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Cryoconcentration by Centrifugation–Filtration: A Simultaneous, Efficient and Innovative Method to Increase Thermosensitive Bioactive Compounds of Aqueous Maqui (*Aristotelia chilensis* (Mol.) Stuntz) Extract

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Abstract: Maqui (*Aristotelia chilensis* (Mol.) Stuntz) is a Chilean berry rich in antioxidants, which are mostly found in the pulp and skin of the fruit. The objective was to evaluate the cryoconcentration process by centrifugation–filtration as a simultaneous, efficient, and innovative method to increase the content of thermosensitive bioactive compounds of aqueous maqui extract. Cryoconcentration separated the concentrated solute from the aqueous maqui extract with an efficiency of more than 95%; it increased the content of total polyphenols and total anthocyanins and antioxidant capacity by 280%, 573%, and 226%, respectively. Although the concentrates obtained by evaporation at 50, 70, and 80 °C increased the content of bioactive compounds, they did so in a lower percentage than the cryoconcentrate. Furthermore, cyanidin 3,5-diglucoside was degraded at 70 and 80 °C. In conclusion, cryoconcentration by centrifugation–filtration as a simultaneous process efficiently separates the solutes from the frozen matrix of aqueous maqui extract, and it maintains and increases the contents of polyphenols and anthocyanins and antioxidant capacity. This method is recommended for concentrating natural berry extracts with thermosensitive compounds.

Keywords: maqui; *Aristotelia chilensis*; cryoconcentration; centrifugation–filtration; simultaneous process; high efficiency

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Citation: Bastías-Montes, J.M.; Vidal-San-Martín, C.; Tamarit-Pino, Y.; Muñoz-Fariña, O.; García-Figueroa, O.; Quevedo-León, R.; Wei, Z.-J.; Lv, X.; Cespedes-Acuña, C.L. Cryoconcentration by Centrifugation–Filtration: A Simultaneous, Efficient and Innovative Method to Increase Thermosensitive Bioactive Compounds of Aqueous Maqui (*Aristotelia chilensis* (Mol.) Stuntz) Extract. *Processes* **2022**, *10*, 25. https://doi.org/10.3390/pr10010025

Academic Editor: Michaela Schmitz

Received: 18 November 2021
Accepted: 16 December 2021
Published: 23 December 2021

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1. Introduction

The demand for health-promoting foods has significantly increased [1,2]. Fruits are perceived as healthy foods because they contain a number of compounds, including vitamin C and polyphenols, which provide high antioxidant power [3]. Fruit consumption, especially of berries, has increased exponentially because there is evidence supporting the health benefits of phytochemicals [4], which are associated with several pathologies, such as cardiovascular disease, diabetes, and cancer [3,5].

Based on the current interest in finding natural phytochemicals derived from plant raw materials to replace synthetic substances, the food industry has developed new types of processing and extraction and concentration techniques for these compounds; it has

primarily focused on the bioactive phytochemicals in fruits [6]. Concentration by freezing is used to recover concentrated solutes from liquid foods in which the solution is concentrated by separating the pure ice crystals. This is compared with evaporation and membrane technologies. It has potential advantages for producing high quality concentrates because the process occurs at low temperatures, resulting in a minimal loss of volatile compounds; the process is temperature sensitive, especially for those compounds that are lost in the concentration by evaporation [7,8]. Its effectiveness in protecting bioactive compounds in various types of fruit products and juices has been demonstrated [9,10]. However, cryoconcentration innovations have focused on a single-stage system (block or progressive); these innovations are characterized by simple procedures as regards equipment operation and construction [11,12] in which the concentrated solution is obtained gravitationally [13,14]. Efficiency is relatively low; assisted techniques, such as ultrasound, vacuum, or centrifugation, are pursued [9–15] to improve the separation process.

Although articles are published on the use of centrifugation to improve the separation of the concentrated phase from the frozen phase, results indicate that this technique offers only 60% separation efficiency of the solute in the process [10]. There is an intensive search for a technique to keep the concentrate separated from the ice after completing the cryoconcentration process and increase process efficiency.

There are studies in other areas of science, such as medicine, that have used centrifuge tubes with filters inside (Millipore Microcon, MW100, 3–5 kD pore size, Merck KGaA, Darmstadt, Germany) to extract DNA and proteins for forensic applications [16,17] or Amicon Ultra 0.5 tubes (Merck KGaA, Darmstadt, Germany) to simplify and improve compound identification by high performance liquid chromatography (HPLC) [18] to efficiently separate the targeted substance from other compounds in the initial sample. Although evidence shows that it is possible to separate solutes from liquid substances by centrifugation–filtration, the process of separating and concentrating bioactive substances in fruit juices using this method is more complex, due to their size and the size of the cellulose filters in the tubes available on the market. However, Bastías et al. [19] reported that cryoconcentration assisted by centrifugation–filtration of aqueous maqui extract (at laboratory scale) produced a concentrate with a high content of soluble solids and 98% separation efficiency of solutes from the concentrated phase. The filter inside the tubes acted as a support to keep the concentrated phase separated from the frozen phase, thus significantly increasing the performance and efficiency of the process.

The objective of this work was to study the effect of cryoconcentration by centrifugation–filtration as a simultaneous, efficient, and innovative method to increase the thermosensitive bioactive compounds of aqueous maqui (*Aristotelia chilensis* (Mol.) Stuntz) extract.

