shoot growth from the main trunk and main stems of the tree. Branches are subdivided by foliage weight, allowing new branches to develop (Silva et al., 2017). Through management techniques, either pruning and/or thinning could be improved and optimize light interception and distribution into the plant (Stephan et al., 2008; Vogel et al., 2016; Doll et al., 2017).

Maqui is a highly vigorous plant species that responds to the pruning; nevertheless, this should not be carried out in the main branches, because the branches must be at least 2 years old to produce fruit (Silva et al., 2017). Secondary and tertiary branches are renewed every year due to the presence of dormant buds that sprout in the presence of light (Doll et al., 2017; Tacón, 2017; Silva et al., 2017). Although maqui grows vigorously after pruning, year to year pruning could weaken the plant causing a negative effect in the plant survival. Therefore, the use of management practices aimed at improving light interception and distribution will certainly influence productivity (Tacón, 2017; FIA, 2017). Under wild conditions, pollinators such as bees (*Apis mellifera* L.) prefer foraging in sunshine (Mora, 1999; Tacón, 2017). Therefore, pruning and/or thinning should promote light entry to increase the production of flowers and fruits, and consequently increase yields (Bonometti, 2000).

2.5.2. Sustainable canopy management plants in wild maqui

Pruning in maqui is usually utilized for structural and productive purposes in orchards. However, pruning management has not been widely implemented in wild plants; only pruning by thinning cut of primary branches has been evaluated to improve canopy light interception (Valdebenito et al., 2003; Chandía and Urra, 2017; Doll et al., 2017; Tacón, 2017; Silva et al., 2017). In wild maqui, thinning by removing branches from base is mostly used, although it is not ideal, making this practice unsustainable for future cultivation and fruit production, as it suppresses the fruit yield of the following seasons (Vogel et al., 2014).

Considering that there is little research on the canopy management by heading cut and/or pruning by thinning cut in wild maqui, the development of management plans in this area can be based on blueberry management, taking into account the similarity in their canopy structure. Most common pruning intensities in blueberry are slight, regular, and severe (Jorquera-Fontena et al., 2014; Retamales et al., 2014; Muñoz-Vega et al., 2017). Pruning can be carried out in summer (green pruning) promoting the vegetative development and canopy growth (Retamales et al., 2014; Kovaleski et al., 2015), or winter (dry pruning) promoting the reproductive development (Correa de Moura et al., 2017). Thus, in blueberry plants with severe pruning, show higher production average and fruit size than slight pruning (Albert et al., 2010; Correa de Moura et al., 2017).

Several pruning systems have been tested in maqui (Table 3). In Chile, the best period to remove low and shaded branches through thinning in the “macal” is from May to August, furthermore, management practices like pruning and/or thinning can be conducted at harvest time in summer (January and February in Chile), enabling fruit picking from pruned branches (Tacón, 2017; Doll et al., 2017; Vogel et al., 2017).

Doll et al. (2017) applied different pruning treatments in cultivated maqui with vegetative material from wild plants and found that pruning by heading cut, pruning by thinning cut (renewal cut) or pruning by thinning cut plus heading cut in spring favors vegetative growth and reproductive shoot development, resulting in higher fruit yields in the following seasons compared with summer pruning. However, given the different conditions in which maqui grows, particularly in terms of available light, it is important to determine the proper time and intensity to prune maqui.

Table 3. Canopy management using pruning by heading and thinning cut in maqui.

Management	Season	Fruit production after management	References
Pruning by heading cut	early spring	561.0 g fresh material/plant	(Doll et al., 2017)
	early summer	177.0 g fresh material/plant	(Doll et al., 2017)
Pruning by thinning cut	summer	172.58 g/plant	(Valdebenito et al., 2013)
	autumn-winter	300 kg/ha	(Silva et al., 2017)

Source: Own elaboration

In order to evaluate the production of wild maqui, Valdebenito et al. (2013) evaluated pruning by thinning cut (renewal cut) in maqui stands and found that canopy thinning in a densely populated “macal” resulted in positive effects on fruit yield, doubling production in several years. They concluded that it is necessary to conduct further research on pruning to manage maqui structure aimed at promoting a more efficient harvest. Maqui plants should not exceed 4 m in height, as it is operationally inefficient at heights of 6-7 m. In addition, it is advisable to thin out maqui trees, leaving stumps of at least 30 cm length to favor the subsequent development of buds, and getting 60% more fruit yield than in trees in which this management technique was not applied.

Doll et al. (2017); Tacón (2017) and Salinas and Parra (2017) suggest pruning by heading cut only tertiary branches with fruits, without cutting main or secondary branches at the base, to avoid damaging the tree and maintain production the following season.

2.6. Conclusions

Maqui is a small tree that grows in the wild and its fruits have an extraordinary antioxidant capacity, and also anti-inflammatory, antimicrobial, antiatherogenic, anticancer, astringent, analgesic and nutritional properties. Their health benefits made it a highly demanded product by the industry however production depend on wild collection. Production needs to be both optimized and enhanced. This necessarily implies conducting research to generate knowledge of maqui's production management, with emphasis on fruiting and harvest techniques to ensure sustainable management of wild plants.

Management enables light availability in the canopy and this is one of the factors that influences the plant-environment interactions and results in secondary metabolites disturbances and/or stimulations.

Current information indicates that it is possible to generate a sustainable canopy management for wild maqui stands by using silvicultural trials oriented to pruning and/or thinning branches or shoots at different intensities whereas it is possible to increase bioactive compounds and reach sustainable production. Future research should address canopy management issues.

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III. CAPÍTULO 3: ACCUMULATION OF DELPHINIDIN DERIVATIVES AND OTHER BIOACTIVE COMPOUND IN WILD MAQUI UNDER DIFFERENT ENVIRONMENTAL CONDITIONS AND FRUIT RIPENING STAGES.

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3.1. Abstract

There is a growing interest in food and bioactive compounds with health benefits. Several studies on wild fruits have reported high anthocyanin contents and antioxidant potential mainly influenced by abiotic stress, particularly in native berries. Maqui (*Aristotelia chilensis* (Mol.) Stuntz), a Chilean native berry, has been referred as a “superfruit” due to its phytochemical composition and antioxidant activity. The content of bioactive compounds in wild maqui, specifically delphinidin, is influenced by collection time, genetic traits, and environmental conditions (location). The objective of this study was to evaluate the phenolic content, levels of anthocyanins and flavonols, and antioxidant capacity of maqui at three fruit ripening stages (unripe, preripe and ripe) in three different locations of central Chile (Coihueco, Cayumanque and Tregualemu) for two consecutive seasons (2019–2020 and 2020–2021). There was an interaction ($p \leq 0.05$) between location and fruit ripening stage in all the bioactive compounds evaluated. Total polyphenol content varied in accordance with the biosynthesis pathway transformation in the different ripening stages, ranging from 2290 (ripe stage) to 1025 mg gallic acid equivalent 100 g^{-1} (fresh weight) (unripe stage). An accumulation of delphinidin derivatives was found at the ripe stage, while these compounds accounted for a significant proportion of around 80% of the total anthocyanins in both seasons. The antioxidant capacity varied depending on the season, location, and fruit ripening stage. The highest values were recorded in Cayumanque, with an average value of 8926 µmol Trolox equivalent 100 g^{-1} (fresh weight) for both seasons. In order to supply raw material of similar quality, which is a key issue for the food and pharmaceutical industries, and allow for sustainable production, seasonal and environmental variations in bioactive compounds as well as chemical characteristics of wild fruit need to be regulated according to edaphoclimatic conditions.

Keywords: *Aristotelia chilensis,* wild berries, phenolic variability, anthocyanins.

