Universidad de Concepción
Dirección de Postgrado
Facultad de Agronomía
Programa de Doctorado en Ciencias de la Agronomía

Determinación de los manejos agronómicos del Calafate (*Berberis microphylla* G. Forst) en la zona centro sur de Chile.

Determination of the agronomic management of Calafate (*Berberis microphylla* G. Forst) in the south-central zone of Chile.

Tesis para optar al grado de Doctor en Ciencias de la Agronomía

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CHILLÁN-CHILE
2022

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DEDICATORIA

A la memoria de Cupertino Atalibar Pinto Cepada, mi padre, quien grabó en mi el amor por la agricultura.

AGRADECIMIENTOS

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Agradecer a Dios Creador y Padre de la humanidad.

A mi Familia, Carolina, Andrés y Daniel.

A la Universidad Adventista de Chile por el apoyo en el perfeccionamiento en este Posgrado.

A mi profesor Guía Dr. Nelson Zapata.

A mi profesor asesor externo Dr. Jorge Retamal.

A mi Directora de Posgrado Dra. María Dolores López.

A todos los docentes del Doctorado en Ciencias de la Agronomía de la Universidad de Concepción, que tuve el privilegio de conocer y aprender en las diferentes asignaturas que cursé.

A la Universidad de Concepción por darme la posibilidad de cursar este excelente doctorado.

Muchas Gracias.

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RESUMEN

El Calafate (*Berberis microphylla* G. Forst) es un frutal silvestre de hoja perenne y bayas azules que crece en forma natural en la Patagonia sudamericana. Posee características nutracéuticas beneficiosas para la salud humana, por este motivo se hace atractivo su domesticación como un frutal que pueda establecerse de manera comercial en la producción agrícola. Sin embargo, la domesticación agronómica de este berry aún no ha sido desarrollada, no se conocen con exactitud sus requerimientos edafoclimaticos y el comportamiento de la planta en huertos establecidos. Para iniciar la domesticación y posterior establecimiento comercial de esta especie, se plantearon dos objetivos, que se desarrollarón en la zona centro sur de Chile. El primer objetivo fue evaluar fecha óptima de cosecha de los diferentes clones silvestres de calafate, en relación al contenido polifenólico, capacidad antioxidante, parámetros de calidad y peso del fruto, durante dos temporadas consecutivas. Para lo cual, se seleccionaron cuatro grupos de clones de calafate silvestres ubicados en la localidad de San Ignacio, Chile. Se estableció un periodo de cosecha desde los 110 a 140 días después de plena floración (DDPF). Entre los principales resultados obtenidos, se pudo destacar que la respuesta a los diferentes parámetros evaluados tuvo un efecto diferenciador el tipo de clon evaluado y la variable fecha de cosecha. El segundo objetivo fue evaluar diferentes concentraciones crecientes de compost sobre la parámetros físico químicos y productivos del fruto de calafate cultivado. Se evaluó el efecto de los tratameintos sobre parámetros fisiológicos, productivos y de calidad en frutos de calafate, durante dos temporadas consecutivas (las temporadas 2018-2019 y 2019-2020). Se observó una respuesta a la fertilización en base a compost en el rendimiento y calidad del fruto, asi como en sus contenidos nutracéuticos, aunque no se mostraron diferencias significativas en los parámetros fisiológicos. Las dosis de compost que mejor resultado obtuvieron entre los parámetros evaluados fueron 10 y 15 Ton ha^{-1} de compost, quienes obtuvieron un rendimeinto de 726 y 924 g planta^{-1} respectivamente y una concentración polifenolica de 764 y 1062 mg de ácido gálico/100 gramos de peso fresco respectivamente.

ABSTRACT

El Calafate (*Berberis microphylla* G. Forst) is a wild evergreen fruit with blue berries that grows naturally in South American Patagonia. It has beneficial nutraceutical characteristics for human health, for this reason its domestication is attractive as a fruit tree that can be commercially established in agricultural production. However, the agronomic domestication of this berry has not yet been developed, its edaphoclimatic requirements and the behavior of the plant in established orchards are not exactly known. To initiate the domestication and subsequent commercial establishment of this species, two objectives were proposed, which were developed in the south central zone of Chile. The first objective was to evaluate the optimal harvest date of the different wild clones of calafate, in relation to the polyphenolic content, antioxidant capacity, quality parameters and fruit weight, during two consecutive seasons. For which, four groups of wild calafate clones located in the town of San Ignacio, Chile, were selected. A harvest period was established from 110 to 140 days after full bloom (DDPF). Among the main results obtained, it was possible to highlight that the response to the different parameters evaluated had a differentiating effect on the type of clone evaluated and the harvest date variable. The second objective was to evaluate different increasing concentrations of compost on the physical, chemical and productive parameters of the cultivated calafate fruit. The effect of the treatments on physiological, productive and quality parameters in calafate fruits was evaluated. for two consecutive seasons (the 2018-2019 and 2019-2020 seasons). A response to compost-based fertilization was observed in the yield and quality of the fruit, as well as in its nutraceutical content, although no significant differences were shown in the physiological parameters. The doses of compost that obtained the best results among the evaluated parameters were 10 and 15 Ton ha^{-1} of compost, who obtained a yield of 726 and 924 g plant^{-1} respectively and a polyphenolic concentration of 764 and 1062 mg of gallic acid/100 grams of fresh weight respectively. although no significant differences were shown in the physiological parameters. The doses of compost that obtained the best results among the evaluated parameters were 10 and 15 Ton ha^{-1} of compost, who obtained a yield of 726 and 924 g plant^{-1} respectively and a polyphenolic concentration of 764 and 1062 mg of gallic acid/100 grams of fresh weight respectively. although no significant differences were shown in the physiological parameters. The doses of compost that obtained the best results among the evaluated parameters were 10 and 15 Ton ha^{-1} of compost, who obtained a yield of 726 and 924 g plant^{-1} respectively and a polyphenolic concentration of 764 and 1062 mg of gallic acid/100 grams of fresh weight respectively.

I. INTRODUCCIÓN GENERAL

El Calafate (*Berberis microphylla* G. Forst), es un arbusto nativo de la Patagonia Chilena y Argentina. En Chile es posible encontrarlo desde la región Metropolitana hasta Punta Arenas (34° 59‘0" Latitud Sur a 53° 28‘33" Latitud Sur). Sin embargo, se concentra en las regiones de Aysén y Magallanes, generando en estas regiones un aumento de la demanda por productos elaborados a partir de Calafate (Pino, 2014). Actualmente, esta planta ha sido foco de estudio dada las propiedades biológicas que presenta, atribuidas principalmente al contenido de polifenoles (Arenas et al., 2013). Al comparar la capacidad antioxidante de de los frutos de *Berberis microphylla* con otros frutos como, por ejemplo, pera, naranja, manzanas, frutillas y arándanos, los frutos de *Berberis* sp. han presentado una capacidad antioxidante 10 veces mayor que las manzanas, naranjas y peras, y más de cuatro veces superior que los arándanos (Rodoni et al., 2014). Particularmente sus propiedades anti-inflamatorias pueden estar asociadas con la protección contra el daño oxidativo. Al evaluar el efecto desintoxicador de *Berberis microphylla* en metabolitos oxidados originados por el antibiótico clorofenicol en sangre humana, contrarrestó el estrés oxidativo generado por el antibiótico. Tanto los eritrocitos como los leucocitos fueron protegidos del efecto estresante, por la capacidad antioxidantes de *Berberis microphylla* (Albrecht et al., 2009). Lo anterior, es relevante al considerarlo en la dieta de niños, y pacientes en general, sometidos a clorofenicol u otras terapias causantes de estrés oxidativo para contribuir a la desintoxicación de metabolitos oxidados (Albrecht et al., 2009). Estudios recientes han detectado 18 antocianinas en los frutos de calafate con una concentración total entre 14,2 y 26,1 µmol g^{-1} de peso fresco, siendo uno de los frutos con niveles más elevados de estos polifenoles. Además, otros compuestos fenólicos y flavonoides han sido descritos para este berry, mostrando interesantes concentraciones de derivados de quercetin y epicatechin (Ruiz et al., 2010; Ruiz et al., 2013). *B. microphylla* forma parte de los pequeños frutos que ahora se consideran como fuente de nutrientes esenciales orgánicos e inorgánico y regulación metabólica, además de las propiedades nutracéuticas como alimentos funcionales, es decir, alimentos que contiene metabolitos específicos que aportan beneficios a la salud (Kuskoski et al.,2005; Arena and Curvetto, 2008).

Los estudios realizados en la especie *B. microphylla* son generados de plantaciones silvestres en la Patagonia de America de Sur, y es sabido que la brotación de hojas y floración bajo esas condiciones ocurre a mediados de primavera (Arena et al., 2003). La producción de fruto se ha visto afectada por la ubicación de los frutos en relación con los vientos predominantes y la altura de la planta y edad de la rama (Arena et al., 1999). La orientación, altura y edad de los brotes afectan significativamente la productividad de los arbustos. El número relativo más alto de brotes frutales, número de frutos y peso de frutos se encontraron en el lado norte del arbusto, seguido de este, oeste y finalmente la orientación sur. Adicionalmente el número de frutos y peso de los frutos es mayor en la mitad más alta de la planta. La edad afecta significativamente el número y peso de los frutos cosechados por brote, los brotes frutales de un año de edad eran significativamente mayores a los de 2 o 3 años (Arena et al., 2003). Un estudio comparativo de la morfología y anatomía de las hojas maduras de Calafate, creciendo en dos condiciones ambientales diferentes mostró que sus hojas cambian su morfología y estructura para adaptase a nuevas condiciones de cultivo, es decir, a temperaturas más altas y menor irradiación (Radice and Arena, 2015). Lo anterior es fundamental, debido a que las variaciones en la morfología de la hoja pueden reflejar la capacidad de la planta a adquirir, usar y conservar recursos (Radice and Arena, 2015). Por lo cual, la capacidad de la planta en tener éxito en nuevos medioambientes puede verse restringido al introducir y domesticar a esta especie a condiciones de sequía y altas radiaciones, como las que se producen en la zona central de Chile.

Las especies frutales, en general, crecen y producen mejor bajo luminosidad relativamente elevada, afectando la diferenciación de los primordios florales. Por otra parte, la luz favorece la coloración del fruto, por la formación de azúcares y pigmentos, siendo indispensable en la síntesis de antocianinas. Con relación al viento, si bien éste cumple un rol determinante como agente polinizador, tiene un efecto perjudicial, ocasionando daños mecánicos en las flores e interfiriendo en el cuajado de los frutos. Además, incide directamente sobre la acidez de los frutos, grosor y calidad (Riedemann et al., 2014), por lo cual, cultivos de Calafate en zonas de condiciones de viento atenuadas respecto a su hábitat natural, podrían no solo mejorar una mayor cuaja de frutos, sino que disminuir daños mecánicos de flores, frutos, hojas y ramas, estimulando así una mayor productividad.

En relación a la maduración de frutos en la Patagonia, el periodo de fructificación de *Berberis microphylla* es alrededor de 18 semanas, mientras que el máximo peso de fruto fresco se obtiene en la semana 12 después de la floración completa (Arena and Curveto, 2008), y según reportes realizados en la Patagonia de plantas silvestres, esta se extendería hasta inicios de febrero (Arena et al., 2003).

Muchos estudios han mostrado que los índices cualitativos y cuantitativos de berries como antocianinas y sólidos solubles son afectado por muchos factores ambientales como temperatura e intensidad luminosa (Bergqvist et al., 2001; Budic-Leto et al., 2006; Yamane et al., 2006). Adicionalmente está bien documentado que la cantidad y calidad de los frutos de calafate son afectado por la fecha de cosecha, métodos de cosecha y métodos de secado (Chandra and Todaria, 1983; Arena and Curvetto, 2008; Fallahi et al., 2011). Referente a los fenoles totales que mostraron diferencias significativas entre las localidades, estudios han mostrado que la síntesis de fenoles es favorecida con condiciones de stress y climas adversos (Mariangel et al., 2013). En este contexto, algunas funciones de los compuestos fenólicos incluyen protección de radiación. Particularmente, antocianinas totales (TA) podría proteger contra un estrés luminoso. Se ha propuesto que la TA actúa como una pantalla y previene daño genético en las células vegetales (O'Neill and Gould, 2003; Mariangel et al., 2013; Moghaddam et al., 2013), también señalan que en la especie *Berberis vulgaris*, los valores más altos en frutos frescos y secos fueron obtenidos en la última fecha de cosecha. Los sólidos solubles, pH y contenido de antocianinas aumentó, sin embargo, la acidez títulable total disminuyó al retrasar el tiempo de la cosecha, lo cual nos indica que la fecha de colecta que optimice la concentración de antocianinas es un factor a considerar en plantaciones comerciales de calafate.

Por otra parte, Arena and Curveto (2008), determinaron que en calafate creciendo en forma silvestre, la mayor concentración de antocianinas y sólidos solubles era el día 126 después de plena floración, con sólidos solubles de 24.98 Brix y concentración de antocianinas de 761.3 mg /100 g peso fruta fresca. Cuando lo frutos presentan la mayor concentración de antocianina y sólidos solubles, el total de acidez valorable estaba en el mínimo valor (2.56%) (Arena and Curvetto, 2008).

Actualmente su recolección es informal, desconociendo sus niveles de comercialización y superficies existentes. Aun así, la rentabilidad del negocio del calafate está lejos de poder establecerse con claridad, debido que hasta ahora no existe información fidedigna que permita conocer sus requerimientos técnicos a nivel de riego, fertilización y aplicaciones fitosanitarias, entre otros, ni menos sus costos de producción a nivel comercial. Por lo tanto, el objetivo de esta investigación fue determinar la fecha más optima de cosecha en un huerto silvestre en la zona centro sur de Chile y establecer los primeros ensayos de domesticación de este frutal silvestre sometiéndolo a una fertlización orgánica en dosis creciente en un huerto comercial. Que nos permita indentificar las variables que influyen de manera positiva en la optimización de un mejor efecto en el desarrollo del cultivo, compuestos fenólicos, peso del fruto y calidad del fruto de calafate.

HIPOTESIS

1. Los parametros físicos-químicos y de calidad del fruto disminuyen según el grado de madurez de la fruta de calafate recolectada.

2. El rendimiento, la calidad del fruto y la fisiología de la planta de calafate es mejorada por las distintas dosis de fertilización orgánica en un huerto comercial.

OBJETIVOS ESPECIFICOS

1. Determinar de la época de cosecha que optimice el contenido polifenólico, capacidad antioxidante y nutricional del Calafate en la zona centro sur de Chile.

2. Determinar el efecto de dosis crecientes de compost sobre tasas de crecimiento, parámetros calidad, productividad y capacidad nutracéutica del Calafate.