2. Materials and Methods

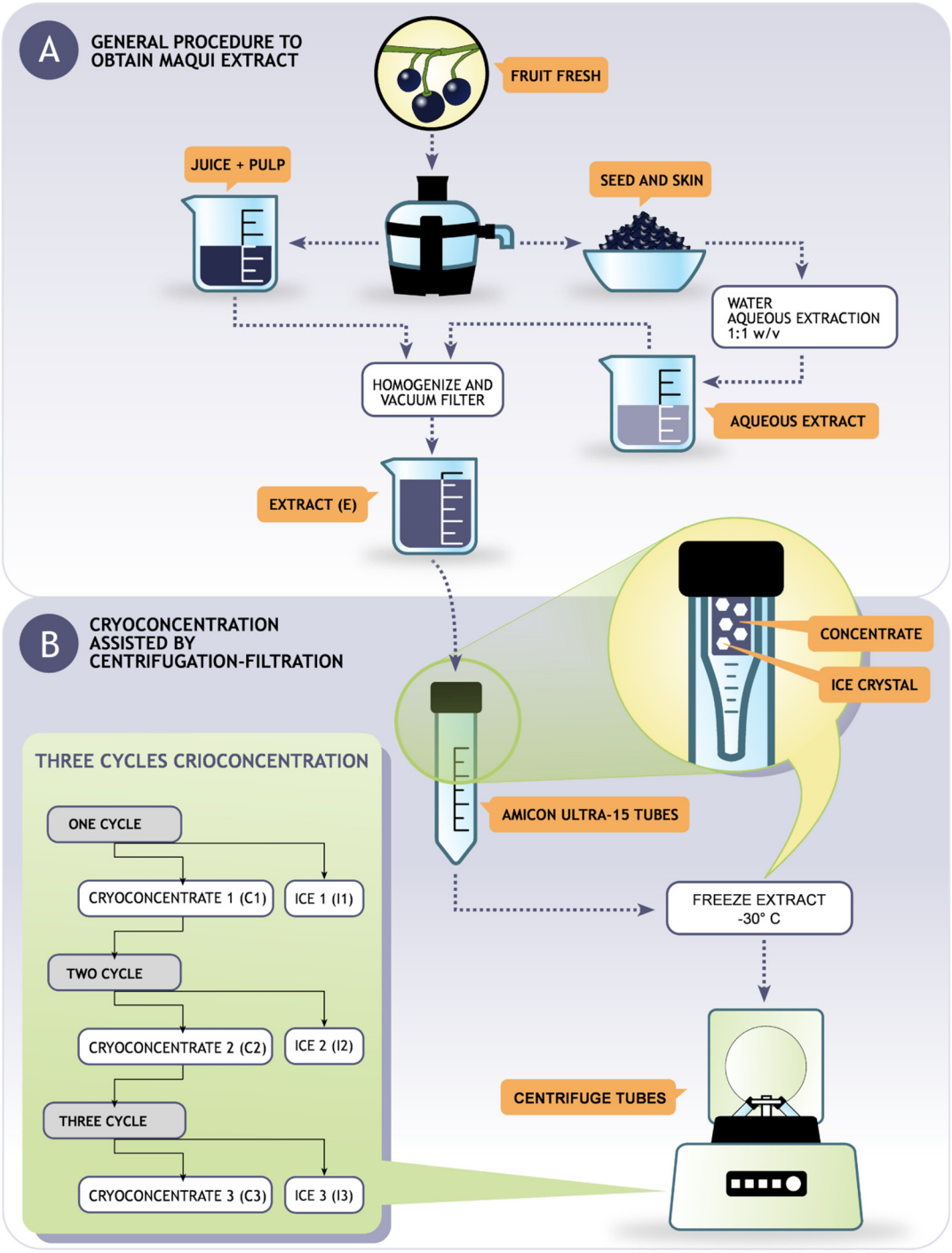
2.1. Chemicals

All chemical products were of analytical reagent grade without further purification. Folin–Ciocalteu reagent, sodium carbonate, gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), DPPH (radical 2,2 diphenyl-1-picrylhydrazyl), trifluoroacetic acid, and acetonitrile were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Production of the Juice and Aqueous Extract

The extract was produced with fresh maqui fruits (undefined variety, semi-domesticated crop) collected in the Fundo las Pataguas in the town of Coihueco, Ñuble Region, Chile. Fresh fruits were cleaned, prepared, and refrigerated (4 °C) until further processing. They were then placed in a pulper (Phillips, model HR-1832, Amsterdam, Nederland) to separate the pulp from the seeds and skin. The soluble compounds in the seed and skin were extracted with water at a 1:1 w/v ratio at 570 rpm for 50 min in an orbital shaker (SCILOGEX SK-018-Pro, Rocky Hill, CT, USA). The fresh maqui juice and aqueous extracts obtained from the extraction process were mixed and vacuum filtered. Finally, the extract

was put into amber-colored bottles with a 100 mL capacity, which were frozen at −30 °C until further analysis (Scheme 1A). The concentration of the total soluble solids of the final extract depended on the initial concentration of total soluble solids in the fruits.

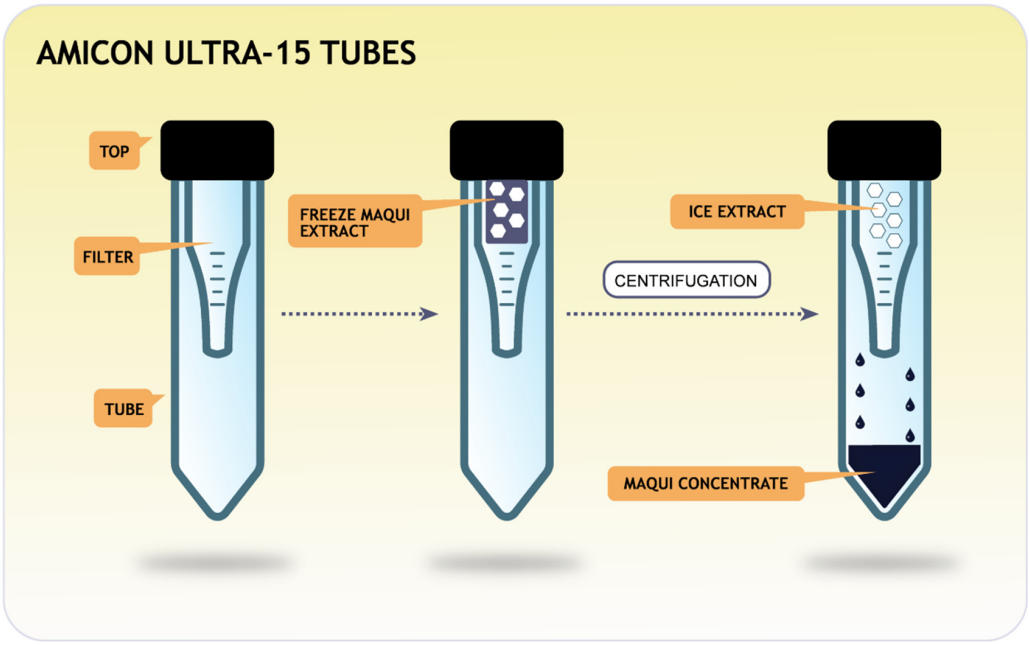


Scheme 1. General procedure to obtain maqui extract (**A**) and cryoconcentration (**B**). Concentrates are C1–C3. Ice obtained after each cryoconcentration cycle are I1–I3.

2.3. Production of Cryoconcentrates by Freezing and Centrifugation-Filtration

The maqui extract sample (15 mL) was placed in the Amicon Ultra-15 filter (Amicon Ultra-15 Centrifugal Filter Devices, MERCK, Darmstadt, Germany) polypropylene centrifugal filter tubes [20] (with nanofilter cellulose membrane removed) (Scheme 2) and frozen at −30 °C. Samples were removed from the freezing chamber and left at ambient temperature

for 5 min before centrifugation (Rotofix 32 A Universal 320, HETTICH, Toronto, Canada) at 4000 rpm for 10 min (20 °C) to force solute separation from the frozen fraction (Scheme 1B). Finally, the concentrate and frozen matrix were collected to determine the concentration of soluble solids with a refractometer (RX-5000α CX, ATAGO, Tokyo, Japan) at ambient temperature with 0.1 °Brix precision.