3.2. Introduction

The demand for food and natural products with health properties has increased in recent years. Bioactive compounds present in fruit and vegetables play an important role in the prevention of various chronic diseases (Zhan et al., 2017; Sidhu et al., 2018). Several studies on wild berries have shown a high accumulation of anthocyanins triggered by oxidative stress and mainly influenced by abiotic conditions. Maqui is an endemic species of the Subantarctic forests of Chile and Argentina, belonging to the Elaeocarpaceae family (Matte and Wilckens, 2019). It predominantly grows in wild stands in sclerophyllous scrub, deciduous and perennial forests, and as a pioneer species under the canopies of adult *Pinus radiata* plantations. These formations extend for more than 2100 km, distributed between latitudes 29°57'12'' and 45°24'10'' south, and longitudes 71°20'22'' and 72°41'30'' west. It grows in the central valley (intermediate depression) of Chile and mountainous areas up to 2500 m.a.s.l. (Coastal and Andes Mountain Ranges), but preferably in the wet soils of the valley (Fuentealba-Sandoval et al., 2021). In summer, maqui produces a small fruit (berry) with novel characteristics and health-promoting benefits due to its high levels of polyphenols, tannins and anthocyanins (Lucas-Gonzalez et al., 2016).

The biosynthesis and accumulation of bioactive compounds is influenced by factors such as fruit ripening stage (development stage), geographical location, climate (rainfall, air temperature), edaphoclimatic conditions, genotype (cultivar), and agricultural practices such as harvesting, pruning and storage (Kulkarni and Aradhya, 2005; Wang et al., 2009; López et al., 2011; Vogel et al., 2014; Farías et al., 2019; Li et al., 2020; Zayed et al., 2020; Zitha et al., 2022). Proanthocyanidins are predominant in immature maqui fruit, while anthocyanins predominate in mature fruit (Das et al., 2021; Fredes et al., 2012; Vvedenskaya and Vorsa, 2004).

The main bioactive compounds found in maqui fruit are anthocyanins, being delphinidin derivatives the most abundant. Anthocyanins are water-soluble flavonoids, which are commonly found in higher plants, particularly berries. Maqui fruits present the richest source of delphinidins known, which are one of the most potent antioxidant anthocyanins because they have the highest number of hydroxyl groups in the B-ring, is maqui fruit (Watson and Schönlau, 2015).

Fruit ripening stage at harvest is the one of the main factors that affect anthocyanin content in maqui. In this sense, it has been reported that total phenolic content declined, and total anthocyanin content increased when maqui berries were harvested at the light red stage (preripe stage), compared with levels observed when harvest was conducted at the unripe stage (Fredes et al., 2012).

In cultivated maqui, an increase in oxygen radical absorbance capacity (ORAC) and total anthocyanin content has been observed as fruit maturity progresses (Fredes et al., 2012). Therefore, as the supply of raw material of the highest functional quality is a key issue for the pharmaceutical industry, determination of the appropriate maturity index for harvesting maqui is essential to obtain higher concentrations of bioactive compounds, like delphinidin derivative content.

The objective of this study was to evaluate total polyphenol content, levels of anthocyanins and flavonols, and antioxidant capacity of maqui at three fruit ripening stages (unripe, preripe and ripe) in three different locations of central Chile (Coihueco, Cayumanque and Tregualemu) for two consecutive seasons (2019-2020 and 2020-2021).

3.3. Materials and methods

3.3.1. Plant material and growth conditions

The experiment was conducted in wild maqui populations in three different locations of the Central Valley of Chile for two consecutive seasons (2019-2020 and 2020-2021). The locations (Coihueco, Cayumanque (Quillón), and Tregualemu (Pelluhue)) are distributed from the foothills of the Andes Mountain Range to the Coastal Range (Fig. 4) and have a Mediterranean temperate climate (Fuentealba-Sandoval et al., 2021).

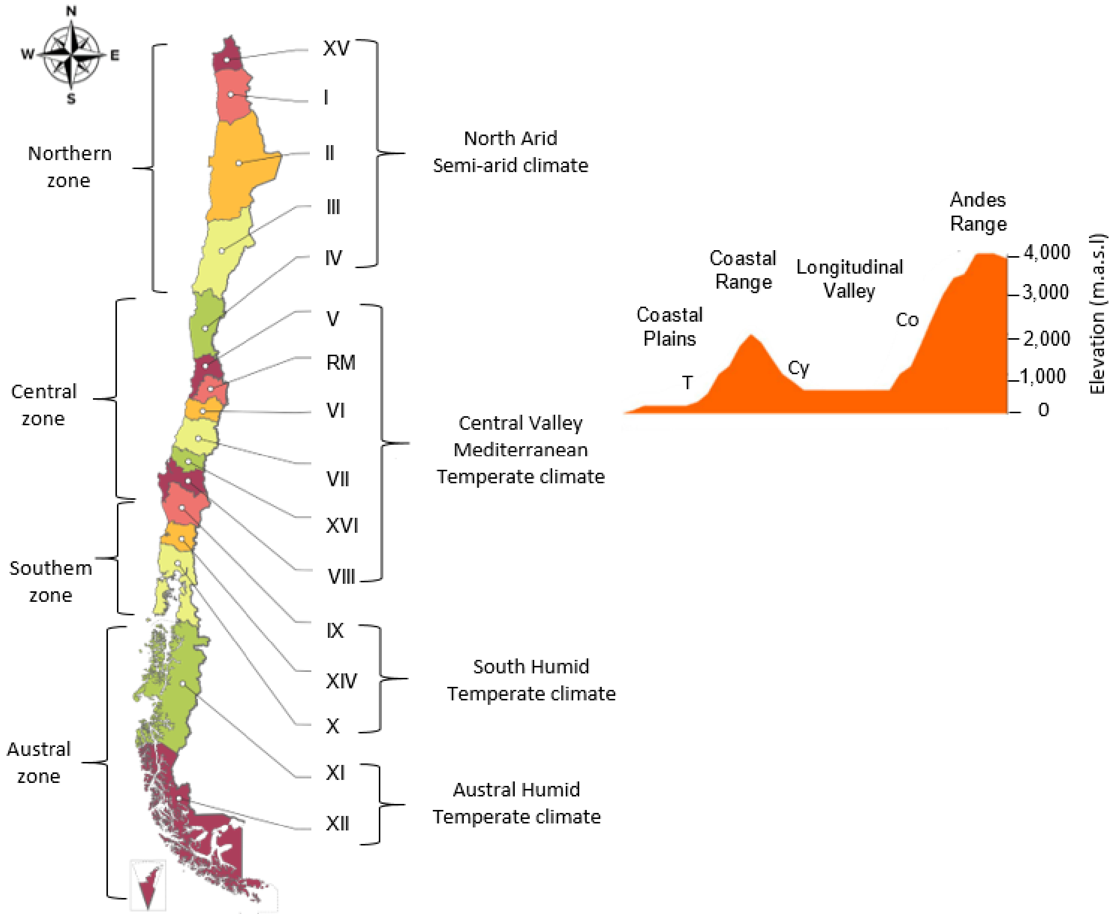

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Figure 4. Map of the zones of Chile, with the three locations where maqui fruit was collected (central zone): Coihueco (Co), Cayumanque (Cy) and Tregualemu (T).

Coihueco (lat. 38°37'60''S; long. 72°13'0''W; 325 m.a.s.l) is located in the Ñuble Region. The soil belongs to the Santa Bárbara series (medial, amorphic, mesic, typic Haploxerands), presenting an undulating topography with complex slopes, good drainage, with an average annual rainfall from 1500 to 2000 mm (Stolpe, 2006). The climate is classified as temperate Mediterranean with dry summers (Sarricolea et al., 2017). Quillón (lat. 36°44'40''S; long. 72°28'36''W, 350 m.a.s.l) is located in the Ñuble Region. The soil belongs to San Esteban series (fine, kaolinitic, mesic, Ultic Palexeralfs), presenting a hilly topography with variable slopes of 15 – 50%, good drainage, and average rainfall ranging from 1000 to 1500 mm (Stolpe, 2006). The climate is classified as temperate Mediterranean, with dry summers (Sarricolea et al., 2017). Pelluhue (lat. 35°48'0''S; long. 72°34'0''W, 134 m.a.s.l) is located in the Maule Region. The soil belongs to the Curanipe series (fine, metamorphic, Malic Haploxeralfs), derived from marine terraces, susceptible to mantle erosion and gully formations; it has a reduced drainage, with an average rainfall of 700-1200 mm (Rodríguez et al., 2003; Arriagada et al., 2019). The climate is classified as Mediterranean (warm summer), with oceanic influence (Sarricolea et al., 2017).