II. CAPITULO I: VARIATION OF PHYSICAL-CHEMICAL PARAMETERS AND PHENOLIC COMPOUNDS IN FRUITS OF FOUR CALAFATE CLONES

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Enviado a revista Agronomy el 2 de julio de 2022.

Abstract: Calafate (*Berberis microphylla* G. Forst) is an evergreen shrub with blue berries that grows naturally in South American Patagonia. It has beneficial nutraceutical characteristics for human health. The objective of the research was to evaluate the different harvest dates of calafate clones in the south-central zone of Chile that optimize the polyphenolic content, antioxidant capacity, quality parameters and fruit yield. To meet this objective during three consecutive years, four wild calafate clones located in the town of San Ignacio, Chile, were selected. Where a harvest period was established from 110 to 140 days after full flowering (DDPF), each of the harvests carried out were used for the following measurements: antioxidant capacity, determination of anthocyanin content, concentration of polyphenols, phenolic compounds, soluble solids, total titratable acidity, pH, fruit yield and quality. Among the main results, it can be highlighted that clone 2 was the one that obtained the highest concentration of soluble solids, at 140 DDPF. Together, it was the one that obtained the highest content of total polyphenols and concentration of anthocyanins, with 1121 g GAE kg^{-1} fw and 714 g cy-3-glu 100 g^{-1} fw, respectively.

Keywords: *Berberis microphylla* G. Forst; nutraceutical properties; berries; antioxidant capacity; polyphenols.

1. INTRODUCTION

Within the native species of berries in Patagonia in South America (Chile and Argentina) that stand out for their high content of polyphenolic compounds and antioxidant activity, there is a wild species called "calafate" (*Berberis microphylla* G. Forst), this is a member of the Berberidaceae family, and grows naturally as an evergreen shrub [1]. We can find it in the Maule region to Tierra del Fuego. Reaching a maximum height of 4 meters, in the south of Chile this species grows on the margins of forests and also in thickets that form in the Magellanic steppe [2]. It has been shown that the calafate fruit is a great source of anthocyanins that correspond to water soluble pigments responsible for the blue coloration of the fruit. This has a great nutraceutical potential, where a wide range of biological factors, pharmacological properties, anti inflammatory, antioxidant and chemoprotective [3]. It has been shown that an ethanolic extract of calafate root has hypoglycemic effects, capturing glucose in liver cells resistant and non-resistant to insulin (hepG2) through activation of monophosphate activated protein kinase (AMPK) [4]. It is important to point out that calafate is collected from bushes that grow wild, thus generating a high demand for it, since it is not only sought for fresh consumption, but also for the production of various products such as candies, jellies, pulps for the production of ice cream, alcoholic and non alcoholic beverages, and also cosmetic products [5], with a high unsatisfied de-mand from the pharmaceutical homeopathic industry, since there are no commercial crops evaluated in production [1,6]. Given this, it is necessary to establish agronomic parameters and the selection of clones that may have great potential as a natural and healthy source of antioxidants, for future intensive crops with high commercial value.

Currently there is a great diversity of native fruit species, which have been developed commercially, and which have outstanding levels of phenolic compounds and antioxidant capacity, such as: blackberries (*Rubus* spp.), blueberries (*Vaccinium corymbosum* L.), raspberries (*Rubus idaeus* L.), strawberries (*Fragaria x ananassa* Duchesne ex Rozier), among others, where values between 0.44 to 1.45 mg L^{-1} of gallic acid have been observed for the total content of polyphenols [7]. However, in various studies, it has been shown that calafate has an antioxidant capacity 10 times greater than other fruit species, such as apples, oranges, pears, and 4 times greater than blueberries.

According to studies carried out on wild plants, calafate could be defined as a super berry, since it could significantly exceed the concentrations of phenolic compounds and anthocyanins of most cultivated berries, as well as other species native to Patagonia in South

America, such as the murtilla (*Ugni molinae*) and the maqui (*Aristotelia chilensis* (Mol.) Stuntz.) [8]. One of the factors that would be influencing the variations of the polyphenolic content and concentration of antioxidants, is the evolution of these during the process of the ripening of fruit until the optimal moment of harvest, not clearly specifying the point that determines the optimization of both the polyphenolic and anthocyanin content, as well as the production and quality of the calafate fruit [9]. Within multiple studies, various levels of quantification of polyphenols and anthocyanins have been reported, both among species of the same genus, as well as in other berries and fruit species [10]. This is corroborated in other native species of berries, such as maqui, where variations in the development of phenolic compounds and antioxidant activity have been reported during the fruit ripening process [11]. On the other hand, Rodarte-Castrejón et al. [12] in blueberries, determined that the optimization of production together with the polyphenolic concentration, is achieved when the fruit reaches 100% blue coverage color. Quite the contrary, as reported in their research by Fredes et al. and Spinoza et al. [11,13], who point out that for the maqui, the optimization of the concentration of polyphenols and antioxidant capacity is achieved in stage three of maturation (dark blue color). On the other hand, in the case of blackberries, in addition to depending on the cultivar, they present a variety of colors during their maturation, which is due to the expression of anthocyanins depending on the pH of the culture medium. Showing its maximum color in acid media with red orange tones, on the contrary, when cultivated in neutral media it high lights the intense red violet color, and in alkaline media red, purple and blue colors are manifested. Therefore, the optimal harvest time in blackberry cannot be assigned transversally to the different culture media, but rather it must be associated with the pH of the soil in the place of establishment [14].

It is important to point out that there are no investigations on the dates or times of harvests that optimize production, polyphenolic concentration and antioxidant capacity of the calafate fruit, being necessary to carry out investigation of this species to establish a base line of the time of harvest. of the fruit, in order to optimize: the content of phenolic compounds, the antioxidant capacity, the nutritional characteristics, productivity and quality of the fruit [11,15]. For which, it is hypothesized that the evolution of nutritional and phenolic parameters will increase after fruit ripening (100% blue fruit coloration) without significantly reducing its quality and yield parameters. It is for this reason that this research aims to evaluate the effect of the different harvest dates of the calafate fruit in the south-central zone of Chile that optimize the polyphenolic content, antioxidant capacity, and quality and yield parameters of the fruit. In order to establish a baseline for future research projects on calafate domestication in the central zone of Chile and subsequent implementation of commercial orchards. To respond to the objective set forth in this study, different selected clones were approached in the central zone of Chile, where the physical chemical, productive and quality parameters of the fruit will be analyzed.

2. MATERIALS AND METHODS

2.1. Plant material, environment and extraction

During three consecutive seasons, surveys were carried out in the Ñuble region (south central zone of Chile) to identify wild clones of *Berberis mycrophylla* G. Forst, as part of the project for the identification and domestication of calafate as a commercial alternative, financed by the Adventist University of Chile. From said prospecting, 4 calafate clones (36.85º S, 71.95º W) similar in size and productive ages were selected and identified. Its taxonomic identification was carried out in the systemic botany laboratory in the Universidad de Concepción, Campus Chillán, Chile. The environmental characteristics of the locality under study were obtained from the Huemul meteorological station, of the Agricultural Research Institute, Chile [16]. The data obtained corresponded to: accumulated rainfall, maximum and minimum air temperatures, accumulation of growing degree days (DGC). The DGC, corresponded to the accumulation of the mean temperature above a base temperature (Tb, $T > 10$ °C) [17].

During the second season, at the beginning of flowering, the populations were individually identified and marked. Poor quality units with damage to twigs, buds, leaves, and flowers were discarded. The calafate fruit samples were collected in isolation from 3 individuals of each group of clones, and collecting the total of ripe fruit, always considering the cover color of 100% dark blue. Samples were collected at 10 day intervals, from 110 days after full bloom (DDPF) to 140 DDPF. The fruits were transferred in a container at 10 °C from the time of harvest, to be stored at -80 °C within the same day. It is important to point out that for each group of clones, three individuals were harvested in isolation (three replicates) for each of the established harvest dates. Next, in each of the repetitions, 100 random fruits were considered to quantify: equatorial diameter, soluble solids (SS), average fruit weight, % carbohydrates, pH and % acidity [18]. The same sample previously selected was used for the chemical analyzes of the fruit in each of the repetitions and harvest dates.

2.2. Determination of the capacity and nutraceutical content of the fruit

The DDPH antioxidant capacity was determined through the decolorization of the 1.1-Diphenyl-2-picrylhydrazyl free radical, according to methodology Pinto-Morales et al. [19]. The antioxidant capacity was expressed in μmol Trolox equivalent (TE) 100 g^{-1} fresh weight.

Total anthocyanins were determined by a differential pH technique, according to the methodology described by Pinto-Morales et al. [19], and data was expressed as mg of cyanidin 100 g^{-1} of fresh weight.

Total polyphenols were determined by colorimetry using the method of Folin Ciocalteu according to the methodology described by López et al. [20]. The results are expressed in mg of gallic acid 100 g^{-1}. All analysis and their procedures, for DDPH, anthocyanins and total polyphenols, were performed in the food chemistry laboratory in the Universidad of Concepcion, Chillán, Chile.

2.3. Yield, physical-chemical parameters and fruit quality

In each harvest, the total weight (g) of fruits per plant were counted, of which 100 fruits were randomly selected for each treatment and each repetition, and for this a Precisa precision balance was used (Precisa instruments AG, Dietikon, Switzerland). To measure the equatorial diameter (D, mm), a digital foot meter was used, with +/- 0.03 mm precision (Electronic Digital Calipter, Atraco, Inc., USA). These same fruits were used to measure soluble solids (°Brix), using a refractometer (PCE – 0.32 holding instruments., Germany). The harvest of fruits was carried out when the covering color reached 100 % dark blue of the fruit [19].

The pH was determined with an Inolab PH7110 digital pH meter. The titratable acidity was performed by manual titration, where a 0.1 N NaOH solution was used. The results were expressed as percentage of acidity (g of citric acid 100 g^{-1}) (Ec.1).

$$\text{\% acidity: } \frac{\text{Vol de NaOH x concentration NaOH x 0.064 (meq citric acid) x 100}}{\text{Weight (g sample)}} \qquad (1)$$

2.4. Experimental design and statistical analysis

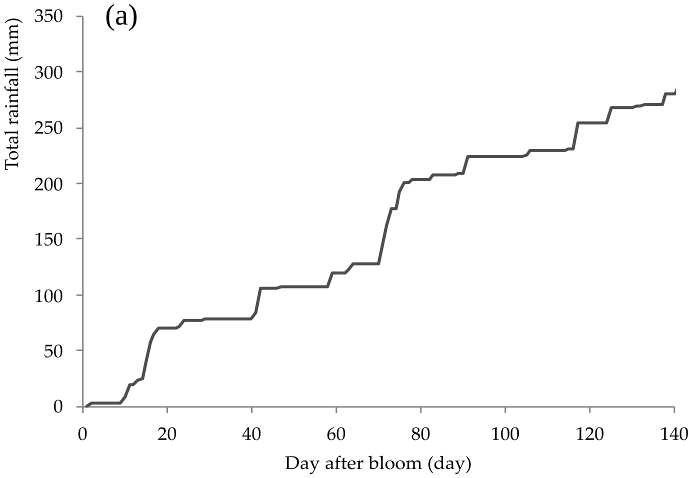
The statistical analysis was carried out using general and mixed linear models, using the INFOSTAT version 2018 software [21]. Differences between means were determined using

Fischer's comparison test ($p < 0.05$). The experimental design used was a completely randomized design, with three replications, with 3 subsamples from each experimental unit.

3. RESULTS

3.1. Environmental descriptions

In Figure 1a, it can be seen that the precipitation was mainly distributed between 20 and 140 DDPF, noting that the highest rainfall was between 20 and 80 DDPF, highlighting that between 70 and 75 DDPF accumulated rainfall was observed close to 100 mm. Resulting in a total accumulated precipitation of 321 mm in the study season, from full bloom to the end of harvest (140 DDPF). The maximum temperatures (Tmax) recorded fluctuated between 8 and 36 °C (figure 1b), where the average Tmax of the fruit development season was 20 °C. It should be noted that the minimum temperatures (Tmin) recorded fluctuated between -3 to 11 °C, observing an average Tmin close to 6 °C (figure 1b). On the other hand, in figure 1c, the accumulation of degree days during the development of the calafate fruit can be observed, highlighting that the DGC necessary from full flowering to the start of harvest (110 DDPF) was 215 DGC, jointly it can be observed that to complete the end of the harvest (140 DDPF) 418 FGDs were necessary, highlighting that half of the total FGDs were obtained between 110 and 140 DDPF (figure 1c).



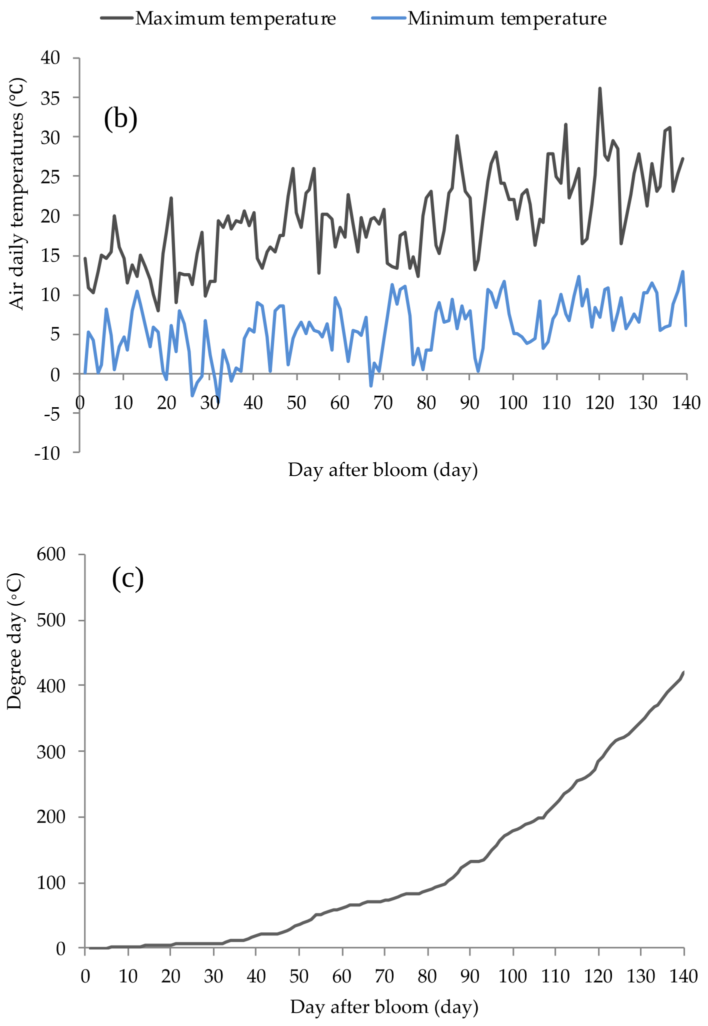


Figure 1. Environmental parameters for the San Ignacio location, Ñuble region, Chile: (a) Accumulated precipitation (mm); (b) Maximum and minimum temperatures; (c) Cumulative growing days grade (DGC). Fuente: Elaboracion propia.