Scheme 2. Amicon Ultra-15 tubes and cryoconcentration process.

2.4. General Procedure for Cryoconcentration Cycles

Twenty kilograms of maqui fruits were pulped to obtain the juice, seeds and skin. Fruit seeds and skin were subjected to aqueous extraction (1:1 w/v) to obtain an extract. The juice and extract were homogenized by mixing the extract in both a vortex and vacuum filtration system to obtain the maqui extract. Material from the first cryoconcentration cycle (C1) was used as raw material for the second cycle (C2). Finally, the second concentrated fraction was used as raw material for the third cryoconcentration cycle (C3) (Scheme 1B) [9]. The cryoconcentrate from C2 was used to analyze bioactive compounds (total polyphenols, total anthocyanin identification and quantification, and antioxidant capacity) and to determine color, referred to as cryoconcentrate (C) for these analyses.

2.5. Evaporation Concentration Process

The maqui extract was placed in a round-bottomed 500 mL capacity BUCHI glass flask (protected from light), which was coupled to a rotary evaporator (R-210/R215, BUCHI, Flawil, Switzerland) for concentration at 50, 70, and 80 °C. The concentration of the soluble solids was determined with a refractometer at ambient temperature. The process ended when the content of soluble solids in the concentrate was the same as in C, which is described in Section 2.3.

2.6. Determination of Cryoconcentration Parameters

Concentration Efficiency

The efficiency of each cryoconcentration cycle was defined as the increase in the concentration of the solution compared with the amount of solids remaining in the frozen fraction. Equation (1) was used to calculate efficiency (n):

$$n(\%) = \frac{\left(C_s - C_f\right)}{C_s} \times 100 \quad (1)$$

where n is efficiency and C_s and C_f are the concentrations of solids (°Brix) in the concentrated solution and frozen fraction, respectively [21].

2.7. Determination of Total Polyphenols, Anthocyanins, Antioxidant Capacity by the Diphenylpicrylhydrazyl (DPPH) and Oxygen Radical Absorption Capacity (ORAC) Methods

2.7.1. Total Polyphenols

The Folin–Ciocalteu method was used for the spectrophotometric determination of total polyphenols [22]. The mixture consisted of 250 µL aliquot of the sample, 1250 µL Folin–Ciocalteu reagent, and 2500 µL 20% sodium carbonate that were added to 50 mL Falcon tubes; the volume was adjusted to 25 mL with distilled water. The tubes were homogenized and protected from light at ambient temperature for 30 min [23]. Sample readings were taken with a UV-Visible spectrophotometer (Orion AquaMate 8100, Thermo Fisher Scientific, Waltham, MA, U.S.A.) at 765 nm wavelength. Results were expressed in mg gallic acid equivalents (GAE) per 100 g of fresh weight (FW) [24].

2.7.2. Quantification and Identification of Anthocyanins

The profile of the anthocyanic components of the maqui extracts produced in the present study well overlaps, from a qualitative point of view, the anthocyanic patterns already reported for similar *Aristotelia chilensis* extracts.

Total anthocyanins were evaluated by the pH differential method described by Giusti and Wrolstad [25] and modified by Gaviria et al. [26]. A mixture was prepared with a sample of 0.2 mL and 1.8 mL potassium chloride buffer (0.025 M at pH 1.0) or sodium acetate buffer (0.4 M at pH 4.5). The mixture was homogenized, and readings were taken with a UV-Visible spectrophotometer (Orion AquaMate 8100, Thermo Fisher Scientific, Waltham, MA, USA) at the 510 and 700 nm wavelengths. All measurements were performed in triplicate, and the results were calculated by Equation (2):

$$\Delta A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5} \quad (2)$$

where ΔA is the absorbance differential determined as pH 1.0 and pH 4.5 and A is absorbance determined at the 510 and 700 nm wavelengths, respectively.

The concentration of total anthocyanins was expressed as mg equivalents of cyanidin 3-glucoside (cya-3-glu) per g FW as expressed in Equation (3):

$$Total\ anthocyanins\left(mg \times g^{-1}FW\right) = (\Delta A \times MW \times FD \times 1000) \times \varepsilon^{-1} \quad (3)$$

where MW is molecular weight (449.2 g $\cdot$ mol^{-1}), ε is the molar extinction coefficient (26.900 L $\cdot$ mol^{-1} $\cdot$ cm^{-1}) [26] and FD is the dilution factor.

The anthocyanin content was determined by the method established by Tanaka et al. [27], with some modifications. Extracts were centrifuged at 14,000 rpm for 10 min and injected in a Waters ALLIANCE 2695 with the Waters 2996 photodiode Array Detector and Empower software. For the chromatographic conditions, an Xterra C18 Water Column (250 × 4.6 mm, 5 µm) was used, the column oven temperature was 30 °C, and the mobile phase consisted of a 0.3% (v/v) trifluoroacetic acid aqueous solution (A) and CH_3CN (B). The gradients were 0 min, 95% (A), 5% (B); 4 min, 95% (A), 5% (B); 4.5 min, 90% (A), 10% (B); 27 min, 85% (A),15% (B); 47 min, 45% (A), 55% (B); 48 min, 10% (A), 90% (B); 50 min, 10% (A), 90% (B); 51 min, 95% (A), 5% (B); and 60 min, 95% (A), 5% (B). The flow rate was set at 0.7 mL/min. The selected detection wavelength was 520 nm. Results were expressed as mg/g FW.

The detection (LoD) and quantification (LoQ) limits are shown in Table 1. The LoD is calculated by Equation (4).

$$LoD\left(\frac{\mu g}{g}\right) = LoD\left(\frac{\mu g}{mL}\right) \times \frac{FD}{g\ sample} \quad (4)$$

Table 1. Detection (LoD) and quantification (LoQ) limits to identify anthocyanins in maqui concentrates.