Measurements of daily temperature and rainfall were compiled using the data of the Instituto de Investigaciones Agropecuarias (INIA) (https://www.agromet.cl) according to the weather station closest to Coihueco, Cayumanque and Tregualemu, corresponding to Santa Rosa (Chillán), Nueva Aldea (Ránquil) and Lomas (Pelluhue) weather stations, respectively (Fig. 5 and Fig. 6).

Samples of maqui berries (100 g fresh weight, FW) were collected from ten trees/bushes grown in the wild of Coihueco, Cayumanque and Tregualemu in spring (November) and summer (December and January), using a randomly experimental design. Fruit collection was conducted at three fruit ripening stages: green (unripe), redish (preripe) and dark violet (ripe), with intervals of about 30 days from one stage to the other. Fruits were stored in cold storage and subsequently frozen at -80°C until analysis.

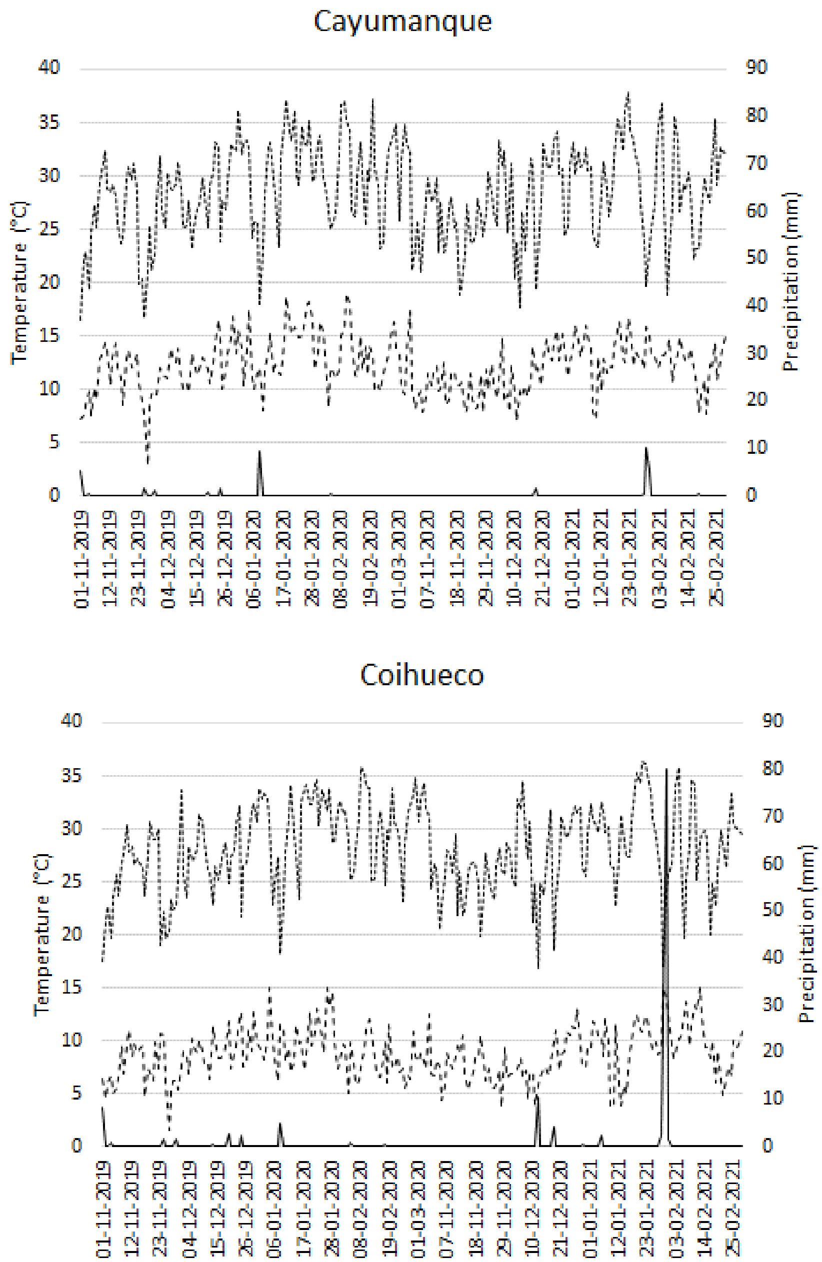

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Figure 5. Measurement of daily precipitation (mm) and maximum and minimum temperatures (°C) recorded in Cayumanque and Coihueco during fruit development in the 2019-2020 and 2020-2021 seasons.

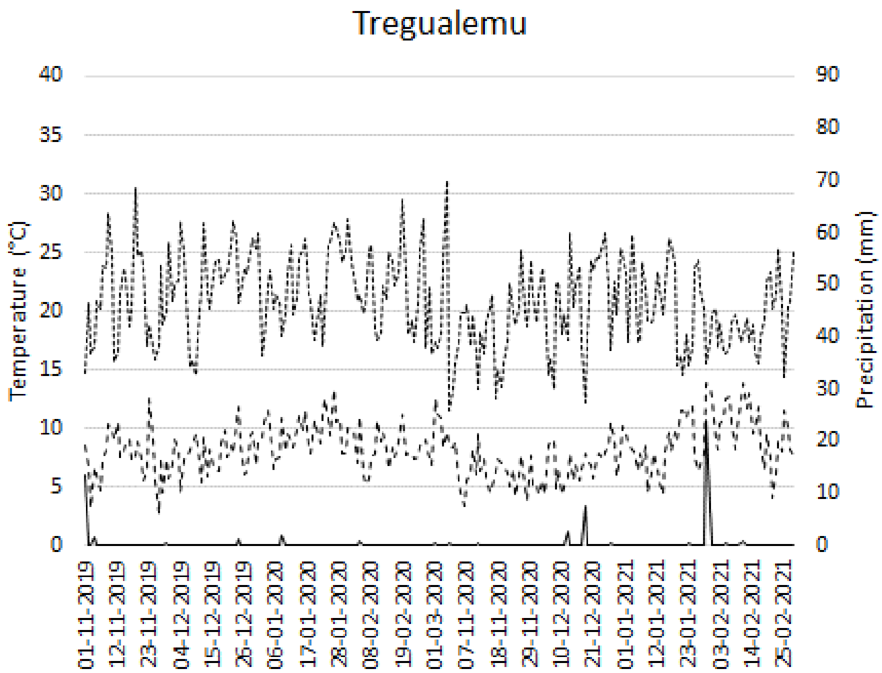

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Figure 6. Measurement of daily precipitation (mm) and maximum and minimum temperatures (°C) recorded in Tregualemu during fruit development in the 2019-2020 and 2020-2021 seasons

3.3.2. Chemical reagents

The analytical-grade reagents (formic acid, acetonitrile, methanol and water) were obtained from Merck (Darmstadt, Germany) as well as 2,2-diphenyl-1-picrylhydrazyl radical (DPPH●), Folin–Ciocalteu reagent, monobasic sodium phosphate, and dibasic sodium phosphate. Meanwhile, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Sigma–Aldrich (MilliporeSigma, St. Louis, MO, USA). Ultrapure water was produced using a Millipore water purification system. Besides, anthocyanins as cyanidin 3-glucoside (99.21%), delphinidin 3-glucoside (98.84%), and flavonols as quercetin dihydrate, kaempferol and myricetin were obtained from Sigma-Aldrich Chemical Co. (MilliporeSigma, St. Louis, MO, USA), all of them with chemical purity >95%.