3.2. Fruit quality and weight parameters

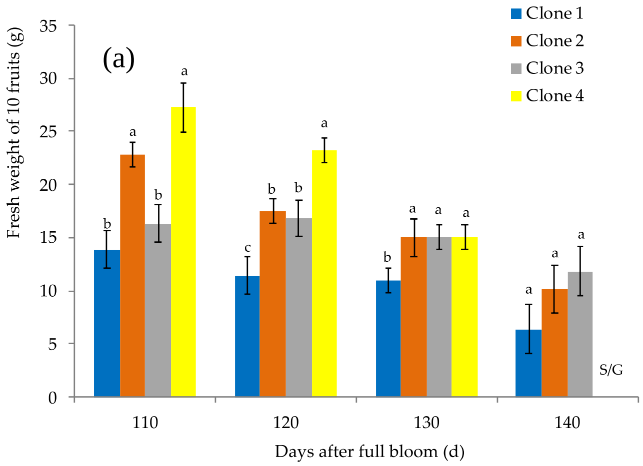
Figure 2a shows the total fresh weight of ten calafate fruits in each of the four clones and harvests carried out, highlighting that in the harvest carried out at 110 DDPF, clone 4 obtained the highest average weight of the fruits, being significantly higher than the rest of the clones for that harvest date, with an average of 2.7 g, observing that the average weight of the 10 fruits of clones 1, 2 and 3, were 1.4, 2.3 and 1.6 g, respectively ($p > 0.05$). For the 120 DDPF, the weight of the fruits for clone 4 was also significantly higher (2.3 g; $p < 0.05$) than the rest of clones 1, 2 and 3, with 1.1, 1.8 and 1.7 g, respectively, clones 2 and 3 were higher to clone 1 ($p > 0.05$). Regarding the harvest date 130 DDPF, there were no significant differences in the weight of the

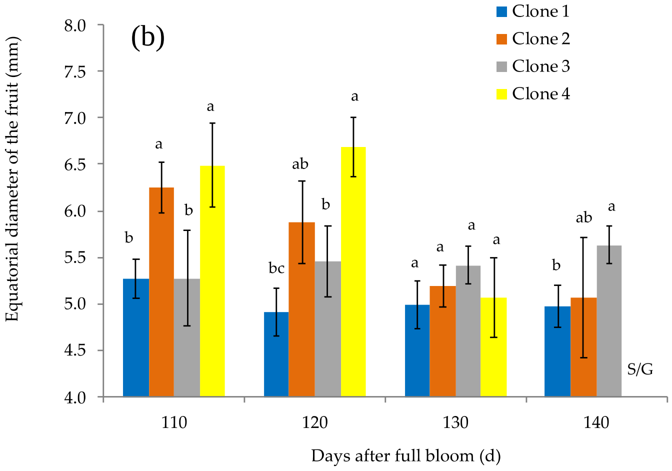
10 fruits of the different clones, being clones 2, 3 and 4 ($p > 0.05$), with values of 1.5, 1.5 and 1.5 g, and higher than clone 1 with 1.1 g ($p < 0.05$).

At 140 DDPF, the weight of the fruit was similar between clones 1, 2 and 3 ($p > 0.05$), it should be noted that clone 4 does not register values of fruit weight, since the fruit was collected in its totality at 130 DDPF. We can point out that as the harvest date progresses, the weight of the calafate fruits decreases by up to 100%, as happens to clone 1 at 140 DDPF (Figure 2a). Together, in figure 2a it can be observed that, when analyzing the behavior of each clone individually for the weight of the fruit through the different harvest dates, the trend was similar in all the clones evaluated, observing a decrease of the weight of the fruit from 110 DDPF to 140 DDPF. It was clone 3, that obtained a lower weight loss with 25%. Clones 1, 2 and 4 had a greater average weight decrease than clone 3, with 57, 56 and 45% decrease in fruit weight (figure 1a), respectively. It should be noted that, despite having a high % weight loss, clone 4 obtained the highest average weight of 10 fruits in all harvests.

Regarding the equatorial diameter of the fruit (Df), in figure 2b, it can be seen that at 110 DDPF, clones 2 and 4 were significantly higher with a Df of 6.3 and 6.5 mm, respectively, than clones 1 and 3, with a Df of 5.3 and 5.3 mm, respectively. At 120 DDPF, the Df of clone 4 was significantly higher, with 6.7 mm, compared to clones 2 and 3, which were equal to each other ($p > 0.05$), with 5.9 and 5.5 mm of Df, respectively (figure 2b). It was clone 1 that obtained the lowest Df at 120 DDPF, with 4.9 mm. With respect to 130 and 140 DDPF, no significant differences were observed between the equatorial diameters of the fruit among the different clones, with average Df values close to 5.0 mm being observed for the different harvest dates (figure 2b). It should be noted that, like the average weight of the fruit, clone 4 was the one which obtained the highest average of the equatorial diameter of the fruit, with 6.1 mm, compared to 5.0, 5.6 and 5.4 mm for clones 1, 2 and 3, respectively.

Figure 2c shows us the concentration of soluble solids (SS), where it is observed that as the fruit ripens from 110 DDPF, the concentration of soluble solids in the fruit increases up to 140 DDPF. Highlighting that clone 2 was the one that obtained the highest concentration of soluble solids towards the end of the study, reaching 38.0 °Brix ($p < 0.05$). Regarding 110 DDPF, clone 1 and 2 reached 15 °Brix ($p > 0.05$), both being higher than clone 3 and 4, whose values reached were 1.6 and 2.7 °Brix ($p < 0.05$). At 120 DDPF, the highest concentrations of soluble solids were recorded by clones 1, 2 and 3, with values close to 23 °Brix ($p > 0.05$), with clone 4 registering the lowest value with 17 °Brix. Brix ($p < 0.05$). At 130 DDPF clone 2 was significantly higher than the rest of the clones with a value of 37.5 °Brix ($p < 0.05$), followed by clone 3, 1 and 4, whose registered values were 26.8, 22.3 and 18.2 °Brix ($p < 0.05$). At 140 DDPF, clone 2 was the one that recorded the highest concentration of soluble solids with 38.0 °Brix ($p < 0.05$), followed by clone 3 and 1, whose values were 19.7 and 16.0 °Brix ($p < 0.05$) (Figure 2c), it should be noted that clone 4 at 140 DDPF does not record data, since the fruit was fully harvested at 130 DDPF.





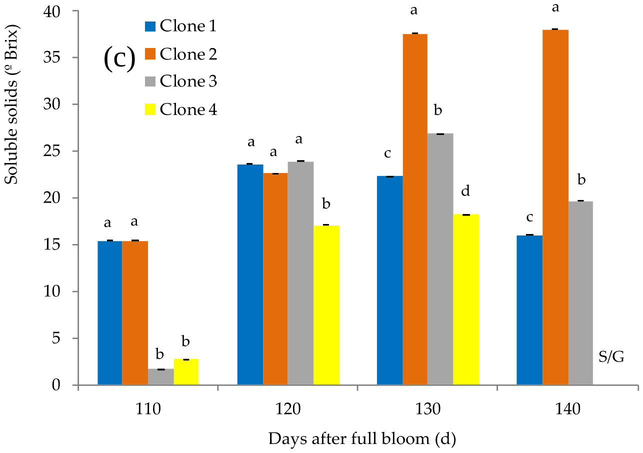
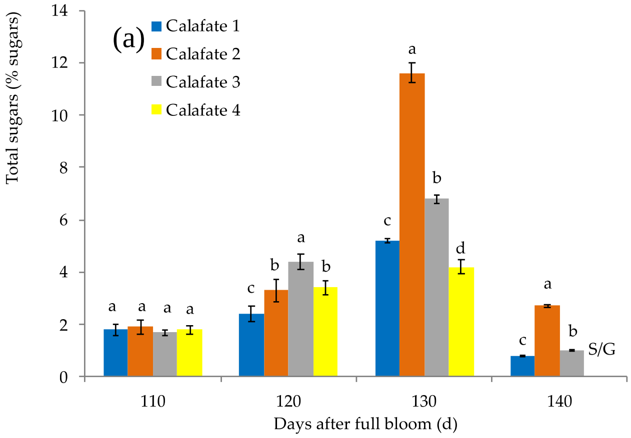
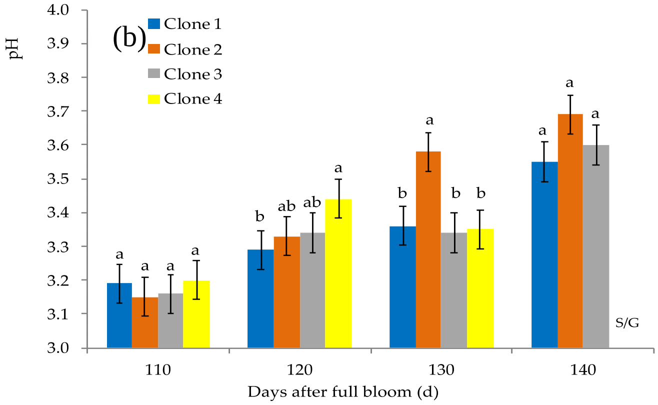


Figure 2. Fruit quality and weight parameters for four calafate clones according to different harvest dates: (a) Fresh weight; (b) equatorial diameter; (c) Soluble solids. Lowercase letters indicate significant differences in comparison between the different harvest dates for the same calafate clone, according to the Fischer test (p < 0.05). S/G = No harvest. Fuente: Elaboracion propia.

Figure 3a shows the proportion of total sugar (AT) in the calafate fruit for the different harvest dates, observing that at 110 DDPF the AT were similar to each other for the different clones evaluated (p > 0.05). This behavior registered variations at 120 DDPF, where the clone of clone 3 was significantly higher in AT (figure 3a) with 4.4%, compared to the rest of the clones, clone 1 < 2 = 4, with average values of 2.4, 3.3 and 3.4% total sugar (p < 0.05), this trend is reversed for 130 and 140 DDPF, with clone 2 having the highest concentration of AT in the fruit with 11.6 and 2.7%, for the 130 and 140 DDPF respectively. It is important to point out that, on average during the different harvest dates, clone 2 was the one that obtained the highest average concentration of TA, with an average of 4.9 % (Figure 3a). On the other hand, figure 3b shows the pH of the fruit for the different harvest dates, not observing significant differences between clones 1, 2, 3 and 4 at 110 and 140 DDPF, whose values fluctuated between 3.2 and 3.7 (Figure 3b). It should be noted that clone 4 was the one that recorded the maximum pH value of 3.7 (p < 0.05) at 120 DDPF, and clone 2 was the one that recorded the maximum pH value of 3.6 (p < 0.05) at 130 DDPF (Figure 3b). In figure 3c, the total acidity (%) of the fruit can be observed, which showed erratic variations and without well defined trends among the clones. However, clone 4 is the one that registers a downward trend in AT (Figure 3c) as the DDPFs advance (Figure 3c). Regarding the recorded AT values, these fluctuated between 1.0 and 1.6% (Figure 3c). It should be noted that there is no evidence of differences in average TA of the fruit between the different clones evaluated, with TA values fluctuating between 1.2 and 1.3%.


(a)
Calafate 1
Calafate 2
Calafate 3
Calafate 4
Total sugars (% sugars)
0
2
4
6
8
10
12
14
a a a a
c b a b
c a b d
c a b
S/G
110
120
130
140
Days after full bloom (d)


(b)
Clone 1
Clone 2
Clone 3
Clone 4
pH
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
a a a a
b ab ab a
b a b b
a a a
S/G
110
120
130
140
Days after full bloom (d)

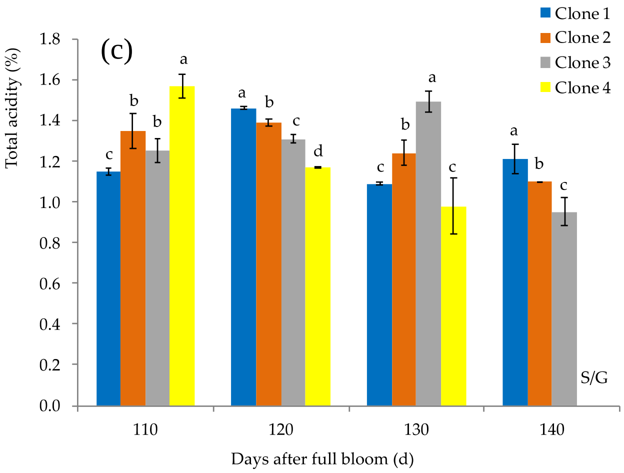

(c)
Clone 1
Clone 2
Clone 3
Clone 4
Total acidity (%)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
c b b a
a b c d
c b a c
a b c
S/G
110
120
130
140
Days after full bloom (d)

Figure 3. Fruit quality and weight parameters for four calafate clones according to different harvest dates: (a) Total sugars; (b) pH; (c) Total acidity. Lowercase letters indicate significant differences in comparison between the different harvest dates for the same calafate clone, according to the Fischer test (p < 0.05). S/G = No harvest. Fuente: Elaboracion propia.

3.3. Evolution of polyphenolic content, anthocyanin concentration and antioxidant capacity of the fruit

Figure 4 shows the concentration of total polyphenols in fruit of different clones. In general, it can be observed as the different harvest dates progress, the concentration of total polyphenols (TPC) remained relatively stable in all the clones evaluated from 110 to 130 DDPF. Emphasizing that at 140 DDPF the TPC increased in all the clones evaluated. For the 110 DDPF, clone 2 was significantly higher than the rest of the clones evaluated (Figure 4) with 898 g GAE kg^{-1} fw, clones 1 and 3 being equal to each other ($p < 0.05$) with 769 and 797 g GAE kg^{-1} fw, respectively. It was clone 4 that obtained a lower TPC at 110 DDPF, with 705 g GAE kg^{-1} fw. It should be noted that at 120 DDPF, clones 1, 2 and 4 were similar to each other ($p > 0.05$), but significantly higher than clone 3, with polyphenolic contents of 779, 741, 788 and 619 g GAE kg^{-1} fw, respectively. At 130 DDPF, the different clones 1, 2, 3 and 4 were equal to each other ($p > 0.05$), with polyphenol concentration values of 820, 923, 963 and 882 g GAE kg^{-1} fw, respectively (Figure 4). For 140 DDPF, no results were obtained for clone 4, since the fruit of the plant fully ripened at 130 DDPF, however, for clone 2, it was significantly superior to clones 3 and 1 ($p > 0.05$), with polyphenol concentration values of 1923, 1697 and 1132 g GAE kg^{-1} fw, respectively (Figure 4). It is important to point out that the highest average value of the total content of polyphenols was obtained for clone 2, with an average value of 1121 g GAE kg^{-1} fw, being the TPC for clones 1, 3 and 4, the concentrations of 875, 1019 and 792 g GAE kg^{-1} fw, respectively.