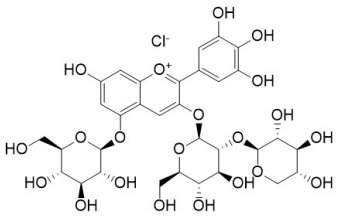
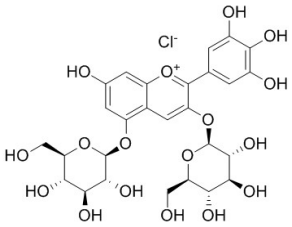
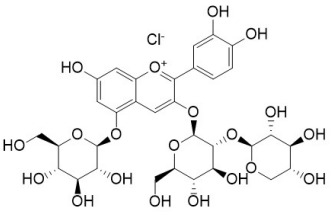
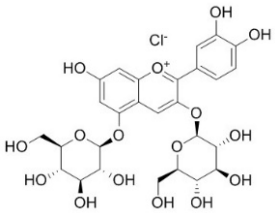
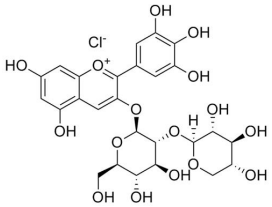
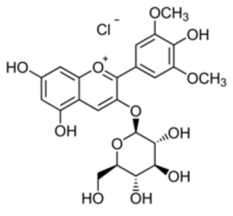
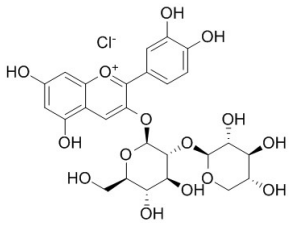
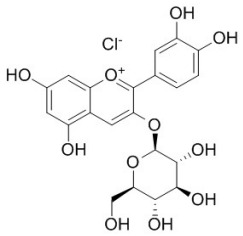
Anthocyanin		LoD (μg/mL)	LoQ (μg/mL)
Delphinidin 3-sambubioside 5-glucoside		19.53	58.6
Delphinidin 3,5-diglucoside		47.39	142.2
Cyanidin 3-sambubioside-5-glucoside		22.18	66.5
Cyanidin 3,5-diglucoside		8.78	26.3
Delphinidin 3-sambubioside		9.76	29.3
Delphinidin 3-glucoside		27.26	81.8

Table 1. *Cont.*

Anthocyanin		LoD (μg/mL)	LoQ (μg/mL)
Cyanidin 3-sambubioside		3.54	10.6
Cyanidin 3-glucoside		4.73	14.2

R^2 = correlation coefficient used in standard curves to determine curve quality; allowed values were >0.99, otherwise determinations were repeated.

2.7.3. Determination of Antioxidant Capacity by the Diphenylpicrylhydrazyl (DPPH) and Oxygen Radical Absorption Capacity (ORAC) Methods

The elimination of DPPH radicals from the maqui concentrates was determined using the methodology described by Brand-Williams et al. [28]. Results were expressed as micromoles of Trolox equivalents per gram (μmol TE/g FW). The antioxidant capacity determined by ORAC was according to a protocol proposed by Huang et al. [29] using a fluorometer (Biotek FLx800 TBID, Angilet, Santa Clara, US). The ORAC assay was conducted using Trolox as a reference. Results were expressed as μmol TE/g FW.

2.8. Determination of Color

Color analysis was performed in the maqui extract and concentrates (obtained by freezing and evaporation) with a spectrophotometer (Konica Minolta Sensing Americans, NJ, US, CHROMA METER, CR-400 with D65 illuminant) previously calibrated to obtain CIELAB colorimetric coordinates. The values for L* (luminosity, black = 0, white = 100), a* (red > 0, green < 0), and b* (yellow > 0, blue < 0) were recorded. Angle tone (h*ab, red = 0°, yellow = 90°, green = 180°, and blue = 270°), chroma (C*ab, 0 is the center of the sphere), and color difference (ΔE*) were calculated by Equations (5)–(7), respectively.

$$C^*_{ab} = \sqrt{a^2 + b^2} \quad (5)$$

$$h^*_{ab} = tan^{-1}\frac{b^*}{a^*} \quad (6)$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (7)$$

2.9. Statistical Analysis

One-way analysis of variance (ANOVA) was performed with the Statgraphics Centurion XVI [30] statistical software. All tests were performed in triplicate and repeated once. The differences between means were evaluated by Tukey's test at a 95% confidence interval.

3. Results and Discussion

3.1. Condition for the Centrifugation–Filtration Process

Figure 1 shows the soluble solids content in cryoconcentrated maqui samples and the cryoconcentration parameters, such as efficiency and impurities found in each centrifugation–filtration cycle. Cryoconcentration technology increases the soluble solids content by 3.3 times the initial concentration obtained in the maqui extract (16.2 °Brix) in only three centrifugation–filtration cycles for 10 min. This value is much higher than results reported by other researchers [13,14,31–33] and is similar to those mentioned by Orellana-Palma et al. [10], who used a similar technique to separate the ice concentrate with a filtration cloth after centrifuging the sample for 15 min. The soluble solids in the ice were low, reaching 3.75 °Brix at the end of C3, which is lower than results reported by Orellana-Palma et al. [10]. It should be noted that cryoconcentration by centrifugation–filtration also showed a high concentration efficiency and attained values greater than 99% in C1. Although it decreased slightly in C3, efficiency was 93% and impurities were less than 7% at the end of C3 (Figure 1).