3.3.3. Extraction of phenolic compounds

The extraction of antioxidants in maqui fruit was performed using the method described by Romero-Román et al., (2021). A ground sample of 0.5 g was treated with 5 mL of H_2O/MeOH/formic acid (24:25:1/v:v:v). It was kept in BRANSON 5800 (Brookfield, Connecticut) brand ultrasound for 1 h and allowed to stand at -20 °C for 24 h. The next day, the mixture was sonicated again for 1 h and then was centrifuged with a Fascio TG1650-WS centrifuge (Varallo, Italy) at 3500 rpm for 15 min.

3.3.4. Total polyphenol contents by Folin-Ciocalteu assay

Total polyphenols (TP) were determined by Folin-Ciocalteu method (Singleton and Rossi, 1965). For this purpose, a calibration curve of gallic acid (0.05-1.0 g L^{-1}) was performed. Subsequently, the absorbance of the samples was measured at a wavelength of 760 nm using a UV/vis spectrophotometer Spectroquant® Prove 300 (Merck, Darmstadt, Germany). For this, 120 μL of Folin Ciocalteu 1 N reagent, 25 μL of the sample, 1.62 mL of water and 340 μL of the 20% sodium carbonate solution were added to each vial and leave to stand for 2 h in the dark. Distilled water was used as the blank.

The results of TP were expressed as mg of gallic acid equivalent per 100 g^{-1} fresh weight (mg GAE 100^{-1} FW).

3.3.5. Identification and quantification of the main anthocyanins and flavonols

Anthocyanins and flavonols in maqui were identified according to their UV spectra and standards as previously described by Romero-Román et al. (2021).

HPLC-DAD analyses were carried out on a Hitachi system (Merck, Darmstad, Germany) equipped with a photodiode array detector.

Anthocyanins and flavonols were quantified using the aliquots as described in the Extraction of Phenolic Compounds Section, filtered through a 0.22 μm PVDF membrane (Millex V13, Millipore, Bedford, MA, USA). The quantitative analysis was carried out under the conditions described by Agulló et al. (2020).

The analyses were performed on a Kromasil 100-5-C18 column (4.6 x 250 mm). The mobile phase consisted of two solvents: (A) 1% (v:v) formic acid and (B) acetonitrile, with a flow rate of 1 mL min^{-1}. The gradient varied with 4% solvent B, reaching 8% at 10 min, 15% at 45 min, and 55% at 60 min, which was maintained until 65 min. Chromatograms were recorded at 320, 360 and 520 nm. Identification was performed by comparison of retention times with standards and UV-Vis spectra. As standards, delphinidin 3-glucoside and cyanidin 3-glucoside were measured at 520 nm, while flavonols such as Quercetin, Kaempferol and Myricetin were measured at 360 nm (Sigma Chemical Co. St. Louis, MO) (Genskowsky et al., 2016; Ruiz et al., 2016).

3.3.6. Determination of antioxidant capacity

Antioxidant capacity (AC) or free radical scavenging activities were determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) methods adapted according to Mena et al. (2011). For this, a volume of 100 μL of the extract and 2.9 mL of the DPPH solution were mixed by shaking vigorously and kept in the dark for 1 h. Finally, the absorbance of the mixture was measured at 515 nm in a UV/vis spectrophotometer Spectroquant® Prove 300 (Merck, Darmstadt, Germany). A blank containing 3 mL of methanol was prepared. The results were expressed as μmol Trolox equivalent 100 g^{-1} fresh weight (μmol TE 100 g^{-1} FW).

3.3.7. Statistical analysis

The statistical analysis was conducted using standard procedures for a randomized complete block design (RCBD) with a split-plot arrangement. The main plot was the location, and the sub-plot was the ripening stage. The two seasons evaluated (2019-2020 and 2020-2021) were considered as a random effect, while the location and fruit ripening stage were considered fixed effects, the former being the principal effect and the latter the secondary effect. Residual mean squares were compared for homogeneity between seasons. As they were heterogeneous, a combined analysis across seasons was not conducted. Comparison of means was conducted using F-protected least square differences (LSD) at a 5% level of significance ($p \leq 0.05$). Data were analyzed using SAS software, University Edition (SAS Institute, 2014).

3.4. Results

3.4.1. Determination of total polyphenol content by Folin-Ciocalteu assay

This method was used to estimate the total phenolic compounds since with the chromatographic profile only the main ones were identified and quantified. High contents of total polyphenols were found in both seasons, but the highest contents were observed at the ripe stage in the second season. Values fluctuated between 2070 and 1145 mg of GAE100 g^{-1}, and between 2291 and 1741 mg GAE 100 g^{-1} (obtained by Folin-Ciocalteu assay) in the 2019-2020 and 2020-2021 seasons, respectively. There was an interaction between the location and maturity stage ($p \leq 0.05$) in both seasons (Table 4).

Table 4. Mean square values for total polyphenols, total anthocyanins and antioxidant capacity for the 2019-2020 and 2020-2021 seasons.

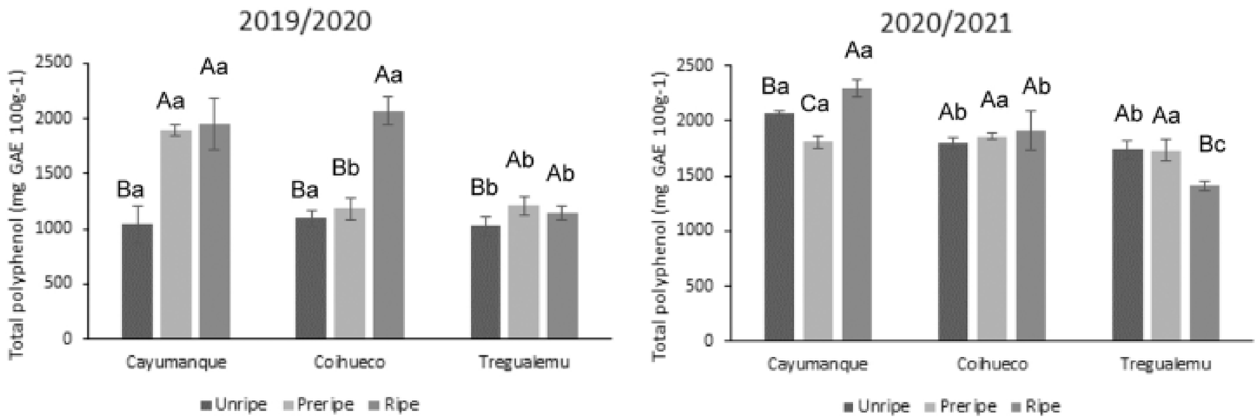
Source of variation	df	Total polyphenols	Total anthocyanins	Antioxidant capacity
		2019-2020		
Rep	2	94,734	1976	121072
Location (loc)	2	1161,199	271,926	55,750,157
Location x Rep	4	76,724	4699	353,146
F. ripening stage	2	2022,407	4342,491	12,782031
L x F		1353,641*	569,326 *	28,639582*
Error		102,600	12,609	423,479
CV,%		6.6	10.9	2.9
		2020-2021		
Rep		16,460	185	354,305
Location		828,669	33,442	17,198247
Location x Rep		17,131	368	472,888
F. ripening stage		29,052	526,424	11,031748
L x F		551,264 *	59,232 *	12,628,900 *
Error		90,660	1812	1249,013
CV,%		4.71	9.54	3.98

Source: Own elaboration

L x F: Location x Fruit ripening stage, * Significant difference ($p \leq 0.05$)

The highest polyphenol levels were recorded at the ripe stage in Coihueco and Cayumanque in both seasons, with values ranging from 2070 to 1951 and from 1911 to 2291 mg GAE 100 g^{-1} in the 2019-2020 and 2020-2021 seasons, respectively. However, lower values were recorded in Tregualemu at the same ripening stage. The lowest levels were observed at the unripe stage in all the study sites only in the 2019-

2021 season. For the 2020-2021 season, similar values were recorded at the different ripening stages (Fig. 7).



Source: Own elaboration

Figure 7. Total polyphenols in maqui grown in Cayumanque, Coihueco and Tregualemu in the 2019–2020 and 2020–2021 seasons. Different lowercase letters indicate significant differences between same stages of maturity in three different location and season ($p \leq 0.05$). Different capital letters are significant difference between the three stages of maturity of same location and season.