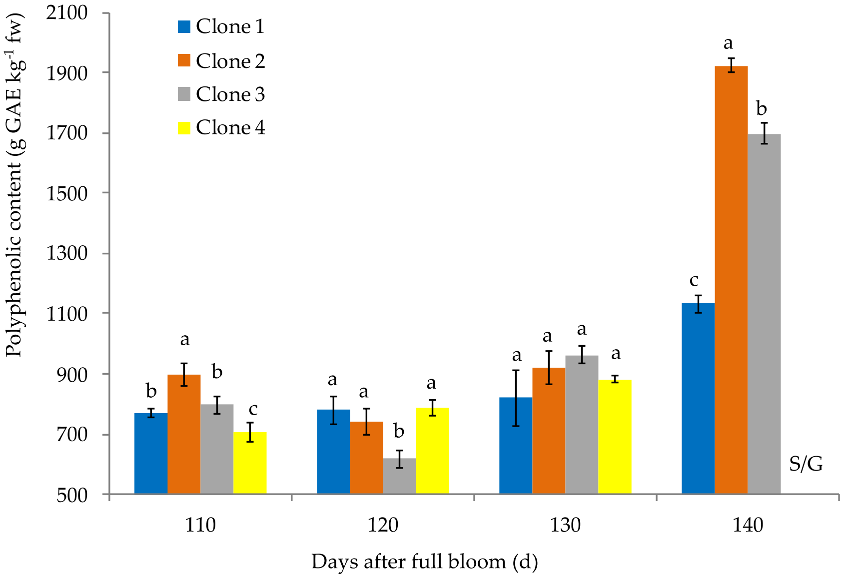


Figure 4. Total polyphenol content for the different calafate clones according to the different harvest dates. Lowercase letters indicate significant differences between the different clones for the same harvest date, according to the Fischer test ($p < 0.05$). S/G = No harvest. Fuente: Elaboracion propia.

On the other hand, in figure 5, it is possible to observe the variation of the concentration of total anthocyanins (TAC) during the different harvest dates for the different clones evaluated. At 110 DDPF it can be seen that the highest TAC corresponded to clone 4 with 394 mg cy-3-glu 100 g^{-1} fw, this was significantly higher than clones 1, 2 and 3, with values of 297, 346 and 316 g cy-3-glu 100 g^{-1} fw ($p > 0.05$), respectively. A similar trend is observed at 120 DDPF, where clone 4 with 601 mg cy-3-glu 100 g^{-1} fw was also significantly higher than the rest of the clones, where the TAC value observed in clone 1= 2 > 3, with 532, 481, 339 mg cy-3-glu 100 g^{-1} fw. It should be noted that clone 2 at 130 DDPF was the one that obtained the highest TAC ($p < 0.05$), with a value of 709 mg cy-3-glu 100 g^{-1} fw, compared to clones 3 and 4, whose values of TAC were 646 and 609 mg cy-3-glu 100 g^{-1} fw, respectively. It was clone 1, which recorded the lowest TAC value at 130 DDPF, with a value of 449 mg cy-3-glu 100 g^{-1} fw. Therefore, at 140 DDPF, clone 2 was the one that obtained the highest TAC value with 1321 mg cy-3-glu 100 g^{-1} fw, followed by clone 3, with a value of 1105 mg cy-3-glu 100 g^{-1} fw ($p < 0.05$). And finally, at 140 DDPF, clone 1 recorded the lowest TAC ($p < 0.05$) with 476 mg cy-3-glu 100 g^{-1} fw. It is important to point out that the highest average value of TAC among all the harvests of the different clones evaluated, corresponded to clone 2, with a TAC of 714 mg cy-3-glu 100 g^{-1} fw, followed by clones

3, 4 and 1, with average TAC values of 601, 535 and 439 mg cy-3-glu 100 g^{-1} fw, respectively.

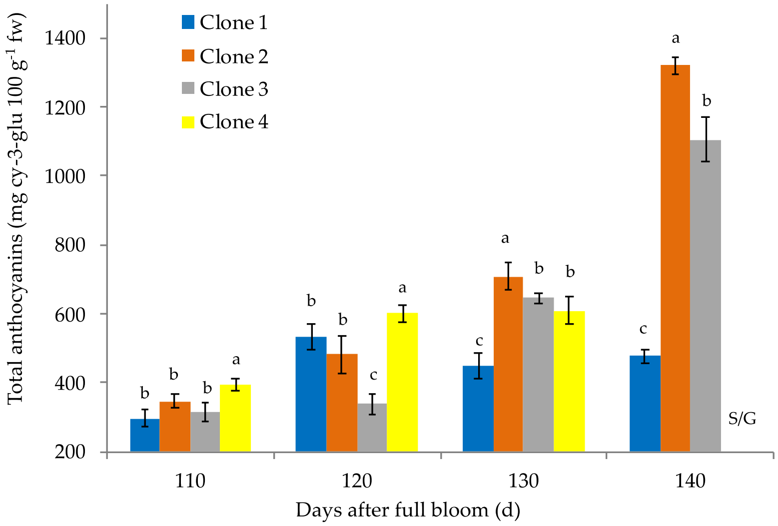


Figure 5. Concentration of total anthocyanins in the fruit for four calafate clones according to the different harvest dates. Lowercase letters indicate significant differences between the different clones for the same harvest date, according to the Fischer test ($p < 0.05$). S/G = No harvest. Fuente: Elaboracion propia.

Finally, Figure 6 shows the antioxidant capacity (AC) expressed as a percentage of inhibition. Highlighting that at 110 and 130 DDPF, all the clones evaluated registered % inhibition close to 94 and 90%, respectively, without significant differences between them within each harvest date. A similar trend was observed at 120 DDPF for clones 2, 3 and 4 ($p > 0.05$), whose values corresponded to 90, 88 and 87%, respectively, with clone 1 being significantly lower, whose inhibition value was 82%. The aforementioned trends were not observed at 140 DDPF, highlighting that only clone 1 was significantly superior to the rest of the clones, with an inhibition value of 85%, and clones 2 and 3 only reached an inhibition 72 and 71%, respectively.

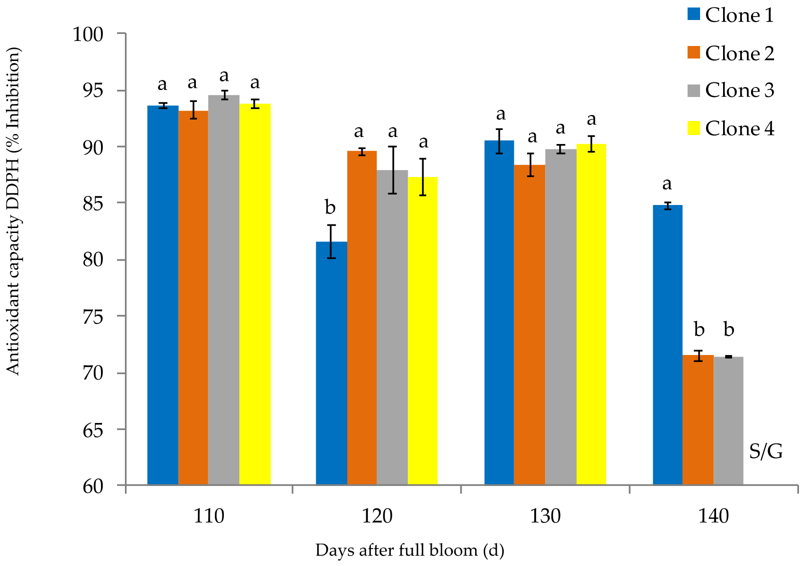


Figure 6. Antioxidant capacity (% inhibition) of the fruit for four calafate clones according to the different harvest dates. Lowercase letters indicate significant differences between the different clones for the same harvest date, according to the Fischer test ($p<0.05$). S/G = No harvest. Fuente: Elaboracion propia.

4. **DISCUSSION**

According to the environmental results observed in the Ñuble region, in general the climatic conditions of the study area varied considerably with respect to the conditions of predominant wild development in Patagonia. This factor is relevant, since it would be affecting the vegetative development of the calafate. In particular, the annual accumulated precipitation (PP) in the study site was 472 mm, well above the precipitation recorded in the same year in the locality of Punta Arenas, which reached 144 mm of annual accumulated precipitation[16]. This would be relevant, since according to Arena and Curvetto [5], for wild calafates the water needs a fluctuation for this species of between 295 to 324 mm of annual accumulated precipitation, this factor could be affecting the vegetative and productive development of the species as a product of the lower availability of the annual water resource, therefore, the domestication of this species in the Ñuble region is propitiated as a viable alternative from the point of view of water resources (Figure 1a). These results could be indicating that the optimal environmental conditions for the development of calafate in Patagonia could be affected by the effects of climate change [22], which from the point of view of PP, the Ñuble region has the characteristics beneficial for the development and domestication for this species.

It should be noted that the accumulation of thermal weather in the study locality reached 321 DGC (figure 1c) with average temperatures above 10 °C, quite the opposite of those observed at the INIA Punta Arenas meteorological station [16], where it reached 31 DGC (base 10 °C), therefore, the clones evaluated in this study should start their vegetative development before the clones from Patagonia, even being able to reach higher productivity due to environmental conditions. , such as temperatures (Figure 1b), which were less extreme throughout the development of the crop [23]. This greater accumulation of thermal time, would be given mainly by the average temperatures recorded in the town of San Ignacio (13.0 °C; Figure 1b), which recorded an average T° higher than 5 °C above the average recorded in Patagonia (8.1 °C) [24], with mean maximum and minimum temperatures for San Ignacio being 20 and 6 °C, respectively. Well below what was recorded in Patagonia for the same year, where temperatures of 11 and 3 °C were recorded, for the maximum and minimum annual average temperature (Figure 1b), respectively.

On the other hand, regarding the average weight of the 10 fruits in this study (Figure 2a), clone 4 at 110 DDPF was superior to the rest of the clones, with an average weight of 2.7 g, and the minimum weight was 1.1 g. These results are lower than other species of *Berberis* spp, where for *B. mycrophilla* the fruit weight was at least 200 % lower than *Berberis heterophylla* Juss., with an average fruit weight between 0.91 - 2.26 g [25]. On the other hand, in blueberries, the highest weight obtained per fruit, according to the research carried out by Rodríguez and Morales [26], was 1.94 g, which also shows a lower average weight of the fruit of *B. microphylla* with other species of berries. In addition to the genetic variations of each species in the potential size of the fruit, there are variations in the biochemical processes of fruit maturation, such as the rates of photosynthates and water accumulation, which could favor high differentials in the final size of the fruit among the aforementioned species [27].

Regarding the equatorial diameter of the fruits evaluated, it was observed that clone 4 is the one with the largest average diameter with a value of 6.7 mm. This result correlates with the average weight of the fruit, since higher average weights correspond to larger equatorial diameters (Figure 2a and 2b). According to the study carried out by Romero et al. [28], which consisted of comparing different wild berries that are distributed in different areas of Chile, and showed that calafate fruits from the Aysén region had a maximum fruit diameter of 10 mm. On the other hand, Dalzotto et al. [29] in the province of Río Negro made various measurements in calafate, where the resulting equatorial diameter of their samples were between 7 and 11 mm. Therefore, the results obtained in this study, although the average weights were lower, the equatorial diameters were similar to the aforementioned studies. It should be noted that the size of the fruit could be expected to be higher in our study compared to the aforementioned locations, mainly due to a higher GDC. However, what was indicated in the previous paragraph may be generated by higher total plant growth and higher total fruit yield (Data not shown).

Figure 3a shows the total content of soluble solids (SS) in the different clones evaluated, where the highest concentration of SS were observed at 130 DDPF, with clone 2 reaching the highest average SS concentration, with a 28.4 °Brix, as the DDPF advance, the color and the SS content advance hand in hand, this is due to the fact that during the ripening of the fruit there is a decrease in the content of organic acids due to respiration and an increase of sugar coming from the synthesis and sap transfer [30]. These parameters are also affected by environmental factors, mainly temperature, solar radiation, rain, shading and nitrogen levels in the soil, all of which can alter the final evolution of the fruit [31]. Similar results were observed by Arenas and Curvetto [5] and Cáceres et al. [32] where the concentrations were 24.8 and 26.8 °Brix, respectively. The case of other wild berries found in Chile is different, such as the murtilla (*Ugni molinae*), which has an average value of 13.1 °Brix, and the white strawberry with 11.6 °Brix [28]. In relation to the concentration of total sugar, clone 2 surpasses the others with an average of 4.9% [33]. In blueberries, the percentage of sugar content ranges between 10 - 14% [34]. In maqui, the values of total sugar concentration are around 41.2 g 100 g^{-1} fw [35]. The content of sugars is influenced by nutrients, plant growth regulators and physical factors that affect transport, metabolism, accumulation and the relationship between them, also maintaining a higher content of sugar in the pulp is a base indicator of maturity and taste quality, it has been described that these contribute to the quality of the fruit, in relation to weight, firmness, color and flavor in calafate [1]. It should be noted that both the concentration of soluble solids and total sugars increase once the fruit is harvested as a product of dehydration, this is due to the degradation of polysaccharides in cell membranes, which contributes to the increase in sugars [36], but as the fruit ripens, and as a result of its respiration, they begin to lose weight as a consequence of the consumption of these sugars, which were observed in our SS and AT results (Figure 2c and 3a) [37].

According to the studies of Arena [38], it was possible to demonstrate that the pH can be a parameter with variable results due to the adaptability conditions of the plant to changes such as light intensity and the level of fertilization where the results were 2.78 and 2.82 for medium and high light intensity. Cáceres observed [32] that in a commercial orchard trial, the average pH was 3.42, maintaining a close similarity with the results of this research where the average pH of wild clones was 3.4.

It is important to highlight that in the total acidity no significant differences were found between the clones, where the average of these were 1.2 and 1.3%, very similar values are found in the fruit of Untusha (*Berberis lobbiana*) where in mature state around 1.56% of total titratable acidity [39], in a study carried out in calafate by Sztarker, it showed the same value in total acidity as the Untusha fruit with values between 1.52% and 1.56% [40]. In blueberries, the total acidity is reflected in less quantity compared to the fruits mentioned above with a value of 0.89% to 1.2% [41]. On the other hand, the camarosa variety strawberry also has a lower total acidity value compared to the Berberideceae family, but very similar to the blueberry, with 0.8% total acidity [42].