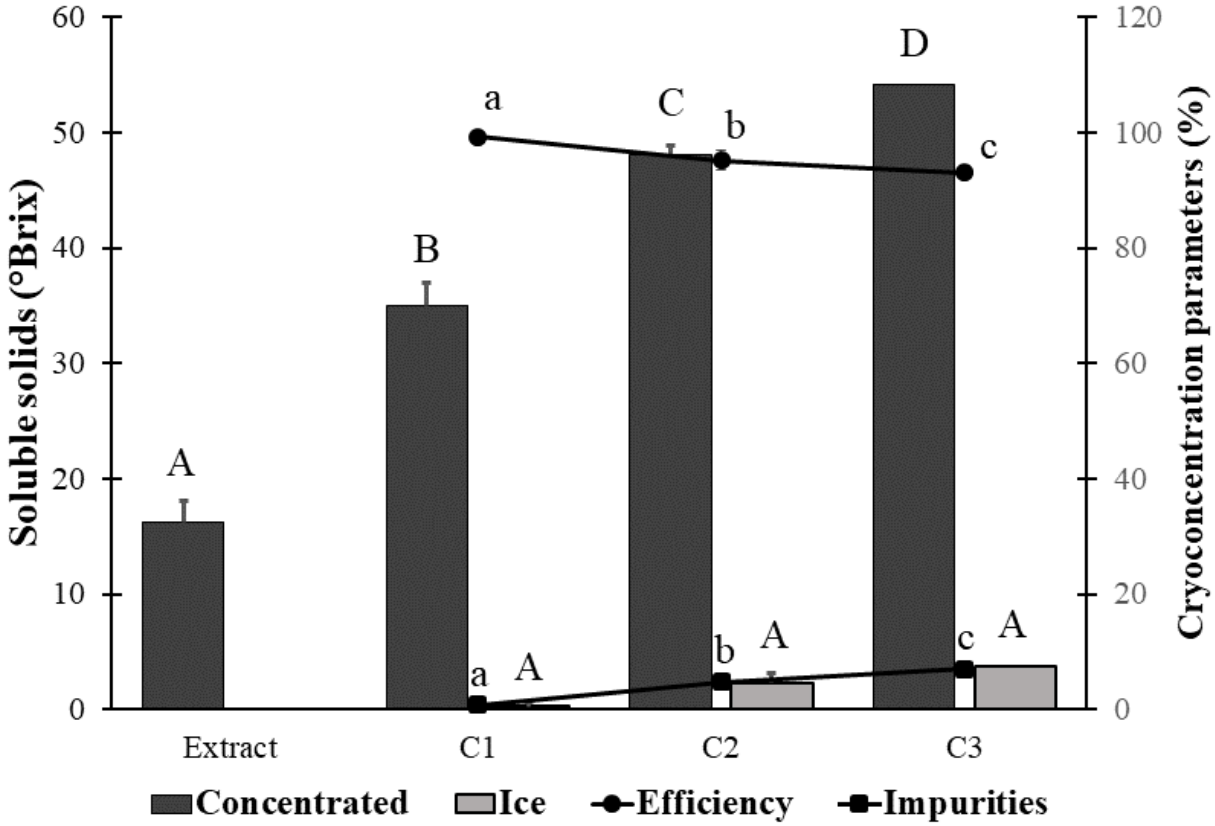


Figure 1. Soluble solid content (concentrate and ice) and cryoconcentration parameters (efficiency and impurities) after each centrifugation–filtration cycle. Extract A; C1: cryoconcentrate obtained from the first cycle; C2—cryoconcentrate obtained from the second cycle; C3—cryoconcentrate obtained from the third cycle. Different capital letters over the bars indicate a significant difference ($p \leq 0.05$ for soluble solid content (concentrate and ice)) and different low letters indicate a significant difference ($p \leq 0.05$ for cryoconcentration parameters (efficiency and impurities)) after each centrifugation–filtration cycle, according to Tukey's test.

High concentration efficiency can be attributed to the method used to separate the ice concentrate. Previous studies have already shown that centrifugation exhibits higher efficiency percentages than other methods (ultrasound and vacuum) as a separation medium [15]. This clearly demonstrates that the efficiency of the process significantly increases when a combination of centrifugation and filtration is used.

It was determined that C2 exhibited optimal process parameters (separation efficiency and impurities); therefore, it was selected to compare bioactive compounds and color with the concentrates obtained by evaporation, because of its high percentage of separation efficiency and low impurity level compared to the three process cycles. It should be noted that as the separation efficiency increases, the sample concentration increases; therefore, a higher content of bioactive compounds is expected [14].

3.2. Determination of Total Polyphenols

The total phenol contents in the maqui extract are shown in Figure 2. The cryoconcentration process maintained and concentrated total polyphenols from 1279 mg GAE/100 g FW in the maqui extract to 3592.7 mg GAE/100 g FW in C. Samples concentrated by evaporation at 50, 70, and 80 °C showed that the total polyphenol content decreased as the process temperature increased.

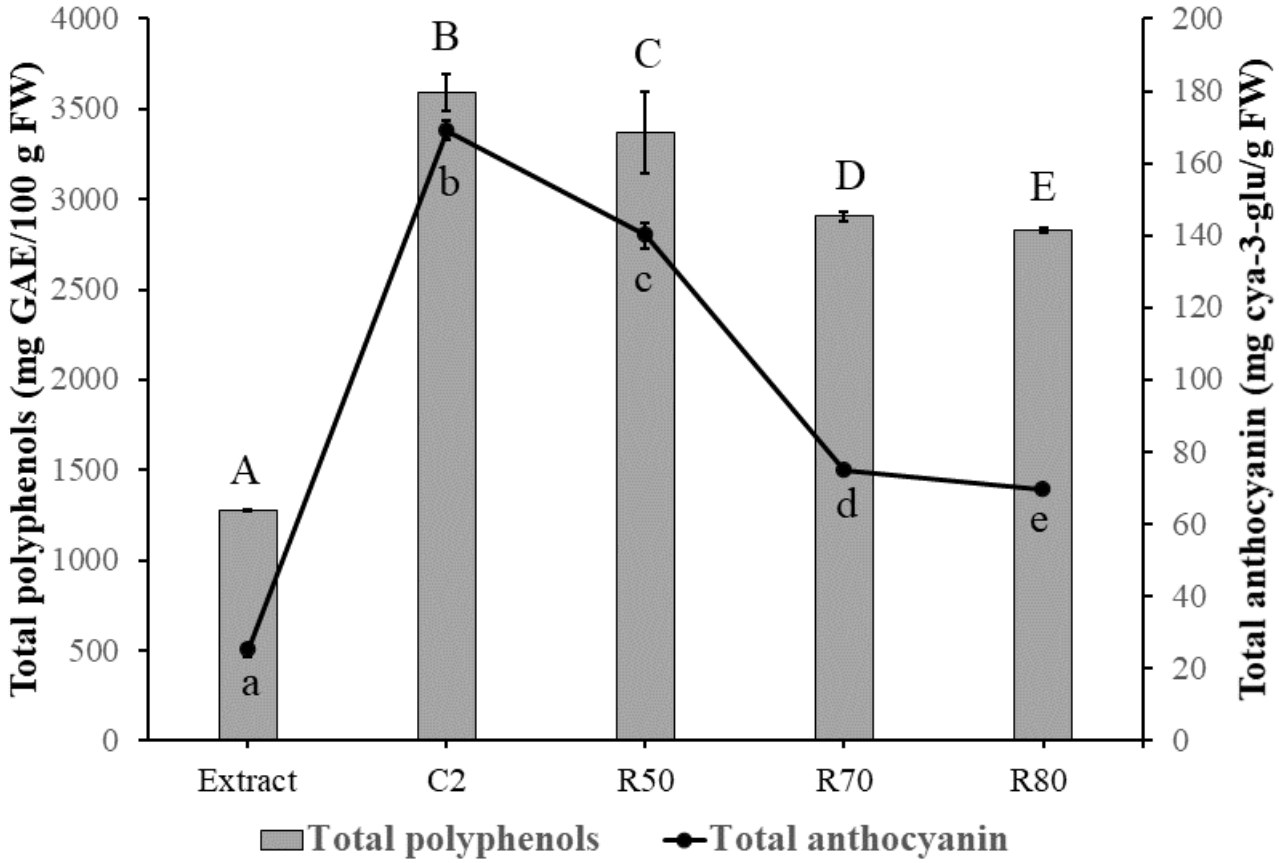


Figure 2. Total polyphenol (mg GAE/100 g FW) and Total anthocyanin (mg cya-3-glu/g FW) content in extract and maqui concentrates. C: cryoconcentrate; concentrates by evaporation at 50 °C (R50), 70 °C (R70), and 80 °C (R80). Different capital letters over the bars indicate a significant difference ($p \leq 0.05$ for total polyphenol content) and different low letters indicate a significant difference ($p \leq 0.05$ total anthocyanin content) in extract and maqui concentrates, according to Tukey's test.