3.4.2. Identification and quantification of anthocyanins and flavonols

The content of anthocyanins and flavonols was influenced by season, location and ripening stage. In the 2020-2021 season, maqui fruit exhibited the highest total anthocyanin content, which averaged 1009.3 mg eq 100 g^{-1} fresh weight (FW) at the ripe stage. In both seasons, there was an interaction ($p \leq 0.05$) between the location and the ripening stage (Table 4). The highest total anthocyanin content was observed at the ripe stage, with similar increases in Coihueco and Cayumanque, reaching an average value of 1171 mg eq 100 g^{-1} FW. However, total anthocyanins had a significant reduction of 62% in Tregualemu in the 2019-2020 season. A similar behavior was observed in the 2020-2021 season, with values of 1183 and 1269 mg eq 100 g^{-1} FW in Coihueco and Cayumanque, respectively. In Tregualemu, total anthocyanins decreased to 576 mg eq 100 g^{-1} FW at the ripe stage, while no anthocyanins were found at the unripe stage in any of the seasons (Fig. 8). The highest accumulation of delphinidins was observed at the ripe stage in both seasons and accounted for a significant proportion of total anthocyanins, reaching 80%, 83% and 75% in Coihueco and Cayumanque and Tregualemu, respectively.

The main anthocyanin compound was dephinidin-3,5 diglucoside, followed by dephinidin-3-glucoside (Table 5).

The concentration of total flavonols varied depending on the location and ripening stage. Levels were similar in both seasons, reaching average values of 23 and 25 mg eq 100 g^{-1} FW in the 2019-2020 and 2020-2021 seasons, respectively. Total flavonols varied slightly in Coihueco in both seasons, with the highest value at the preripe stage with 25.5 mg eq 100 g^{-1} FW. In Cayumanque, the highest concentration reached 32 mg quercetin 3-glucoside eq 100 g^{-1} FW the 2019-2020 season. In Tregualemu, the highest levels were observed at the preripe and unripe stages for the 2019-2020 and 2020-2021 seasons, with 24 and 40 mg quercetin 3-glucoside eq 100 g^{-1} FW, respectively (Fig. 9).

Fifteen flavonol compounds were found in maqui fruit by comparing the relative retention times and spectral data for both seasons. Eight of them were detected in all the fruit ripening stages evaluated, five compounds were identified during unripe and ripe stages, while two of them (M- rutinoside, and Q-3- galactoside) were identified at the unripe stage (Table 6 and Table 7). The main flavonols were myricetin-3 galactoside. The maximum and minimum concentrations were achieved at the ripe stage in the 2020-2019 season, with values of 6.11mg eq 100 g^{-1} FW (Cayumanque) and 2.16 mg eq 100 g^{-1} FW (Tregualemu), respectively (Table 6 and Table 7).

Table 5. Anthocyanin compounds (mg eq 100 g^{-1} FW) in maqui grown in Coihueco, Cayumanque and Tregualemu in the 2019-2020 and 2020-2021 seasons.

Season	Location	Fruit ripening stage	D 3,5-diglucoside	C 3,5-diglucoside	D 3-sambubioside	D 3-glucoside	C 3-sambubioside	C 3-glucoside	*% Total Delphinidin derivatives*
2019-2020	Coihueco	Unripe							
		Preripe	26.35 ± 0.04	4.15 ± 0.05	7.56 ± 0.01	22.51 ± 0.14	1.96 ± 0.01	3.63 ± 0.06	85.27
		Ripe	482.22 ± 4.81	85.04 ± 0.61	147.86 ± 0.24	351.36 ± 0.38	44.25 ± 0.21	68.20 ± 0.13	83.24
	Cayumanque	Unripe							
		Preripe	46.99 ± 0.69	7.76 ± 0.05	14.27 ± 0.03	31.19 ± 0.02	3.73 ± 0.05	5.54 ± 0.01	84.44
		Ripe	423.40 ± 4.66	114.53 ± 0.81	120.35 ± 0.38	377.80 ± 0.79	45.93 ± 0.18	80.14 ± 0.77	79.29
	Tregualemu	Unripe							
		Preripe	36.58 ± 0.24	9.59 ± 0.07	7.54 ± 0.03	21.68 ± 0.07	2.99 ± 0.01	6.34 ± 0.02	77.66
		Ripe	174.22 ± 0.18	67.48 ± 1.14	33.79 ± 0.69	114.27 ± 0.39	20.04 ± 0.42	35.58 ± 0.34	72.36
2020-2021	Coihueco	Unripe							
		Preripe	23.66 ± 0.05	4.90 ± 0.16	7.98 ± 0.01	18.73 ± 0.19	3.00 ± 0.02	4.38 ± 0.04	80.39
		Ripe	534.27 ± 5.90	90.79 ± 2.34	149.84 ± 0.31	310.92 ± 2.20	40.50 ± 0.23	57.05 ± 0.98	84.08
	Cayumanque	Unripe							
		Preripe	27.00 ± 2.43	5.24 ± 0.19	7.89 ± 0.10	20.76 ± 0.39	2.21 ± 0.03	4.42 ± 0.02	82.42
		Ripe	570.79 ± 5.11	87.99 ± 0.62	138.50 ± 0.16	378.65 ± 0.60	32.43 ± 0.01	60.53 ± 0.09	85.73
	Tregualemu	Unripe							
		Preripe	24.94 ± 0.38	5.46 ± 0.08	6.92 ± 0.12	18.21 ± 0.27	2.29 ± 0.02	4.10 ± 0.06	80.86
		Ripe	224.71 ± 0.61	60.22 ± 1.58	53.06 ± 0.68	167.15 ± 0.08	21.88 ± 0.18	48.64 ± 0.33	77.28

Source: Own elaboration

D 3,5-diglucoside as delphinidin-3,5-diglicoside; C 3,5-diglucoside as cyanidin-3,5-diglucoside;

D 3-sambubioside as delphinidin-3-sambubioside; D 3-glucoside as delphinidin-3-glucoside; C 3-sambubioside as cyanidin-3-sambubioside;

C 3-glucoside as cyanidin-3-glucoside

Table 6. Flavonol compounds (mg eq 100 g^{-1} FW) in maqui grown in Coihueco, Cayumanque and Tregualemu in the 2019-2020 and 2020-2021 seasons.