In this investigation, there was evidence that the highest average polyphenolic content was for clone 2, with 1121 mg GAE 100 g^{-1} fw, these results coinciding with previous studies, where Pinto-Morales et al. [19] observed that the commercial cultivation of calafate under different organic fertilization management, the average values of polyphenolic content were 1100 mg GAE 100 g^{-1} fw. On the other hand, Speisky et al. [7], reported the polyphenolic content of 27 species in Chile, where they indicated that their average content for calafate was 1201 mg GAE 100 g^{-1} fw, which agrees with our results (Figure 4), but lower than the polyphenolic content of murta with 863 mg GAE 100 g^{-1} fw and blackberry 671 mg GAE 100 g^{-1} fw, and blueberry with 529 mg GAE 100 g^{-1} fw. On the other hand, in studies where 3 types of wild fruits of the genus *Rubus* sp., which have nutraceutical benefits like calafate, were compared, average values of polyphenolic content of 285 to 592 mg of GAE 100 g^{-1} fw were observed [43], showing that the calafate fruit has higher concentrations of total polyphenols than other species.

During the maturation of the calafate fruit, the highest concentration of total anthocyanins was reached at 140 DDPF in clone 2 with 1321 g cy-3-glu 100 g^{-1} fw. A comparable data found in the study by Arena and Curvetto [5] carried out in the city of Ushuaia, where at 126 DDPF the concentration of anthocyanins reached a value of 800 mg cy-3-glu 100 g^{-1} fw. And in our study at 120 DDPF the highest concentration corresponds to clone 4 with 601 g cy-3-glu 100 g^{-1} fw. Radice et al. [23], in the city of Moreno, Argentina, worked with plants brought from Patagonia where he carried out chemical studies and the concentration of anthocyanins was 118 mg cy-3-glu 100 g^{-1} fw, highly variable data when compared to those found in this investigation [44].

In relation to the antioxidant capacity, the highest value was obtained by clone 1 with an average 85% inhibition, surpassing the other clones in all the harvests carried out, these concentrations found, go hand in hand with the high presence of polyphenols present in calafate, significantly surpassing other fruits, such as blueberries, where the antioxidant capacity expressed in the percentage of inhibition was 13.89%, 19.82% in elderberry (*Sambucus nigra*), 25.6% in maqui and 34% in cassis (*Ribes nigrum*) in the research carried out by Busso et al.

[45]. Arenas et al. [46] in 2017, they carried out a study where they analyzed the behavior of calafate in different light irradiances where the antioxidant capacity obtained was from 56% to 66.8% under the high light treatment, whose results were lower than the results in this study (Figure 6).

5. CONCLUSIONS

According to the results obtained in this study, on the different wild calafate clones evaluated in the commune of San Ignacio, Ñuble region, Chile, the duration of the period from flowering to harvest fluctuated between 110 to 140 DDPF. To complete this period of vegetative development, the accumulation of thermal time between 215 and 418 GDC was necessary.

Clone 4 was the one that obtained the highest fruit size and weight at 110 DDPF (beginning of harvest), but its highest content of total polyphenols and antioxidant capacity was achieved at 120 DDPF, without a significant decrease in size and weight of the fruit.

Among the different clones evaluated, clone 2 was the one that obtained the highest concentration of soluble solids, at 140 DDPF. Together, it was the one which obtained the highest content of total polyphenols and concentration of anthocyanins, with 1121 mg GAE kg$_{-1}$ fw and 714 g cy-3-glu 100 g^{-1} fw, respectively. However, the antioxidant capacity drops by more than 25% at 140 DDDF. Therefore, the recommended date that optimizes the antioxidant capacity, together with the polyphenolic content and concentration of anthocyanins, is at 120 DDPF.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, J.R-S. and F.P-M.; methodology, J.R-S. and R.V-R.; software, F.P-M.; validation, M.D.L., N.Z. and J.R-S.; formal analysis, R.V-R., D.P., F.P-M. and J.R-S.; investigation, F.P-M., J.R-S., D.P. and R.V-R.; resources, J.R-S- and F.P-M.; data curation, D.P. and F.P-M.; writing—original draft preparation, F.P-M. and D.P.; writing—review and editing, M.D.L., N.Z. and J.R-S.; visualization, R.V-R. and F.P-M; supervision, N.Z., M.D.L. and J.R-S.; project administration, R.V-R. and J.R-S.; funding acquisition, J.R-S. and F.P-M. All authors have read and agreed to the published version of the manuscript.”.

Funding: The present work benefited from the project of research Nº 97 and 99, granted by the Universidad Adventista de Chile, Chile.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the students of Universidad Adventista de Chile, SebastianCamilo Ospino, Natalia Cáceres, Felipe Quintana and Geber Adaos, for actively participate in data collection.

Conflicts of Interest: The authors declare no conflict of interest.

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III. CAPITULO II: THE USE OF COMPOST INCREASES BIOACTIVE COMPOUNDS AND FRUIT YIELD IN CALAFATE GROWN IN CENTRAL SOUTH OF CHILE

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Publicado en revista Agriculture 2022, doi:10.3390/agriculture12010098

ABSTRACT

Different concentrations of compost as organic fertilizer can modify productive, quality, and chemical parameters in several fruit tree species. The objective of the study was to determine the effect of increasing applications of compost on physiological, productive, and quality parameters in the Calafete fruit during the seasons of 2018-2019 and 2019-2020. The study was conducted on a commercial Calafate orchard using a randomized complete block design with 4 treatments (CK: With no compost application, T1: 5 Ton ha^{-1}, T2: 10 Ton ha^{-1} and T3: 15 Ton ha^{-1}), each with 4 repetitions. The results did not show statistical significance for stomatal conductance (Gs), PSII quantum yield, or photosynthetic active radiation (PAR) within treatments. As for fruit yield, statistical difference was found between control treatment and T1, which were lower than T2 and T3 in both seasons. The trees reached a higher leaf area index with T2 in both seasons. The highest antioxidant capacity was obtained with T3 and T2 for first and second season respectively. Polyphenols and total anthocyanins production showed statistical significance, with a higher content at the second season with T2. It is concluded that the dose under which yield, quality, and nutraceutical content of Calafate fruit are optimized is the one used in T2, 10Ton ha^{-1}.

Key words: Polyphenols; Berberis; Negative Fruits, Organic Agriculture.

1. INTRODUCTION

Calafate (*Berberis microphylla* G. Forst), is a bush native of the Chilean and Argentine Patagonia. In Chile it can be found from the Metropolitan Region to Punta Arenas (34° 59‘0" South to 53° 28‘33" South). However, its existence is concentrated in the Aisén and Magallanes regions. In these regions it has been observed an increasing demand for products made from calafate [1,2]. Currently, this plant is being subject of study due to its biological properties, attributed mainly to the content of polyphenols present in it [3]. The antioxidant capacity of *B. microphylla* compared with other species, has shown to be up to 10 times more than apples, oranges and peers, and more than 4 times higher than blueberries [4]. Different studies have detected 18 anthocyanins in the calafate fruit, with a total concentration between 14.2 and 26 μmol g^{-1} of fresh weight [5,6]. These polyphenols (PF) substantially reduce the presence of degenerative, cardiovascular, carcinogenic diseases, among others [7]. This, because they have properties that are believed to be associated with protection against cellular oxidative damage, therefore, they are beneficial compounds for human health [8].

Most studies of B. microphylla have been developed in the so-called Austral Zone of Chile, and all of them using wild calafate, which vegetative growth takes place in midspring [9].

Furthermore, a comparative study about morphology and anatomy of mature leaves of calafate, growing under two different conditions, showed that leaves change their morphology and structure to adapt to new environmental conditions, in this case, to higher temperatures and less radiation [10]. This makes it important to analyze behavior and/or adaptation of *B. microphylla* focusing on both, physiology and phenology since they were introduced to agroclimatic zones different from its natural habitat. Multiple studies have described that a change on PAR radiation availability can be a limiting factor on crops yield, as result of a reduction on photosynthesis rate, which could also lead to negative effects of the quality of the product [11,12].

In citruses and some berries, it has been demonstrated that photosynthetic capacity can be enhanced by decreasing PAR, reducing photo inhibition caused by excess of radiation, consequently increasing the photochemical efficiency of photosystem II [13,14]. Thus, a question that the present research addresses is: Could the introduction of *B. microphylla* into an intensive commercial environment, different from the original, generate changes on productive and quality parameters of the fruit? [15].

Currently, there is an unsatisfied demand for this fruit that wild species have not been able to achieve [16], generated by educated consumers and eager for a more natural and nutritious alimentation to have a healthier life. This has increased safe and environmentally friendly food production [17]. Because of the last, it would be interesting to introduce and domesticate healthy species like calafate, with proper agronomic management [2].

Calafate is a species capable of growing under several environments [5], however, no agronomic research as been conducted under intensive commercial conditions that allow to optimize productivity and polyphenolic content in the fruit [10]. There is also no studies on *B. microphylla* aiming to evaluate yield response to different fertilization doses based on organic sources [10]. Despite the fact that compost application is a widely used technique of organic fertilization to use bio residues, its implementation in developing countries has not been studied [18]. However, it is known that compost usage has beneficial effects on both quality and soil fertility, but also on the environment [19].

Compost, as organic fertilizer has shown in other species to enhance fruit quality, as pointed out by Vásquez and Maravi [20], where applications of 10 Ton ha^{-1} of compost in *Morus alba* L. significantly increased yield and quality of the fruit. Similarly, in a study on strawberries (*Fragaria* × *ananassa* Duch) cvs. Allstar y Honeoye in grown in pots with an organic fertilization (0%, 50% and 100% compost), the concentration of anthocyanins, phenolic content and antioxidant capacity of the fruit, increased with the increasing compost doses [21].

Furthermore, in Macadamia (*Macadamia integrifolia*), compost application to the soil increased its total cationic exchange capacity, organic matter, potassium (K), Calcium (Ca), Magnesium (Mg), among other micronutrients [22]. Compost also modifies physical properties in the soil, such as: total porosity and water retention capacity. The last has also been observed on studies on wine grapes (*Vitis vinifera* cv. Chardonnay) improving nitrogen mineralization and its availability to the studied crop [23]. Also, on a research conducted for 21 years of organic applications to eroded soils, nutritionally deficient and with low pH, it significantly increased: soil pH, organic carbon content, total nitrogen, phosphorus, potassium, available nitrogen and biological activity [24].

According to the stated above, the objective of this research was to determine the effect of different doses of compost on productive and physiologic parameters, including polyphenolic composition and antioxidant activity of the fruit of calafate, grown under an intensive agronomic management in the central zone of Chile.

2. MATERIALS AND METHODS

2.1. General Characteristics of the site of study and orchard establishment

The The study was conducted at Universidad Adventista de Chile (UnACh), located in Kilometer 12 on the way to Tanilvo, province of Chillán, Region of Ñuble (36º31'S; 71º54'W), Chile. The site of study has a volcanic soil (Melanoxerand) (Stolpe, 2006), temperate Mediterranean climate, hot and dry summers, cold and humid winters, with an annual precipitation of 815 mm, concentrated in Winter and beginning of spring [25]. The study was conducted in a commercial calafate orchard established in august of 2017, using two-year old plants with an average height of 70 cm. The plants were multiplied from seeds in 2015. The plant population density was 1 m on row and 3 m between row, planted on berms 1 m. wide and 20 cm high. After establishing the orchard, the soil was physically and chemically characterized at a depth of 0 to 40 cm, where most part of the roots are found [9] (Table 1). These analyzes were carried out by the chemistry and physics of soils of the Agricultural Research Institute of Chile (INIA Quilamapu, Chillán, Chile).

Table 1. Physical-chemical analysis of the soil before treatments.

Analysis	Unit	Result
Organic Matter	%	9,70
Water pH		6,40
Nitrogen availability	mg kg^{-1}	19,00
Phosohorus availability	mg kg^{-1}	15,30
Potassium availability	mg kg^{-1}	496,00
Sulfur availability	mg kg^{-1}	24,00
Exchangeable calcium	cmol+ kg^{-1}	8,70
Exchangeable magnesium	cmol+ kg^{-1}	1,60

Exchangeable potassium	cmol+ kg^{-1}	1,30
Exchangeable sodium	cmol+ kg^{-1}	0,01
Sum of bases	cmol+ kg^{-1}	11,60
Interch. aluminum	cmol+ kg^{-1}	0,02
CIC	cmol+ kg^{-1}	11,59
Aluminum saturation	%	0,14
Boron	mg kg^{-1}	0,40
Copper	mg kg^{-1}	1,63
Zinc	mg kg^{-1}	0,90
Iron	mg kg^{-1}	44,00
Manganese	mg kg^{-1}	3,02

[1] Samples were obtained at the beginning of the study in august of 2017, 0-40 cm depth. Fuente: Elaboracion propia.

At establishment, a base fertilization was applied into the plantation hole, of 150 g of urea (45% N), 200 g of triple superphosphate (46% P_2O_5) and 200 g of Potassium sulfate (50 % K_2O) [19]. Also, hydraulic replenishment was standardized for all treatments, and estimated according to the daily potential evapotranspiration of the crop (ETCc), using the methodology suggested by Romero *et al*. [26]. This, with the objective of maintaining optimums humidity levels in the soil during the entire development of the crop. Weed control was also standardized for all treatments and consisted in the manual elimination of them when establishing the orchard, plus three times a year equally distributed according to the annual cycle of the crop. In parallel, the same phytosanitary management was applied to all treatments, which consisted in six annual applications during the growing season, alternating two active ingredients, which were Tebuconazole (Orius 43 SC), at a concentration of 25.8 grams per hectoliters, and cuprous oxide (Cuprodul WG) at a concentration of 180 grams per hectoliter of active ingredient.

The experimental design used for the study was a randomized complete block design with a total of 4 treatments of compost doses, with 4 repetitions per treatment. Each treatment and repetition consisted on 4 plants, in which only the 2 central plants were evaluated. Additionally, there were border rows to help diminish border effect. Compost treatments consisted in: 1) Control treatment (CK), with no compost application, 2) 5 tons per hectare (Ton ha^{-1}) of compost (T1), 3) 10 Ton ha^{-1} of compost (T2) and 4) 15 Ton ha^{-1} of compost (T3). All treatments were applied each year in august, to each experimental unit, doing the first application at plantation.

The compost used in the study was commercially produced by the composting and recycling center of the Universidad Adventista of Chile, which was elaborated from chicken manure, produces at the same institution, and oat bales. The manure and bales were mixed at a rate of 3:1 (Vol/Vol). The elaboration process lasted 5 months, controlling during that time temperature, humidity and ventilation [27–29]. The Physical-chemical charactarization of the compost used for this study, is detailed in Table 2.

Table 2. Physical-chemical análisis of compost used for this study.

Análisis	**Unidad**	**Resultado**
Humidity (dry basis)	%	22,20
Apparent density (dry basis <16 mm)	Kg m^{-3}	NS^{1*}
Porosity (sample <16 mm)	mg kg^{-1}	NS^{1*}
pH in water 1:5		7,41
Electric conductivity 1:5	dS/m	0,19
Organic matter	%	21,60
Organic carbon	%	12,00
Total nitrogen	%	0,87
Nitrogen-ammonia (N-NH_4)	mg kg^{-1}	0,50

Nitrogen- Nitric (N-NO_3)	mg kg^{-1}	59,64
Carbon / Nitrogen Ratio	--	13,78
Ammonium / Nitrate Ratio	--	0,008

* Undetermined. Fuente: Elaboracion propia.