There are few studies about polyphenols in cryoconcentrated products. Petzold et al. [9] used cryoconcentration to concentrate wine in which the total polyphenols increased 1.7 times the initial concentration; this is lower than the results of the present study for maqui extract in which total polyphenols were concentrated 2.8 times. Regarding the use of high temperatures in juices, Jaeger et al. [34] reported that pasteurization and concentration were the most common heat treatments used for fruit juices. These treatments used processing temperatures ranging from 75 to 90 °C for long periods of time; this could alter fruit quality due to thermal and oxidative damage to the most heat-sensitive

components [35]. It was reported that there was a loss of sensory properties and nutritional compounds in juices at temperatures greater than 50 °C [36]. Tadapaneni et al. [37] observed that polyphenolic compounds decreased only when pasteurization was applied in strawberry juices at a high temperature for a short time. For berries, Brauch et al. [38] produced maqui fruit juice pasteurized at 85 °C for 2 min, and they reported that the total polyphenol content decreased by approximately 60% compared with the initial fresh juice content. Capanoglu et al. [39] used evaporation to treat grape juice, and total polyphenols decreased by approximately 85% on reaching a concentration of 70 °Bx. This decrease in phenolic compounds was previously indicated by Buckow et al. [40], who described that bioactive phytochemicals were more sensitive to degradation when the processing temperature increased. Saeeduddin et al. [41] found a greater decrease in total polyphenols at 95 °C as opposed to 65 °C for pasteurized pear juice. It should be noted that the effect of a high temperature treatment is variable because it depends on the processing temperature and time and specific factors, such as the food matrix and the sensitivity of different phytochemicals to the process [42]. Therefore, processing juices with a high content of phenolic compounds concentrated at low temperatures maintains the physicochemical characteristics of fresh fruit, especially those of thermosensitive compounds; this makes cryoconcentration interesting from a biological point of view.

3.3. Determination of Total Anthocyanins and Identification by High Performance Liquid Chromatography (HPLC)

The cryoconcentrate (C) had the highest anthocyanin content (Figure 2) and increased 6.7 times (573%) the initial maqui extract content. Although the concentrates obtained by evaporation increased total anthocyanin content by 5.57, 2.98, and 2.77 times when concentrating at 50, 70, and 80 °C, respectively, content clearly decreased as the evaporation temperature increased.

Maqui berries contain a high total anthocyanin content. It was reported that its content varies because fruits from southern Chile contain a higher percentage of anthocyanins [38,43,44]. This depends on multiple factors, including growing conditions, geographic area, genotype [45] and botanical characteristics of the fruit [46]. As a result, anthocyanins are mainly accumulated in the epicarp and fleshy parts of the fruit [47].

The development of products derived from fruits with a high proportion of seeds and low pulp content has led to the extraction of compounds from the skin and fleshy parts of the fruit. The aqueous extraction process can extract hydrophilic plant pigments that are widespread in berries that mitigate oxidative stress and can help prevent a number of diseases [48]. However, both the collection of fruits [49] and their processing can reduce the content of compounds, such as anthocyanins [50].

These are critical stages of the process and can cause a significant degradation of anthocyanins [51] and other phenolic compounds [44] that provide anti-inflammatory, anti-adipogenic [52], anti-atherogenic [53] and cardioprotective activity [54] typical of maqui fruits, which would cause a significant decline in interest from the biological point of view.

In the present study, water-soluble compounds, including anthocyanins, were extracted by grinding and in an aqueous extraction of soluble compounds in the seeds and skin of the fruit, which significantly increased the anthocyanin content in the extract; this significantly increased the anthocyanin content when compared with maqui fruits in other studies [40]. However, anthocyanins are thermolabile and can decrease when subjected to heat treatment [55].

Brauch et al. [38] reported that pasteurizing maqui juice reduces the anthocyanin content by approximately 55%. Martínez et al. [55] indicated that the greatest impact on agraz juice (*Vaccinium meridionale* Sw.) heated at different temperatures occurred at 90 °C and reduced the anthocyanin content by approximately 90%. Similar behavior was found in grape juice concentrated at 70, 80, and 90 °C, which exhibited a decrease of 53%, 58%, and 86%, respectively; this concurs with our results for maqui concentrate.

The present study is useful for future research aimed at protecting thermolabile compounds with antioxidant capacity in foods, given that most studies are focused on process parameters that occur when soluble solids are separated from the frozen matrix [32,56,57] rather than determining the behavior of these phytochemicals in the technological processing of raw materials. Some studies have indicated that anthocyanin stability depends on several factors, such as chemical structure, pH, concentration, temperature, presence of oxygen, and water activity, which affect the total content of these compounds [58,59].

Anthocyanin contents and HPLC chromatograms are presented in Table 2 and Figure 3, respectively. Eight different anthocyanin peaks were identified (based on chromatographic retention time only) in the products: delphinidin-3-sambubioside-5-glucoside, delphinidin-3,5-diglucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside-5-glucoside, delphinidin-3-sambubioside, delphinidin-3-glucoside, cyanidin 3-sambubioside, and cyanidin 3-glucoside. Retention times for these anthocyanins ranged from 17 to 32 min and were similar for all analyzed samples. However, in the concentrates by evaporation at 70 and 80 °C, the retention time for cyanidin 3,5-diglucoside (22.001 min) had no peak associated with it. All concentrates by both freezing and evaporation showed that delphinidin derivatives (80% to 90%) were higher than cyanidin derivatives (10% to 20%), while delphinidin 3-sambubioside-5-glucoside (43% to 65%) had a higher proportion among the other identified anthocyanins.