Season	Location	Fruit ripening stage	M-rutine	M-3-galactoside	M-3-glucoside	Q-hexoside	Q-3-rhamnoside	Q-galoil-galactoside	Q-3-rutinoside
2019-2020	Coihueco	Unripe	2.37 ± 0.37	1.35 ± 0.54	3.45 ± 0.33	0.76 ± 0.27	-	0.94 ± 0.02	1.74 ± 0.01
		Preripe	-	0.60 ± 0.19	0.74 ± 0.23	1.46 ± 0.98	1.42 ± 0.03	0.32 ± 0.03	0.82 ± 0.08
		Ripe	-	3.62 ± 0.03	1.80 ± 0.01	0.88 ± 0.04	0.41 ± 0.01	0.92 ± 0.01	0.35 ± 0.01
	Cayumanque	Unripe	2.02 ± 0.02	2.80 ± 0.08	3.13 ± 0.01	1.75 ± 0.01	-	0.72 ± 0.33	5.88 ± 0.24
		Preripe	-	1.37 ± 0.01	1.33 ± 0.11	1.75 ± 0.01	0.85 ± 0.01	0.91 ± 0.02	0.75 ± 0.03
		Ripe	-	5.66 ± 1.16	2.57 ± 0.07	1.72 ± 0.01	1.10 ± 0.01	0.84 ± 0.21	0.97 ± 0.08
	Tregualemu	Unripe	2.52 ± 0.09	6.41 ± 0.35	4.00 ± 0.01	0.97 ± 0.03	-	0.99 ± 0.05	0.36 ± 0.01
		Preripe	-	2.76 ± 0.02	2.46 ± 0.00	1.55 ± 0.01	1.31 ± 0.01	0.59 ± 0.01	0.65 ± 0.01
		Ripe	-	2.59 ± 0.31	1.13 ± 0.10	1.46 ± 0.02	0.23 ± 0.13	0.41 ± 0.06	0.40 ± 0.09
2020-2021	Coihueco	Unripe	1.28 ± 0.50	3.47 ± 0.05	1.02 ± 0.04	1.14 ± 0.06	-	0.46 ± 0.01	2.56 ± 0.26
		Preripe	-	3.31 ± 0.02	2.61 ± 0.02	0.80 ± 0.07	1.58 ± 0.01	1.12 ± 0.56	0.45 ± 0.14
		Ripe	-	2.89 ± 0.58	2.60 ± 0.06	1.29 ± 0.02	0.67 ± 0.01	0.89 ± 0.02	0.61 ± 0.02
	Cayumanque	Unripe	2.74 ± 0.13	3.47 ± 0.64	2.40 ± 0.01	1.53 ± 0.06	-	1.78 ± 0.32	4.52 ± 0.17
		Preripe	-	1.48 ± 0.16	1.92 ± 0.05	0.76 ± 0.02	0.59 ± 0.41	0.56 ± 0.03	0.51 ± 0.11
		Ripe	-	6.11 ± 0.37	2.05 ± 0.02	1.32 ± 0.03	0.72 ± 0.01	0.85 ± 0.01	0.76 ± 0.01
	Tregualemu	Unripe	4.31 ± 0.05	3.67 ± 0.01	4.42 ± 0.03	1.67 ± 0.01	-	0.82 ± 0.02	8.48 ± 0.10
		Preripe	-	3.12 ± 0.01	2.69 ± 0.06	1.56 ± 0.02	0.90 ± 0.01	0.77 ± 0.03	0.69 ± 0.01
		Ripe	-	2.16 ± 0.05	2.66 ± 0.02	1.70 ± 0.06	1.74 ± 0.02	0.72 ± 0.02	0.68 ± 0.12

Source: Own elaboration

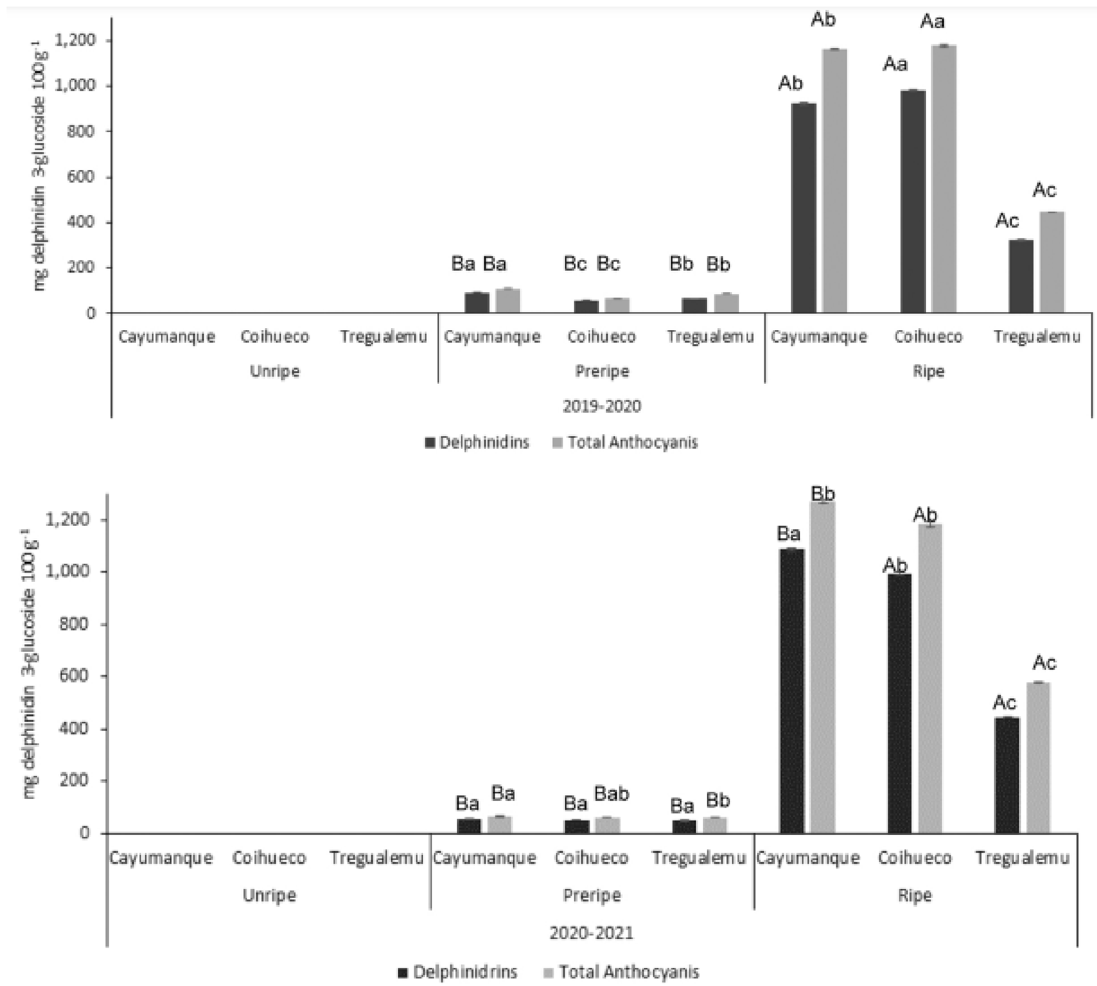
M: Myricetin; Q: Quercetin

Table 7. Flavonol compounds (mg eq 100 g^{-1} FW) in maqui grown in Coihueco, Cayumanque and Tregualemu in the 2019-2020 and 2020-2021 seasons.

Season	Location	Fruit ripening stage	M-3 pentoside	Q	Q-3-galactoside	Q-3-glucoside	K- hexoside	Q- pentoside	DimethoxyQ	M
2019-2020	Coihueco	Unripe	-	-	0.99 ± 0.02	0.06 ± 0.02	-	0.46 ± 0.01	6.00 ± 0.08	-
		Preripe	0.73 ± 0.06	4.61 ± 0.22	-	0.65 ± 0.01	0.88 ± 0.02	1.58 ± 0.02	6.47 ± 0.09	2.54 ± 0.04
		Ripe	0.85 ± 0.18	1.07 ± 0.01	-	0.17 ± 0.00	0.80 ± 0.02	0.61 ± 0.00	3.90 ± 0.01	1.69 ± 0.00
	Cayumanque	Unripe	-	-	3.87 ± 0.70	2.77 ± 0.40	-	1.65 ± 0.09	7.86 ± 0.11	-
		Preripe	0.51 ± 0.01	0.75 ± 0.10	-	1.72 ± 0.20	0.85 ± 0.04	1.04 ± 0.01	7.03 ± 0.06	0.29 ± 0.00
		Ripe	1.27 ± 0.42	3.28 ± 1.22	-	1.48 ± 0.25	3.35 ± 0.05	0.67 ± 0.02	5.19 ± 0.02	1.45 ± 0.07
	Tregualemu	Unripe	-	-	0.73 ± 0.01	0.09 ± 0.00	-	0.46 ± 0.00	5.66 ± 0.02	-
		Preripe	0.84 ± 0.00	2.53 ± 0.01	-	1.12 ± 0.01	1.48 ± 0.00	1.40 ± 0.00	6.36 ± 0.01	0.26 ± 0.00
		Ripe	0.41 ± 0.15	4.63 ± 0.21	-	1.24 ± 0.19	1.50 ± 0.33	1.11 ± 0.00	5.14 ± 0.04	1.07 ± 0.20
2020-2021	Coihueco	Unripe	-	-	1.94 ± 0.37	0.34 ± 0.12	-	1.10 ± 0.03	5.26 ± 0.01	-
		Preripe	0.23 ± 0.27	2.04 ± 0.59	-	1.63 ± 0.48	1.26 ± 0.51	0.93 ± 0.03	9.31 ± 0.06	0.26 ± 0.04
		Ripe	0.66 ± 0.02	1.23 ± 0.10	-	1.14 ± 0.12	0.84 ± 0.12	0.85 ± 0.00	4.89 ± 0.03	1.28 ± 0.02
	Cayumanque	Unripe	-	-	4.49 ± 0.26	1.51 ± 0.15	-	1.96 ± 0.02	6.78 ± 0.08	
		Preripe	0.74 ± 0.03	0.65 ± 0.07	-	3.38 ± 0.17	3.13 ± 0.09	1.25 ± 0.03	5.75 ± 0.07	0.37 ± 0.12
		Ripe	0.96 ± 0.01	1.55 ± 0.01	-	0.42 ± 0.01	0.39 ± 0.00	0.95 ± 0.00	4.95 ± 0.02	1.19 ± 0.00
	Tregualemu	Unripe	-	-	3.41 ± 0.01	2.61 ± 0.02	-	1.64 ± 0.00	8.77 ± 0.01	-
		Preripe	0.84 ± 0.01	2.39 ± 0.02	-	1.12 ± 0.23	0.62 ± 0.00	1.20 ± 0.01	7.61 ± 0.12	0.36 ± 0.02
		Ripe	0.78 ± 0.11	3.76 ± 1.24	-	1.13 ± 0.22	1.82 ± 0.53	1.58 ± 0.03	6.05 ± 0.02	0.95 ± 0.05