2.1. Characterization of physiologic and environmental conditions of the plant

2.1.1. PAR radiation and leaf area index

For the purposes of environmental records, the photosynthetically active photon density (PPFD, µmol m^{-2} s^{-1}) was quantified at five times of the day: 09:00, 11:00, 13:00, 15:00 and 17:00 h, in ambient conditions of a completely sunny day. The radiation parameters correspond to: direct, diffuse, residual and reflected photosynthetically active radiation, soil and plant throughout the development of the crop, and with these parameters the intercepted PAR was estimated. For this, an AccuPAR LP-80 ceptometer (Decagon Devices Inc., Washington, USA) was used, which delivers the average of 80 quantum sensors. The readings of the leaf area index (IAF) were made at noon, and were measured in post-harvest (January), when the growth of the plant had already stopped, with the same instrument and in parallel to the PAR radiation measurements. [30].

2.1.2. Chlorophyll fluorescence and stomatal conductance

The maximum intensity of fluorescence (Fm) was measured, as well as the minimum intensity of fluorescence (Fo) of chlorophyll, this by using a portable fluorimeter model OS-5p (Opti-Sciences, USA) during a clear day, at five times of the day respectively: 09:00, 11:00, 13:00, 15:00 and 17:00 [31].

Both Fo and Fm were determined after a period of 30 minutes in which the leaves were adapted to darkness. [31,32], For this, foliar clips that included a mobile obturation plate were used. With these parameters, the maximum photochemical efficiency of photosystem II (Fv / Fm) was quantified, using the following relationship proposed by Kooten and Snell and Maxwell and Johnson [33,34]: $Fv/Fm= (Fm-F0)/Fm$.

At the same time, stomatal conductance measurements (Gs, mmol m^{-2} s^{-1}) were performed, for this a portable porometer model SC^{-1} (Decagon Devices INC, Washington, USA) was used. The Gs measurements were performed on fully illuminated leaves of the same plant, shoots, location and frequencies used in the chlorophyll fluorescence measurements. Using the same equipment and the same frequencies as for Fv / Fm, a record of the leaf temperature (Tf; ° C) was kept. In order for the data collected to be representative, these were taken on leaves exposed to the sun and in the second third of a branch of the season. [14].

2.2. Yield and chemical parameters of the fruit

2.2.1. Calafate fruit productivity

The harvest was carried out 130 days after full flower, for both study seasons. The harvest was done manually, in which process the total weight (g) of fruits per plant was quantified.

2.2.2. Determination of total polyphenol concentration

Total polyphenols were determined by colorimetry using the method of Folin Ciocalteu, in the food chemistry laboratory of the Universidad de Concepcion, Chillán, Chile. To calculate the polyphenol content, a calibration curve with Gallic Acid was used, with concentrations between 0 to 1000 mg L^{-1} of gallic acid according to the methodology proposed by Yildirim et al. [35]. The results are expressed in mg of Gallic Acid 100 g^{-1} [10].

2.2.3. Determination of anthocyanin content

Total anthocyanins were determined by a differential pH technique. The determination of the anthocyanin content is based on the Lambert-Beer Law ($A = \varepsilon * C * L$), where A corresponds to the absorbance that is measured with a spectrophotometer, ε corresponds to the molar absorbance, a constant physics for molecular species in a solvent at a given wavelength; C is the molar concentration; and L the length of the route, expressed in cm. Molar absorbance values for purified pigments were obtained from the literature. The concentration in mg L^{-1} was determined multiplying by the molecular weight (MW) of the pigment. To calculate the anthocyanin content, the molecular weight and molar absorbance of the anthocyanin pigment present in the highest proportion were used. [5]. The calculation of the anthocyanin concentration was carried out with the equation shown below, the data were expressed as mg of Cyanidin 100 g^{-1} of fresh weight:

$$\frac{A \times 1000 \times 449{,}2}{26900} \times \frac{3000}{100} \times \frac{5}{1000 \times g\ sample} \times 100 \qquad (3)$$

2.2.4. Determination of antioxidant capacity

The DDPH antioxidant capacity was determined through the decolorization of the 1,1-Diphenyl-2-picrylhydrazyl free radical, proposed by Brand-Williams et al [36]. The DPPH radical is reduced in the presence of antioxidants, manifesting a color change in the solution over time. To quantify the inhibition, a calibration curve was elaborated using the TROLOX reagent in methanol achieving concentrations of 25, 50, 75, 100, 150, 200, 250, 300, 350 and 400 ppm. A methanol solution was used as a blank coonand all solutions were incubated in the dark for 30 min, their absorbance being measured in the spectrophotometer at 515 nm after 60 minutes. The antioxidant capacity was expressed in μmol Trolox equivalent (TE) 100 g^{-1} fresh weight.

2.3. Statistical analysis

The effect of the treatments was estimated by ANOVA, and the Fischer LSD test, with a level of statistical significance of 0.05; and for this, the INFOSTAT software was used (Infostat, Cordoba, Argentina, 2015).

3. RESULTS

3.1. Edaphoclimatic and physiological parameters of calafate

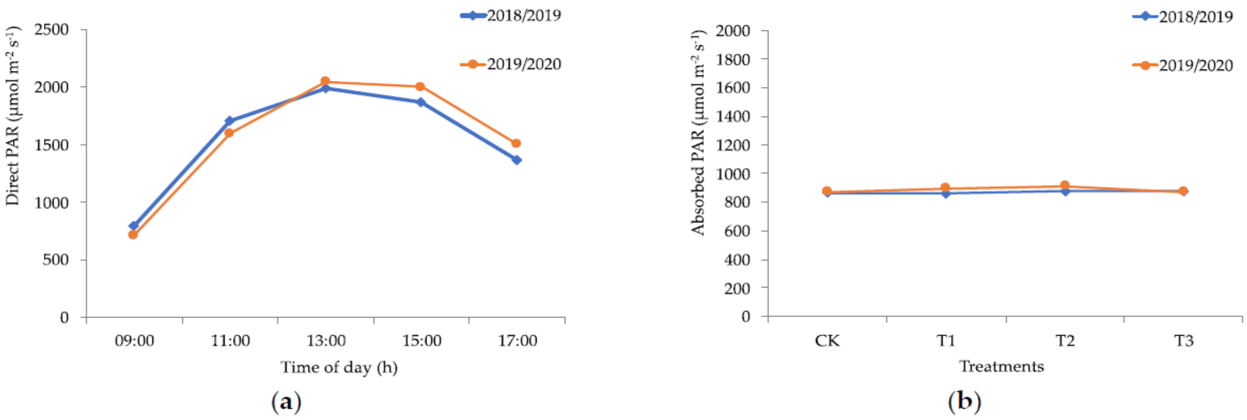
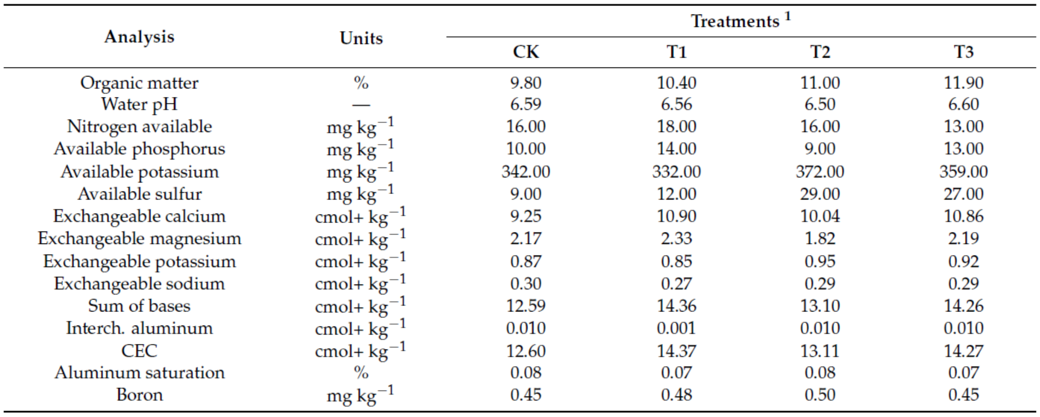


Figure 1. Average Photosynthetically Active Radiation (PAR); a) direct PAR (µmol m^{-2} s^{-1}) and b) absorbed PAR (µmol m^{-2} s^{-1}) for the different treatments, for the 2018-2019 and 2019-2020. 2019–2020. For Figure 1b: according to Fischer's LSD test ($p < 0.05$), there are no significant differences between treatments; the experimental error was very small, so the error bars were not observed. Fuente: Elaboracion propia.

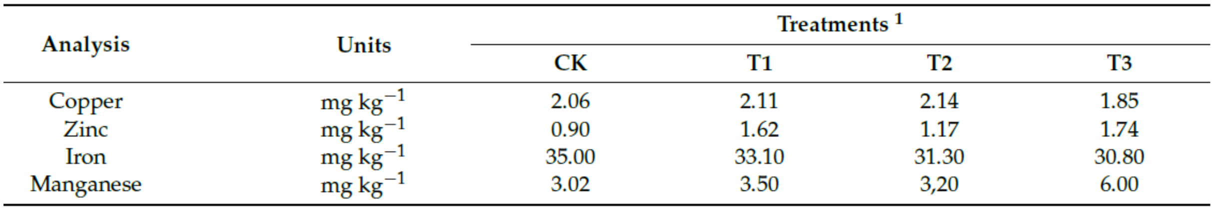
In Figure 1a, the average of direct photosynthetically active radiation (µmol m^{-2} s^{-1}) can be observed in the 2018-2019 and 2019-2020 seasons, quantified at five times of the day: 09:00, 11:00, 1:00 p.m., 3:00 p.m. and 5:00 p.m. Presenting the lowest PAR values at 09:00 h for both seasons, with values close to 750 µmol m^{-2} s^{-1}. In both seasons, the same trend of increasing PAR was observed from the first hours of the day, until reaching the maximum values, close to 2000 µmol m^{-2} s^{-1}. To reach the end of the day, with values close to 1500 µmol m^{-2} s^{-1}, in both seasons (Figure 1a). Together, in figure 1b, the absorbed photosynthetically active radiation is observed, which did not show significant differences between the different treatments for each of the seasons under evaluation, registering similar values ($p < 0.05$) in both seasons, which they were on average 866, 878, 893 and 873 µmol m^{-2} s^{-1}, for CK, T1, T2 and T3, respectively.

Table 3. Physical-chemical analysis of the soil 2019/2020 season, at the end of the study.



Analysis	Units	Treatments [1]			
		CK	T1	T2	T3
Organic matter	%	9.80	10.40	11.00	11.90
Water pH	—	6.59	6.56	6.50	6.60
Nitrogen available	mg kg^{-1}	16.00	18.00	16.00	13.00
Available phosphorus	mg kg^{-1}	10.00	14.00	9.00	13.00
Available potassium	mg kg^{-1}	342.00	332.00	372.00	359.00
Available sulfur	mg kg^{-1}	9.00	12.00	29.00	27.00
Exchangeable calcium	cmol+ kg^{-1}	9.25	10.90	10.04	10.86
Exchangeable magnesium	cmol+ kg^{-1}	2.17	2.33	1.82	2.19
Exchangeable potassium	cmol+ kg^{-1}	0.87	0.85	0.95	0.92
Exchangeable sodium	cmol+ kg^{-1}	0.30	0.27	0.29	0.29
Sum of bases	cmol+ kg^{-1}	12.59	14.36	13.10	14.26
Interch. aluminum	cmol+ kg^{-1}	0.010	0.001	0.010	0.010
CEC	cmol+ kg^{-1}	12.60	14.37	13.11	14.27
Aluminum saturation	%	0.08	0.07	0.08	0.07
Boron	mg kg^{-1}	0.45	0.48	0.50	0.45

Table 3. Cont.



Analysis	Units	Treatments [1]			
		CK	T1	T2	T3
Copper	mg kg^{-1}	2.06	2.11	2.14	1.85
Zinc	mg kg^{-1}	0.90	1.62	1.17	1.74
Iron	mg kg^{-1}	35.00	33.10	31.30	30.80
Manganese	mg kg^{-1}	3.02	3.50	3,20	6.00

[1] CK = Control Tratment (without compost application); T1 = Treatment 1 (5 Ton ha^{-1}); T2 = Treatment 2 (10 Ton ha^{-1}); T3 = Treatment 3 (15 Ton ha^{-1}). CEC = cation exchange capacity of soil; all samples were obtained at the end of the

study in August 2020 at a depth between 0–40 cm. All samples were obtained at the end of the study in August 2020, at a depth between 0-40 cm. Fuente: Elaboracion propia.

Table 3 shows the variations in the physicochemical parameters of the soil at the end of the study, for the different compost treatments. It should be noted that the percentage of organic matter increased in the different treatments as the volume of compost applied increased, being CK <T1 <T2 <T3, with values of 9.8%; 10.40%; 11.00% and 11.90%, for each of the treatments respectively. For the pH parameter, no significant modifications were observed between the different treatments towards the end of the study, corresponding to 6.59, 6.56, 6.50 and 6.60 for CK, T1, T2 and T3 respectively. It should be noted that the nutritional levels of the soil tended to improve with increases in the dose of compost applied, mainly in the case of P, K, Ca, S, Mg (Table 3). Together, the cation exchange capacity increased close to 12% in the treatments with the application of compost (Table 3). On the contrary, the concentration of iron and manganese decreased, by 12 and 89%, respectively.