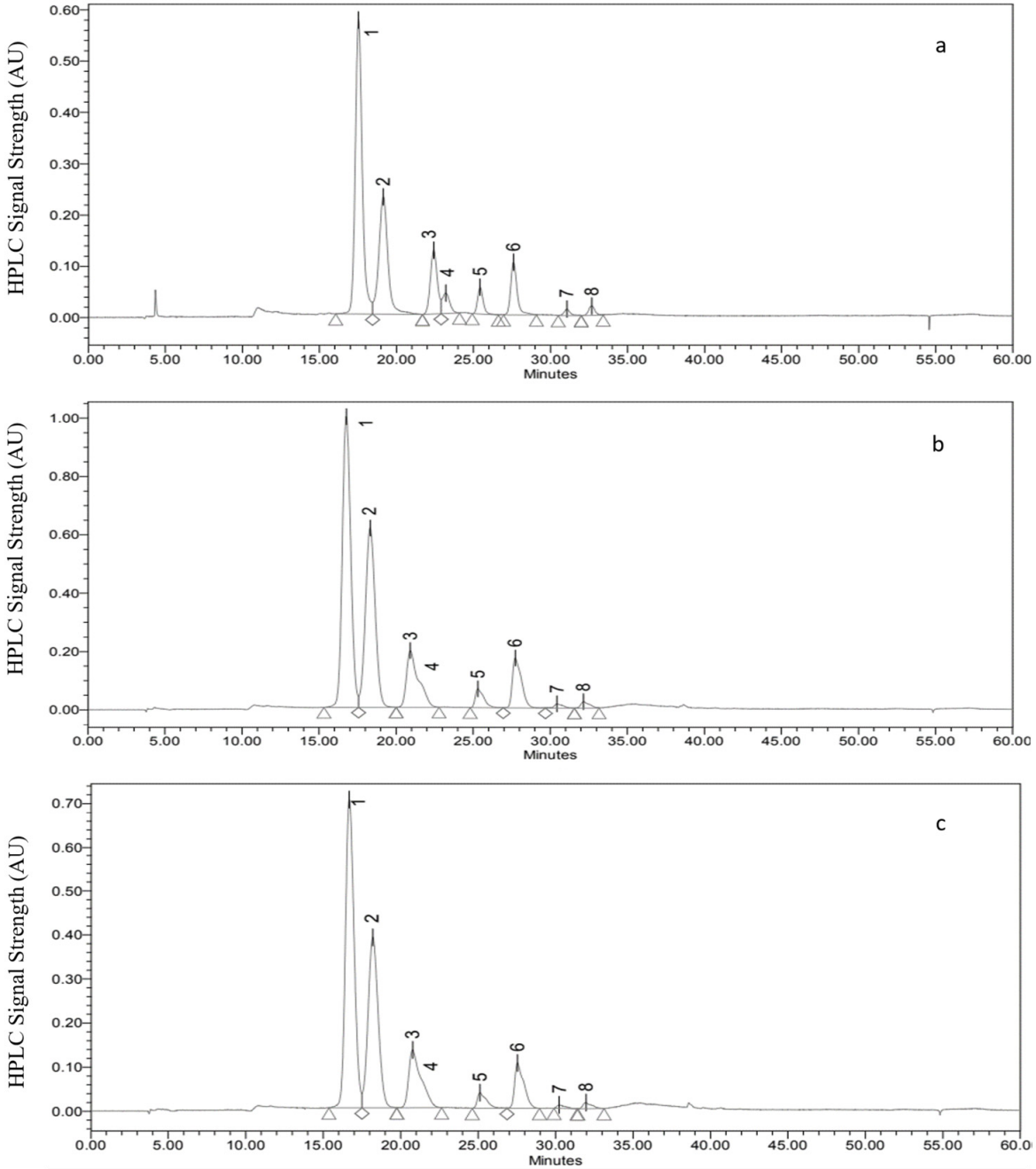
Table 2. Anthocyanin content (mg cyanidin 3-glucoside/g) of maqui (*Aristotelia chilensis* (Mol.) Stuntz) extract, cryoconcentrate, and concentrates by evaporation at 50, 70, and 80 °C.

Anthocyanin	Extract	C	R50	R70	R80
Delphinidin 3-sambubioside 5-glucoside	9.68 ± 1.68 ^{c}	79.60 ± 0.8 ^{a}	92.07 ± 22.76 ^{a}	32.77 ± 0.06 ^{b}	33.95 ± 2.38 ^{b}
Delphinidin 3.5-diglucoside	7.24 ± 0.96 ^{c}	41.90 ± 0.1 ^{a}	16.31 ± 6.31 ^{cb}	23.71 ± 0.09 ^{b}	21.93 ± 1.52 ^{b}
Cyanidin 3-sambubioside-5-glucoside	2.79 ± 0.63 ^{c}	18.98 ± 2.1 ^{a}	17.79 ± 0.65 ^{a}	9.07 ± 0.02 ^{b}	6.27 ± 3.25 ^{b}
Cyanidin 3.5-diglucoside	1.73 ± 0.61 ^{b}	5.43 ± 1.91 ^{a}	1.14 ± 0.14 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}
Delphinidin 3-sambubioside	0.76 ± 0.05 ^{b}	6.17 ± 0.27 ^{a}	2.32 ± 0.32 ^{b}	2.15 ± 0.01 ^{b}	1.33 ± 0.58 ^{b}
Delphinidin 3-glucoside	2.33 ± 0.20 ^{d}	13.16 ± 0.31 ^{a}	9.08 ± 1.09 ^{b}	6.03 ± 0.02 ^{c}	5.25 ± 0.33 ^{c}
Cyanidin 3-sambubioside	0.25 ± 0.20 ^{b}	1.48 ± 0.08 ^{a}	0.50 ± 0.05 ^{b}	0.47 ± 0.03 ^{b}	0.27 ± 0.11 ^{b}
Cyanidin 3-glucoside	0.33 ± 0.24 ^{b}	2.29 ± 0.04 ^{a}	0.76 ± 0.06 ^{b}	0.72 ± 0.02 ^{b}	0.45 ± 0.32 ^{b}
Total Anthocyanin content	25.11 ± 4.57 ^{c}	169.01 ± 5.61 ^{a}	139.97 ± 31.38 ^{a}	74.92 ± 0.25 ^{b}	69.45 ± 8.49 ^{b}

Extract: maqui extract. C: cryoconcentrate. Concentrates by evaporation: R50 at 50 °C, R70 at 70 °C, and R80 at 80 °C. $^{(a–d)}$ Different letters in the same row indicate significant differences between sample means according to Tukey's test ($p \leq 0.05$).

Unlike the evaporation concentrates, C had the highest anthocyanin content (Figure 2). In addition, cyanidin 3,5 diglucoside was not detected in the evaporation concentrates at 70 and 80 °C (R70 and R80) (Figure 3b,c). Escribano-Bailón et al. [59] reported that maqui extracts contain two types of anthocyanins, delphinidin and cyanidin, which can be linked to different sugars such as 3-glucosides, 3,5-diglucosides, 3-sambubiosides, and 3-sambubioside-5-glycosides; these findings coincide with the anthocyanins identified in the present study. These authors identified eight anthocyanin monomers in maqui by high performance liquid chromatography-photodiode array detector-mass spectrometry (HPLC-PAD-MS). The anthocyanin monomers were identified as delphinidin 3-sambubioside-5-glucoside, delphinidin 3,5-diglucoside, cyanidin 3-sambubioside-5-glucoside, cyanidin 3,5-diglucoside, delphinidin 3-sambubioside, delphinidin 3-glucoside, cyanidin 3-sambubioside, and cyanidin 3-glucoside; the delphinidin derivatives (73%) prevailed over the cyanidin derivatives (37%) and delphinidin 3-sambubioside-5-glucoside was the main anthocyanin (34%) [51]. Céspedes et al. [60] also identified eight anthocyanins in maqui berries collected in the Andes near Temuco, Chile, which were the same as those identified by Escribano-Bailón et al. [59] and those found in the present study. We detected that the delphinidin derivatives were significantly higher (74.3%) than the cyanidin derivatives (25.7%), and delphinidin 3-sambubidium-5-glucoside showed the highest content (35.1%).

Brauch et al. [38] identified the same eight monomeric anthocyanins in the Aysén Region, Chile; however, delphinidin and cyanidin 3,5-diglucoside were only classified as delphinidin diglucoside and cyanidin diglucoside, and the cyanidin derivatives showed the highest concentration (52%). These authors mentioned that the same anthocyanins identified in the fruits were in the maqui juice, but with a lower concentration due to the pasteurization process. Delphinidin derivatives slightly decreased when the fruit was thermally processed, while cyanidin derivatives showed a drastic reduction of 86%.