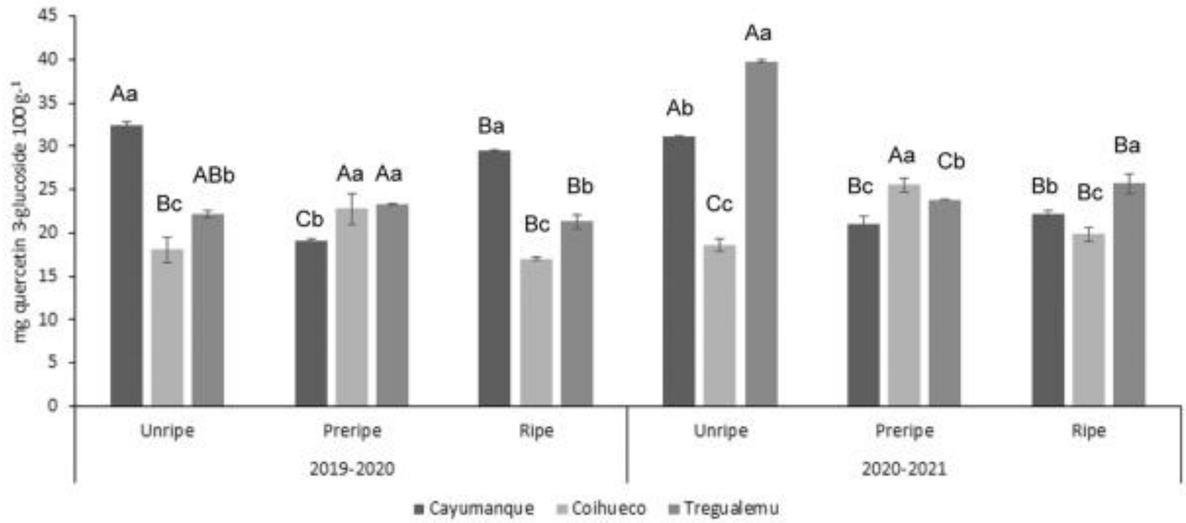
Source: Own elaboration

M: Myricetin; Q: Quercetin; K: Kaempferol



Source: Own elaboration

Figure 8. Total anthocyanins and delphinidin content in maqui grown in Cayumanque, Coihueco and Tregualemu in the 2019-2020 and 2020-2021 seasons. Different lowercase letters indicate significant differences between same stages of maturity in three different location and season ($p \leq 0.05$). Different capital letters are significant difference between the three stages of maturity of same location and season.



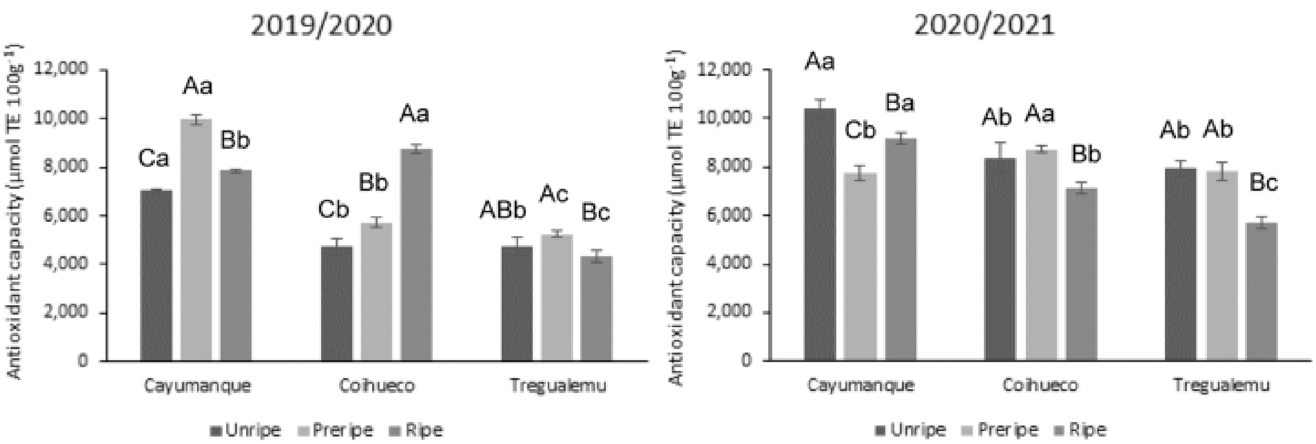
Source: Own elaboration

Figure 9. Total flavonols in maqui grown in Cayumanque, in Coihueco, Cayumanque and Tregualemu in the 2019-20 and 2020-21 seasons. Different lowercase letters indicate significant differences between same stages of maturity in three different location and season ($p \leq 0.05$). Different capital letters are significant difference between the three stages of maturity of same location and season.

3.4.3. Determination of antioxidant capacity

The antioxidant capacity determined by DPPH in the harvested fruit varied between the two seasons evaluated, with higher levels in the 2020-2021 season. An interaction between location x fruit ripening stage was observed in both seasons (Table 4). Cayumanque recorded the highest antioxidant capacity in both seasons, but not at the same fruit ripening stage (Fig. 10). In Coihueco and Tregualemu, the antioxidant capacity was similar at unripe and preripe stages in the 2019-2020 season. In the 2020-2021 season, the antioxidant capacity observed at the unripe stage was higher in Cayumanque compared to values obtained in the other two locations at the same ripening stage. Values were similar in unripe and preripe fruit grown in Coihueco and Tregualemu.

These results show that antioxidant capacity in maqui varies depending on the site (location) where plants grow (Fig. 10).



Source: Own elaboration

Figure 10. Antioxidant capacity in maqui grown in Coihueco, Cayumanque, and Tregualemu in the 2019–2020 and 2020–2021 seasons. Different lowercase letters indicate significant differences between same stages of maturity in three different location and season ($p \leq 0.05$). Different capital letters are significant difference between the three stages of maturity of same location and season.