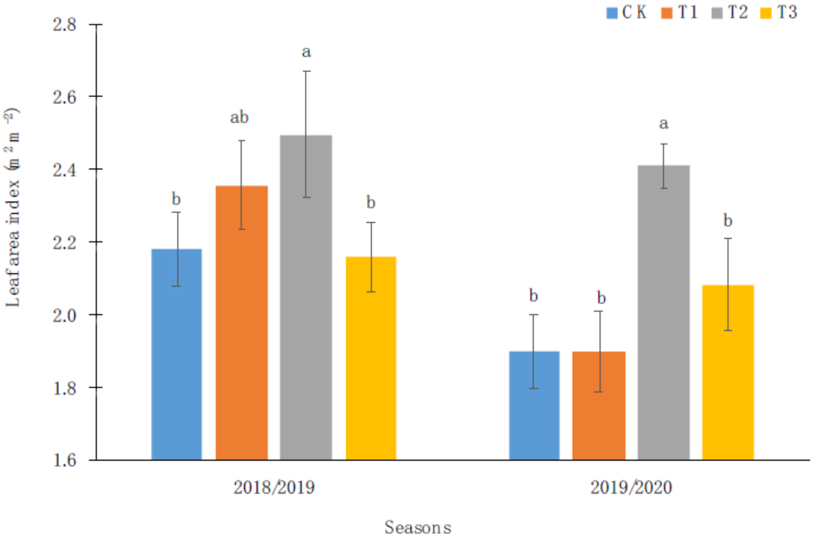
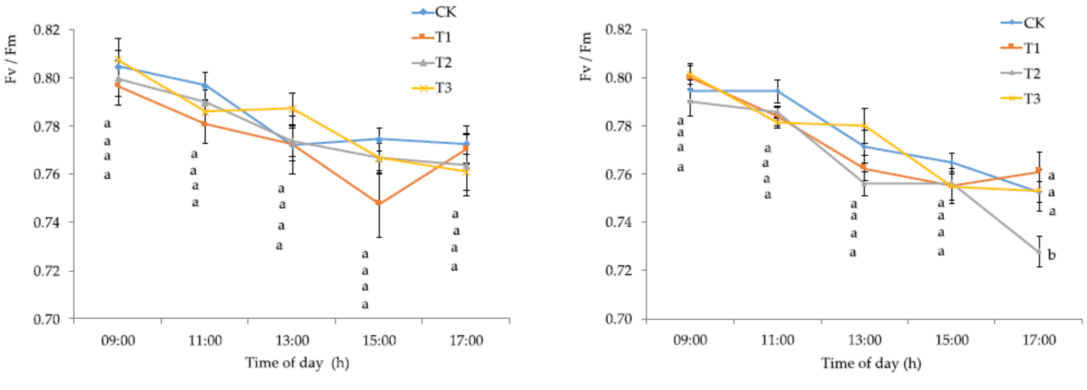


Figure 2. Effect on the fruit leaf area index (LAI) of Calafate (*Berberis mycrophilla* G. Forst), for the different compost treatments, for the 2018-2019 and 2019-2020 seasons. For each figure: CK = Control treatment (without compost application); T1 = Treatment 1 (5 Ton ha^{-1}); T2 = Treatment 2 (10 Ton ha^{-1}); T3 = Treatment 3 (15 Ton ha^{-1}). For each season, different lowercase letters indicate significant differences for the leaf area index between the different treatments, according to the LSD-Fischer test ($P < 0.05$). The bars correspond to the experimental error of each treatment. Fuente: Elaboracion propia.

In figure 2, the results obtained for the leaf area index parameter can be observed. In the first season, the treatment that registered the highest LAI value was T3 (IAF: 2.5), significantly higher than CK and T1; and without significant differences with T1 (Figure 2). It should be noted that for the second season the same trend as the first season was observed, with T2 showing the highest LAI value (2.41; $P < 0.05$) compared to treatments CK, T1 and T3, which did now show statistical significance ($P > 0.05$), 1.9, 1.9 and 2.08 LAI, respectively.



(a) (b)

Figure 3. Variation of the maximum quantum yield of photosystem II (Fv / Fm) in calafate plants *(Berberis mycrophilla* G. Forst), for the different Compost treatments, evaluated at different times of the day, 09:00, 11:00, 13:00 , 3:00 p.m. and 5:00 p.m., corresponding to the figure: (a) Season 2018-2019; (b) Season 2019-2020. For each figure: CK = Control Treatment (without compost application); T1 = Treatment 1 (5 Ton ha^{-1}); T3 = Treatment 2 (10 Ton ha^{-1}); T3 = Treatment 3 (15 Ton ha^{-1}). For each hour of the day, different lowercase letters indicate significant differences between each of the treatments, according to Fischer's LSD test ($P < 0.05$). The bars correspond to the experimental error of each treatment. Fuente: Elaboracion propia.

The results corresponding to the maximum quantum yield of photosystem II (Fv / Fm), can be observed in figure 3a and 3b, for the 2018/2019 and 2019/2020 seasons, respectively. Emphasizing that, in the first season, all treatments presented similar values at the beginning of the day ($P < 0.05$), close to 0.8. Similar results were observed at 11:00, 13:00, 15:00 and 17:00, where the average values recorded for Fv / Fm were 0.79, 0.78, 0.76 and 0.77 respectively. It should be noted that all the treatments were decreasing the Fv / Fm values as the day passed (Figure 3a), without significant differences between the treatments ($P < 0.05$). For the second season, the same trend and values recorded in the first season were observed on average, with only a lower average value of Fv / Fm, towards the end of the day, in the second season under evaluation (Figure 3b).

In Figure 4, it is possible to observe the registered values of the stomatic conductance (mmol m^{-2} s^{-1}) of the calafate leaf for different hours of the day and for the different compost dose treatments. Highlighting that for the 2018-2019 season, no significant differences were observed between the different treatments, with the average values of the day being 260, 289, 308 and 291 mmol m^{-2} s^{-1} ($P > 0.05$), for CK, T1, T2 and T3, respectively. However, for the control treatment, after 11:00 and until the end of the day, Gs values were always maintained above 260 mmol m^{-2} s^{-1}, unlike the rest of the treatments, which after obtaining the maximum values of stomatal conductance, their values decreased by up to 40% (data not shown). It should be noted that for the 2019/2020 season, the same trend was observed as in the 2018/2019 season. However, the recorded values were slightly lower than the previous season, with average values of Gs, for CK, T1, T2 and T3, of 227, 214, 257 and 230 mmol m^{-2} s^{-1} ($P > 0.05$), respectively.

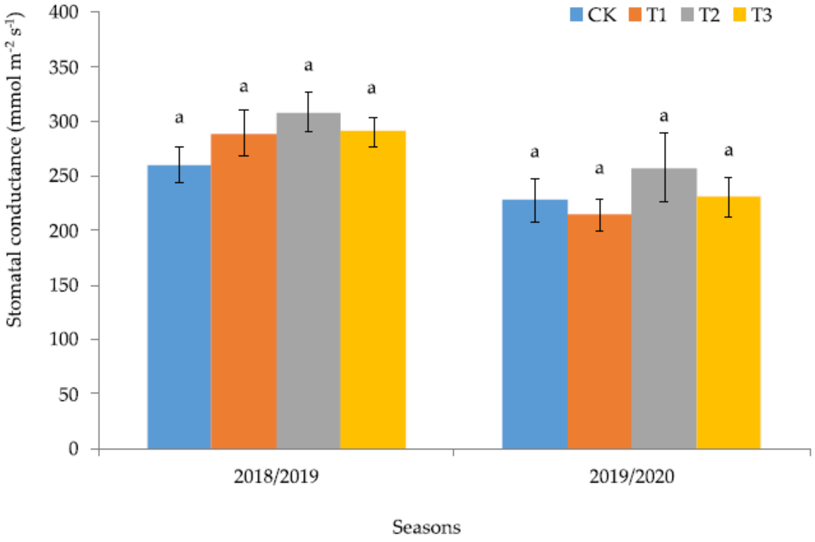


Figure 4. Effect of different doses of compost on the stomatal conductance (mmol $m^{-2}s^{-1}$) of the Calafate leaf (*Berberis mycrophilla* G. Forst) for the 2018-2019 and 2019-2020 seasons. CK = Control Treatment (without compost application); T1 = Treatment 1 (5 Ton ha^{-1}); T2 = Treatment 2 (10 Ton ha^{-1}); T4 = Treatment 3 (15 Ton ha^{-1}). For each treatment, different lowercase letters indicate significant differences in both seasons, according to Fischer's LSD test ($P < 0.05$) The bars correspond to the experimental error of each treatment. Fuente: Elaboracion propia.

3.2. Productive and quality parameters of the calafate fruit

In figure 5, the antioxidant capacity of the calafate fruit can be observed, for the 2018-2019 and 2019-2020 seasons. For the first season under study, the highest value of DDPH antioxidant capacity was recorded at the dose of 15 Ton ha^{-1} (T4; $P < 0.05$), with 4900 µmol TE / 100 g pf. Followed by treatments CK, T1 and T2, with 3961, 4130 and 4172 µmol TE / 100 g pf, respectively, being similar to each other ($P > 0.05$). It should be noted that for the second season a greater effect of the treatments was observed, where T2 was the one who reported the highest antioxidant capacity of the calafate fruit, with 4905 µmol TE / 100 g pf ($P < 0.05$), followed by treatments T1 and T3, with values of 4406 and 4435 µmol TE / 100 g pf, both without significant differences. Finally, the control treatment was the one that showed the lowest DDPH antioxidant capacity of all the treatments ($P > 0.05$), with a value of 3958 µmol TE / 100 g pf (Figure 5).

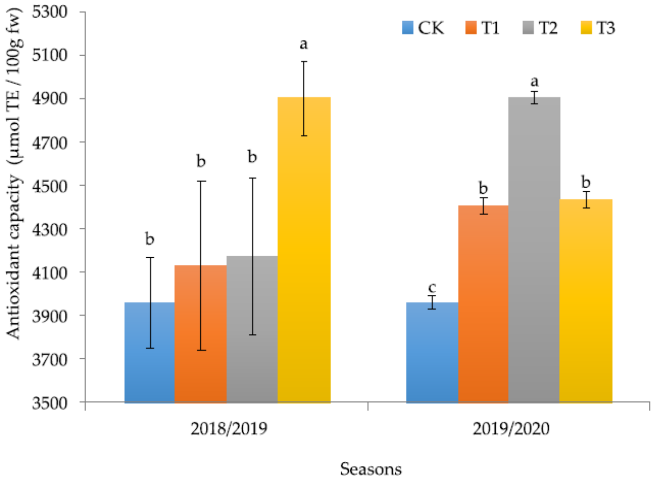


Figure 5. Effect of different doses of compost on the antioxidant capacity of the Calafate fruit (*Berberis mycrophilla* G. Forst) for the 2018-2019 and 2019-2020 seasons. CK = Control treatment (without compost application); T1 = Treatment 1 (5 Ton ha^{-1}); T2 = Treatment 2 (10 Ton ha^{-1}); T3 = Treatment 3 (15 Ton ha^{-1}). For each treatment, different lowercase letters indicate significant differences in both seasons, according to Fischer's LSD test ($P < 0.05$). The bars correspond to the experimental error of each treatment. Fuente: Elaboracion propia.

In figure 6, shows the total content of polyphenols in the calafate fruit grown under different doses of compost, for the 2018-2019 and 2019-2020 seasons. In the first season, no significant differences were observed between the different treatments. Ihowever, for the 2019/2020 season, the average polyphenolic content of the fruit decreased by 25% compared with CK. T2 and T3 were the ones that contributed the highest CPT values ($P < 0.05$), these being 764 and 718 mg of gallic acid / 100 g of fresh weight, respectively (Figure 6). However, T3 did not show significant differences with T1 (645 mg of gallic acid / 100 g of fresh weight). CK being the one who registered the lowest PFT content (543 mg of gallic acid / 100 g of fresh weight) ($P < 0.05$) among all treatments.

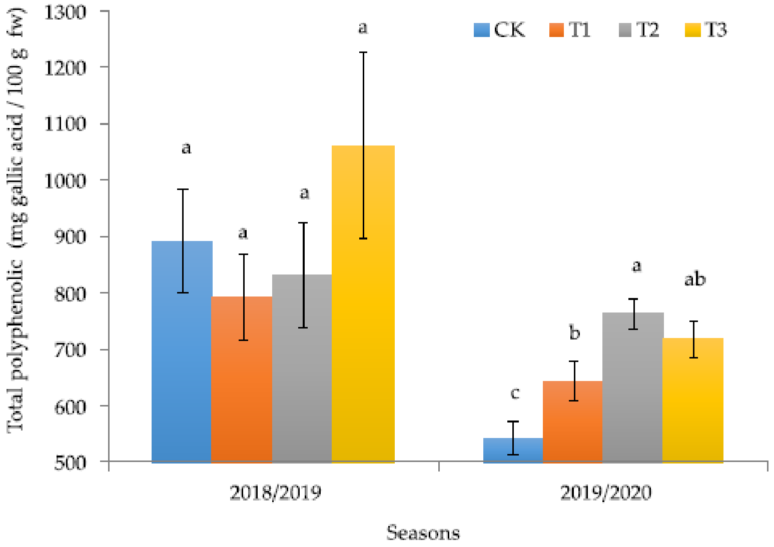


Figure 6. Effect of different doses of compost on the total polyphenolic content of the Calafate fruit (*Berberis mycrophilla* G. Forst) for the 2018-2019 and 2019-2020 seasons. CK = Control Treatment (without compost application); T1 = Treatment 1 (5 Ton ha^{-1}); T2 = Treatment 2 (10 Ton ha^{-1}); T3 = Treatment 3 (15 Ton ha^{-1}). For each treatment, different lowercase letters indicate significant differences in both seasons, according to Fischer's LSD test ($P < 0.05$). The bars correspond to the experimental error of each treatment. Fuente: Elaboracion propia.

Figure 7 shows the total anthocyanin content (TAC) in the calafate fruit for the different compost treatments, in the 2018-2019 and 2019-2020 seasons. For the first season under evaluation, the same behavior as the CPT was observed, not registering significant differences between CK, T1, T2 and T3, whose values averaged 573 mg cyanidin-3-glucoside / 100gr ms. For the second season, a more noticeable effect of the treatments was observed, although there was a decrease in the TAC in most of the treatments, the T2 was the one that registered the highest TAC ($P < 0.05$), with 545 mg cyanidin -3-glucoside / 100gr ms. Followed by T1 and T3, without significant differences between them, registering values of 445 and 431 mg cyanidin-na-3-glucoside / 100gr ms, respectively. And finally, the one who registered the lowest TAC with the rest of the treatments ($P < 0.05$) was CK, with a value of 363 mg cyanidin-na-3-glucoside / 100gr ms (Figure 7).

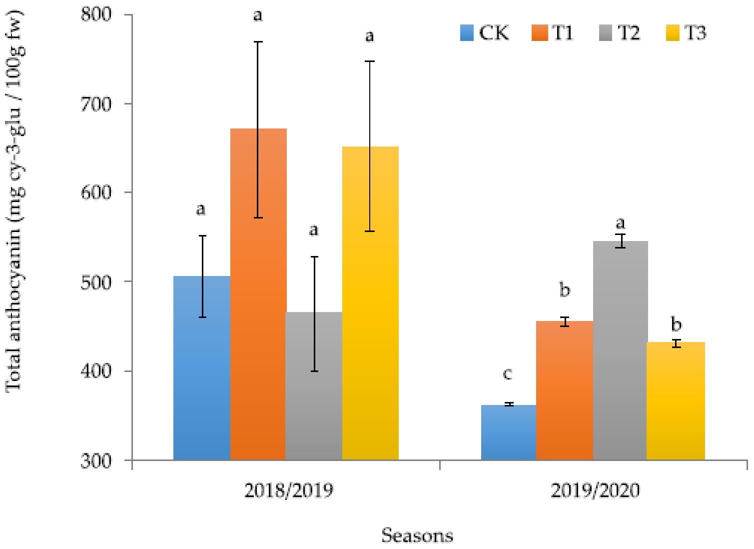


Figure 7. Effect of different doses of compost on the total anthocyanin content of the Calafate fruit (*Berberis microphylla* G. Forst) for the 2018-2019 and 2019-2020 seasons. CK = Control treatment (without compost application); T1 = Treatment 1 (5 Ton ha^{-1}); T2 = Treatment 2 (10 Ton ha^{-1}); T3 = Treatment 3 (15 Ton ha^{-1}). For each treatment, different lowercase letters indicate significant differences in both seasons, according to LSD test ($P < 0.05$). The bars correspond to the experimental error of each treatment. Fuente: Elaboracion propia.

In figure 8, the yields of fresh calafate fruit (g plant^{-1}) grown under different doses of compost in the 2018-2019 and 2019-2020 seasons are observed. For the first season, the treatments that recorded the highest fruit production were T2 and T3 ($P < 0.05$), without significant differences between them, which values were 629 and 561 g plant^{-1}, respectively. On the contrary, the treatment that registered a lower production was CK, with an average fruit yield of 249 g plant^{-1} ($P < 0.05$). In the second season, there was an increase in performance in most of the treatments. Highlighting that T3 and T2 were the ones who registered the highest average fruit production of 924 and 726 g plant^{-1} ($P > 0.05$), respectively, and without significant differences in themselves. Finally, the treatments that registered a lower fruit production were T1 and CK, with average productions of 424 and 370 g plant^{-1} ($P > 0.05$), respectively.

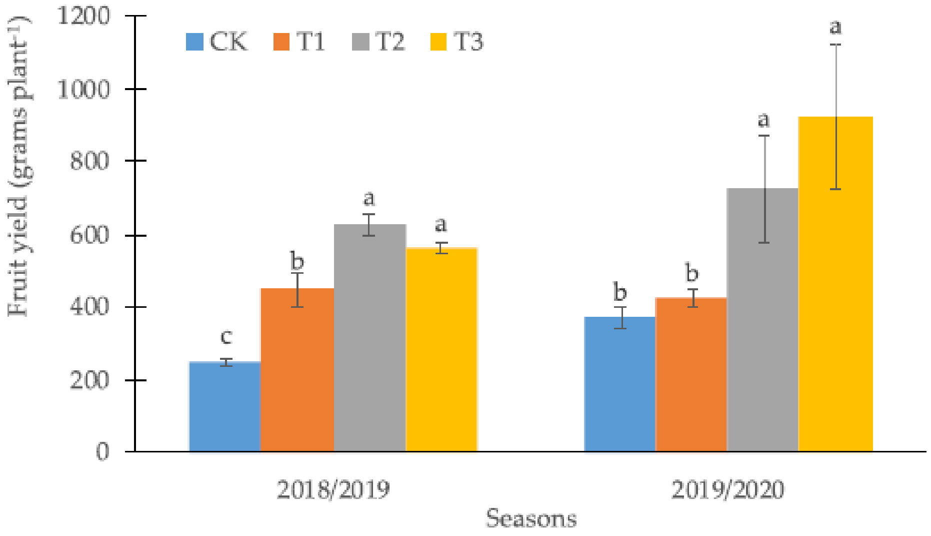


Figure 8. Effect of different doses of compost on the yield of the Calafate fruit (*Berberis microphylla* G. Forst) for the 2018-2019 and 2019-2020 seasons. CK = Cntrol treatment (without application of compost); T1 = Treatment 1 (5 Ton ha^{-1}); T2 = Treatment 2 (10 Ton ha^{-1}); T3 = Treatment 3 (15 Ton ha^{-1}). For each season, different lowercase letters indicate significant differences in the different treatments, according to Fischer's LSD test ($P < 0.05$). The bars correspond to the experimental error of each treatment. Fuente: Elaboracion propia.

4. DISCUSSION

Despite that photosynthetically active radiation (Figure 1a) was 26% higher in the study place, with respect to the habitat of origin of the plants (1600 µmol m^{-2} s^{-1}; Valdivia, Chile), no symptoms of excess radiation were evidenced. This was possibly due to the great structural and physiological plasticity that this species possesses. [15,37–42], managing to adapt to higher ambient temperature conditions, as indicated by Radice and Arena [10]. The above is confirmed by Romero-Román et al. [43], who observed that productive and some physiological parameters of the calafate plant were not influenced by extreme temperatures, however, these environmental conditions could be influencing the chemical parameters of the fruit [43], in response to higher levels of radiation, such as those observed at the study site (1a). Which despite of being high, did not show higher levels of absorbed PAR radiation between the different treatments and seasons (Figure 1b), this could be showing that the calafate, despite having morphological plasticity, has low variability of the light saturation point. The light saturation point of Calafate is 800 µmol m^{-2} s^{-1} [44], and at values higher than 1000 µmol m^{-2} s^{-1} the photosynthesis rates would be constant. Therefore, higher levels of PAR radiation could be generating photo oxidative damage due to excess of radiation. Studies developed by Arenas et al. [15], point out that low levels of irradiation improve plant development and nutrient content in the calafate leaf. In this study, however, no improvements were observed in indirect parameters, which are indicators of photosynthetic performance, such as stomatal conductance (Figure 4), where the results showed that this species does not respond strongly to changes in fertilization levels. in relation to Gs, the values being between 200 and 300 mmol m^{-2} s^{-1} between the different treatments and evaluation seasons ($P> 0.05$). These results are in contrast to those found in a study carried out on blueberries, where Gs was affected by the fertilization doses together with the water regime, with a correlation between the fertilization dose and the available moisture content in the soil. [45]. As the soil moisture was constant in all the treatments of this study, it could be strongly influencing so that no significant differences in Gs are observed between treatments. [45].

On the other hand, the application of compost to the soil increased the levels of organic matter content (OM) in all the treatments (Table 1 and 2), which could be affecting the moisture retention capacity, aeration, porosity, and soil carbon content, as reported in multiple studies [46–48]. Said OM modifications in the soil could be having an impact not only on the physical parameters of the soil, but also on the nutritional status and biomass of the plant, and consequently on the foliar area of the plant. [45,46]. In the present study, the FAI was higher in both seasons in Q3 (Figure 2), showing an increase in the second season of more than 20% compared to CK and T1 ($P <0.05$) and close to 10% higher than T3 ($P <0.05$). These results are consistent with other authors, who point out that the application of compost not only increases the vegetative development of the plant, but also the total chlorophyll content of the leaf. [15,46].

In a study carried out on vine (Vitis vinifera cv. Chardonnay), during 9 years of compost applications, equal yields were observed with an inorganic fertilization, however, fertilization with compost significantly increased the levels of organic matter in the soil, in addition, there was a substantial increase in the concentrations of mineralizable nitrogen in the soil [23]. The former, could have happened in this study, since the applied levels of nitrogen reached 60 mg kg^{-1} of soil (Table 2), but they were not strongly affected until the third year from the implementation of the study. (Table 3). Interestingly, nitrogen levels in the soil fluctuated between 13 and 16 mg kg^{-1}, in all treatments; this response of low nitrogen availability in the soil could be being generated by the high levels of nitrogen that the plant is extracting, to satisfy the greater vegetative development [49] (Figure 2) in conjunction with the higher levels of productivity [46] (Figure 8), as those observed in T2 and T3, which were 100% higher in both seasons for the treatments of 10 and 15 ton ha-1 of compost, compared to the control treatment. Treatments that obtained the best productive results, both at the be-ginning and at the end of the study, were the treatments with the highest doses of compost, corresponding to 10 and 15 tons ha^{-1} and with productivity levels close to 1000 grams per plant (Figure 8), coinciding with the results of other authors [45,48,549]. These results suggest that the longterm application of compost to the soil, in addition to improving the physical and chemical properties of the soil, as indicated above, could improve its biological activity. [24]. Although, the aforementioned is an uncertainty in this new species for commercial purposes, since there are no studies related to the microbial activity of the soil and the rhizosphere, therefore, this study opens the doors to future investigations that propose to understand and/or analyze the interaction of agronomic management with the activity and microbiological diversity of the soil, and the response of the calafate plant [15,42].

In the present study, it can be observed that the evaluated nutraceutical parameters were positively influenced by the dose of compost application when compared with the control treatment (Figures 5, 6 and 7). Regarding the content of polyphenols (Figure 6) and total anthocyanins of the calafate fruit (Figure 7), these decreased con-siderably in the second evaluation season by 25% on average between the different treatments. It should be noted that the compost treatment of 15 Ton ha^{-1} was the one that contributed the greatest decrease in polyphenolic content in the second season, however, the average total polyphenol contents observed in this study (764 mg of gallic acid / 100 g mp) were below the polyphenolic contents observed in wild plants in studies developed by other authors [50], However, the results obtained in the compost treatments were superior to the control treatment, this has been corroborated in other species, such as strawberries where the effect of an organic fertilization based on compost, increased the contents of anthocyanins, phenolic contents and antioxidant capacity, also in the Rhubarb (*Rheum rhabarbarum* L.) crop, where organic fertilization also improved the levels of polyphenolic content and antioxidant capacity of the fruit [48,51]. In a study conducted by Cojocaru et al., no increase in fruit yield was observed, as it was observed in this study (Figure 8), probably associated with the low doses of compost (2.4 Ton ha^{-1}) used in their study [51], associated with the high levels of extraction given by the levels of fruit production.

Regarding the contents of total anthocyanins, despite the fact that these plants are being cultivated and subjected to intensive agronomic management, the total concentrations of anthocyanins observed were higher than the results obtained in wild calafate plants in different locations in Usuahia (Argentina) and Buenos Aires (Argentina) [10], with values close to 118 and 316 mg cyanidin-3-glucoside / 100 gr, respec-tively. As well as, values lower than the results observed in Chile [16,43,52], with val-ues over 1000 mg cyanidin-3-glucoside / 100 gr of fresh weight. Said differences in concentrations of anthocyanins and total polyphenols could be being stimulated by multiple factors, which would be affecting the biosynthesis of bioactive compounds, such as different light intensities, ultraviolet radiation, extreme temperatures, availability of nutrients and water, among other factors specific to each environment where this species grows [15,53]. However, it is suggested that among the factors that could be most influencing the differences in results between the different studies, is the great variability in the opportune moment of harvest, which is influenced by the aforementioned parameters. It is important to mention that in this study the harvest time corresponded to 130 days after full flower, being higher than the harvest dates observed in the other polyphenolic evaluation studies of wild calafate fruits, fluctuating from 98 to 126 days later. full flower [40,54].

5. CONCLUSIONS

The use of increasing doses of compost turned out to be beneficial on the physiological, productive and quality parameters of Calafate, during the studied seasons. Treatment T2 at a rate of 10 Ton ha^{-1}, was the one that obtained the highest index of foliar area, antioxidant capacity, total polyphenols and total anthocyanins in the second study season. The compost application rates of 10 and 15 Ton ha^{-1} were those that ob-tained the highest fruit production per plant, with a production of 3300 kilos per hectare. On the other hand, treatments at increasing compost doses generated an increasing increase in organic matter in the soil and nutritional content of the soil. Therefore, the dose that optimizes the yield, fruit quality and nutraceutical content of the calafate fruit is set at a rate of 10 Ton ha^{-1}. However, this study opens the doors to future research in this line, to answer questions regarding the behavior of soil microbial activity and its interaction between agronomic management and the calafate plant, which could be altering the nutraceutical properties of calafate fruits.

Author Contributions: Conceptualization, J.R.-S.; methodology, J.R.-S., F.P.-M. and N.Z.; software,
J.R.-S., A.P.-P. and R.V.-R.; validation, J.R.-S.; formal analysis, J.R.-S. and F.P.-M.; investigation, F.P.-M. and J.R.-S.; resources, F.P.-M. and J.R.-S.; data curation, M.D.L. and J.R.-S.; writing—original draft preparation, F.P.-M. and J.R.-S.; writing—review and editing, R.V.-R., N.Z., M.D.L. and A.P.-P.; Project administration, J.R-S.; funding acquisition, J.R.-S. and F.P.-M. All authors have read and agreed to the published version of the manuscript.

Funding: The present work benefited from the project of research Nº 106, granted by the Univer-sidad Adventista de Chile, Chile. We are grateful to Fondecyt 1191141 ANID Chile for the scientific instruments provided for this research.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Corresponding author.

Acknowledgments: Agradecemos el apoyo prestado a los alumnos de la Universidad Adventista de Chile, por participar activamente en la recolección de datos: Andres Pinto; Samuel Contreras, Juan Pablo Molina and Sebastiancamilo Ospino.

Conflicts of Interest: The authors declare no conflict of interest.

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IV. CONCLUSION GENERAL

De los estudios realizados en clones silvestres de calafate en la región de Ñuble, comuna de San Ignacio, se pudo observar, que para completar el ciclo productivo del calafate fueron necesarios 418 grados días de cercuimento en base a 10 ºC. Entre los distintos clones evaluados de calafate, el clon 2 fue quien obtuvo la mayor concentarcon de polifenolesy concentraciónde antocianians, 1121 g GAE kg^{-1} fw y 714 g cy-3-glu 100 g^{-1} fw, respectivamente, por lo cual el clon 2, podría ser seleccionado para posterior clonación y/o cruzamiento para un programa de mejoramiento genético del calafate. Conjuntamente, se determinó que la fecha de cosecha optima para el fruto de calafate es a los 120 DDPF, donde el fruto alcanzo el mayor contenido de polifenoles y concentración de antocianianas totales, asi como la capacidad antioxidante del fruto.

El uso de dosis crecientes de compost resultó ser benefico sobre los parámetros fisiológicos, productivos y de calidad del Calafate, durante las temporadas estudiadas. El tratamiento T3 a razón de 10 Ton ha^{-1}, fue el que obtuvo un mayor indice de área foliar, capacidad antioxidante, polifenoles totales y antocianinas totales en la segunda temporada de estudio. Las tasas de aplicación de compost de 10 y 15 Ton ha^{-1}, fueron las que obtuvieron mayores produccion de fruto por planta, con una producción de 3300 kilos por hectarea. Por otro lado, los tratamientos a dosis creciente compost, generarón un aumento creciente de la materia orgánica en el suelo y contenidos nutricionales del suelo. Por lo cual, la dosis que optimiza el rendimiento, calidad del fruto y contenido nutraceútico del fruto de calafate fue a razón de 10 Ton ha^{-1}. Sin embargo, este estudio abre las puertas a futuras investigaciones en esta línea, para responder la interrogante del comportamiento de la actividad microbiana del suelo y su interacción entre el manejo agronómico y la planta de calafate, que podrían estar alterando las propiedades nutracéuticas de los frutos del calafate. Ademas se hace necesario seguir evaluado y sometiendo este frutal a otros manejos ténicos y científicos referidos a; riego, control de malezas, densidad de plantación, podas y manejos fitosanitarios.

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