3.5. Discussion

Content variations of bioactive compounds with the season, location and fruit ripening stage has also been described in berries, while such variations have been attributed to environmental effects (López et al., 2019; Noriega et al., 2021; Romero-Román et al., 2021). Similar variations were observed in the concentration of bioactive compounds between the locations in the present study. The higher values obtained in the 2020-2021 season could be explained by the differences observed between temperatures during fruit ripening (end of December and January) compared to the previous season. Plants synthesize bioactive compounds to protect themselves from the oxidative stress produced by environmental factors (Yoon et al., 2017;

Farías, 2019; Ferreres et al., 2009). Higher levels of polyphenols, particularly phenolic compounds including anthocyanins and flavonols, were recorded in maqui fruit grown in Cayumanque and Coihueco, where average maximum temperatures (around 30º C) were higher than those recorded in Tregualemu. In fact, the latter site recorded an average of 21º C, with a lower variation between maximum day and night temperatures (12.8° C) in the 2020-2021 season. More differences were observed in Coihueco and Cayumanque in the same season, reaching 19.8° C and 16.4° C, respectively. In this sense, a study on blueberries conducted by Spinardi et al. (2019) showed that larger differences between day and night temperatures during color break and ripening in "Duke" resulted in a higher accumulation of anthocyanins.

The synthesis of polyphenols increases when the plant is affected by environmental stress since they protect the plant due to their participation in the scavenging of reactive oxygen species. Under temperature stress, there is an increase in the activity of enzymes, such as phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS). Both are key enzymes in the flavonoid synthesis (Borges et al., 2017; Toscano et al., 2019).

The similar polyphenolic content obtained in the 2020-2021 season in the three maturity stages in Coihueco could be attributed to the rainfall during maturity. A negative correlation but with no significance between rainfall and total polyphenolic content was observed in *Ugni molinae* fruits and sour cherry fruits varieties, when in growing season increase rainfall (Alfaro et al., 2013; Viljevac et al., 2017).

Maqui fruit has a high content of total anthocyanins, even higher than other fruits (Vázquez-Espinosa et al., 2019). Environmental factors play a crucial role in the qualitative and quantitative accumulation of antioxidant compounds in many types of fruits, being temperature, solar radiation, water stress, and soil the major elements affecting anthocyanin content in fruit (Stevenson and Scalzo, 2012; Viljevac et al., 2017; Gündeşli, et al., 2019). Furthermore, altitudinal gradient can also affect the concentration of these compounds. A study on *Vaccinium angustifolium* showed that a higher accumulation of anthocyanins was induced by altitudinal gradient (White et al., 2012). This agrees with the present study since Coihueco and Cayumanque, which are located about 350 m.a.s.l., recorded higher levels of these compounds, while values obtained from berries grown in the location closer to the Pacific Ocean (Tregualemu) were lower. Others studies on water and temperature stress tolerance have described the key role of climatic conditions associated with different altitudes in the concentration of anthocyanins (Kovinich et al., 2014; Gould et al., 2018; Naing et al., 2018; Gambetta et al., 2020; Cerezo et al., 2020).

Previous studies under water and temperature stress on maqui fruit grown in the central zone of Chile have described the rapid increase in the accumulation of anthocyanins from ripening (between preripe and ripe stages). In addition, the increase in color and accumulation of sugars is directly related to the anthocyanins found in the fruit (Fredes et al., 2012; Gonzalez et al., 2015). In blueberries, a progressive increase in anthocyanins was also observed in fruits from the green to the ripe stage when anthocyanin accumulation is strongly regulated by development stage and genotype as well as environmental factors associated with altitude gradient (Spinardi et al., 2019).

Berries are rich sources of anthocyanins for pharmaceutical uses (Vázquez-Espinosa et al., 2019; Aboonabi et al., 2020). Delphinidin derivatives accounted for around 80% of the total anthocyanin content in the three locations evaluated for both seasons, which is significantly high compared to concentrations found in other berries. In blueberries, Cho et al. (2004) reported that the accumulation of delphinidins reached 40% of total anthocyanins, while a recent study recorded a much lower level of 27% (Chai et al., 2021). Furthermore, a concentration of 22% was found in *Mahonia aquifolium* berries (Coklar et al., 2021). In the present study, the highest accumulation of delphinidins was recorded in Cayumanque and Coihueco. This could be explained by the differences in solar radiation, which reached average amount values of 26.2, 27.1 and 28.3 Mj m^{-2} in Coihueco, Cayumanque and Tregualemu, respectively. In fact, high light intensity increases the accumulation of anthocyanins in fruit, while light quality affects anthocyanin content (Ma et al., 2019). A study conducted by Samkumar et al. (2021) found that red light induced a higher induction of specific genes of anthocyanins and delphinidin biosyntesis. In addition, the lower concentration of delphinidins found in the maqui berries grown in Tregualemu could be associated with postharvest conditions. There is evidence that storage, including a freezing period (initial step) before processing, results in variations in the concentration of delphinidins in blueberries (Michalska et al., 2015). In blueberry, a pasteurization of 70°C for 15 s inactivates polyphenol oxidase involved in anthocyanins degradation, indicating that temperature plays a key role in the stability of anthocyanins (Cesa et al., 2017). The maqui fruit harvested from the three locations evaluated were stored in a cooler at a temperature of 4° C while being transported from the field to the laboratory. As Tregualemu is located at a longer distance from the laboratory, transport time was longer (double) than that of the other two locations.

The total antioxidant activity was influenced by the bioactive composition in the different environments and maturity stages. Variation in antioxidant capacity in other fruits and berries were related to environmental factors and gene expression. In wild *Vaccinium myrtillus*, there was a variation in total polyphenol content and antioxidant capacity influenced by the population-year interaction where genotype could significantly affect them (Ciulca et al., 2021). Grape (*Vitis vinifera* L.) berry ripening of Cabernet Sauvignon was influenced by climate and differed depending on the location and gene expression. Some of these genes may be potentially controlled in different ways by the vinegrower to adjust final berry composition. Since in our study there was observed similar behavior, future studies should include gene expression.

The high content of anthocyanins in ripe maqui can account for the high total antioxidant activity observed in the fruit. However, the particularly high values of total antioxidants recorded in unripe y and preripe fruit collected from Cayumanque in the 2019-2020 season and from the three locations in 2020-2021 season could be explained by the presence of other phenolic compounds that promote this activity (Lee et al., 2015; Noriega et al., 2021; Subbiah et al., 2021).

3.6. Conclusions

There was an interaction between location and fruit ripening stage in all the bioactive compounds evaluated in maqui. The highest contents of polyphenols, anthocyanins and flavonols were found at the ripe stage. The antioxidant capacity varied depending on the season, location, and fruit ripening stage. These results show that environmental conditions (mainly temperature and water stress), and fruit ripening stage influence the phytochemical profile of maqui berry. In terms of total anthocyanins, the proportion of delphinidins increased in higher altitude. This confirms that maqui is the main source of this anthocyanin, being an interesting plant for pharmaceutical industries. The results obtained contribute to a better understanding of the anthocyanin profile in maqui, particularly delphinidins, providing valuable data for the supply of raw material and their quality associated to the edaphoclimatic conditions of the location where plants grow.

3.7. Acknowledgments

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IV. CONCLUSIONES GENERALES

Para el manejo de maqui en condiciones silvestres y una producción sustentable, se debe considerar la intervención de la canopia en madera del año anterior. En esta se debe procurar aumentar el ingreso de luz fotosintéticamente activa y una distribución homogénea de la misma.

Se requiere generar un protocolo para el manejo de la canopia, abordando el momento, y la intensidad de la poda o raleo en plantas de maqui silvestre, que permita una mejor intercepción y distribución de la luz aumentando la productividad y facilitando la cosecha.

Las características edafoclimáticas de la localidad, desde donde se obtienen los frutos, afecta la producción de los compuestos fenólicos (antocianinas y polifenoles totales), asociándose principalmente a condiciones de mayor temperatura, menor humedad y mayor radiación solar.

El maqui es una fuente sobresaliente de antocianinas, con un potencial de uso para la industria farmacéutica. Una mejor comprensión del perfil de antocianinas, particularmente de las delfinidinas, proporciona datos valiosos para el suministro de materia prima y su calidad asociada a las condiciones edafoclimáticas del lugar donde crecen las plantas.

Al realizar un manejo de canopia mediante poda y raleo se logra mejorar la productividad de la planta de maqui, aumentando el rendimiento de frutos en aquellas plantas con un raleo y una poda en conjunto con raleo al momento de la cosecha. La respuesta en rendimiento es dependiente de la localidad donde se encuentran los macales.