Peak	Anthocyanins	Peak	Anthocyanins
1	Delphinidin 3-sambubioside5-gl	5	Delphinidin 3-sambubioside
2	Delphinidin 3.5-diglucoside	6	Delphinidin 3-glucoside
3	Cyanidin 3-sambubioside-5-gluc	7	Cyanidin 3-sambubioside
4	Cyanidin 3.5-diglucoside	8	Cyanidin 3-glucoside

Figure 3. High performance liquid chromatography (HPLC) identification of anthocyanins at 520 nm in the cryoconcentrate (C) (**a**) and concentrates by evaporation at 70 °C (R70) (**b**) and at 80 °C (R80) (**c**).

These results confirm that conservation or concentration treatments using high temperatures in fruit juices with a high content of bioactive compounds cause significant degradation of thermosensitive compounds, thus degrading anthocyanins and reducing their content. Therefore, cryoconcentration is a technology that helps to preserve the compounds in the fruits because it uses freezing temperatures to avoid altering the fruit composition before subjecting the fruits to a concentration treatment.

3.4. Determination of Antioxidant Capacity by the Diphenylpicrylhydrazyl (DPPH) and Oxygen Radical Absorption Capacity (ORAC) Methods

Figure 4 shows that C has the highest antioxidant capacity for both the percentage of DPPH radical inhibition and ORAC content. The cryoconcentrate (C) exhibited an antioxidant capacity that was 226% to 240% higher than the aqueous maqui extract and 39.8%, 53.3%, and 89.1% higher than the concentrates obtained by evaporation at 50, 70, and 80 °C, respectively.

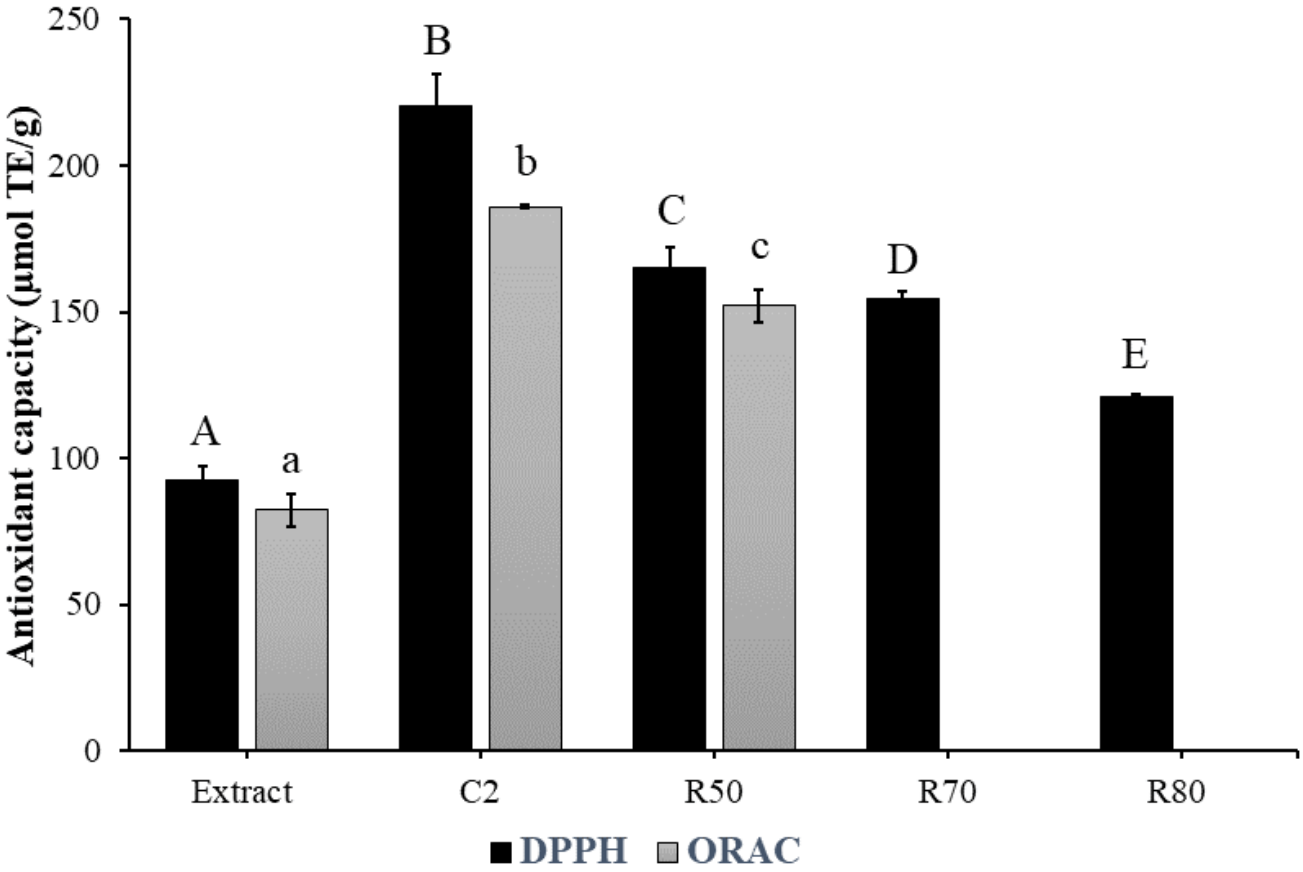


Figure 4. Antioxidant capacity by DPPH and ORAC in concentrated maqui products (cryoconcentrated and evaporated). Extract: C—cryoconcentrate; evaporation at 50 °C (R50), 70 °C (R70), and 80 °C (R80). Different capital letters over the bars indicate a significant difference ($p \leq 0.05$ for DPPH content) and different capital letters over the bars indicate a significant difference ($p \leq 0.05$ ORAC content) in concentrated maqui products (cryoconcentrated and evaporated), according to Tukey's test.

The antioxidant capacity determined by the ORAC method was studied in only three maqui products. Although maqui extracts obtained by evaporation at 50, 70, and 80 °C increased the content of total polyphenols and anthocyanins (Figure 2), they did so in a significantly lower proportion, which also decreased the antioxidant activity of the analyzed products. This was evident when studying the antioxidant capacity by the DPPH inhibition percentage (Figure 4), which significantly decreased as the concentration temperature

increased to evaporate the samples. Meanwhile, the present study demonstrated that cryoconcentration is an effective method to increase the antioxidant potential of maqui extract. When comparing the concentration level of these compounds with the concentrates obtained by evaporation at 50, 70, and 80 °C, the concentration temperature at 50 °C already showed a significantly lower antioxidant capacity than C.

Studies of antioxidant capacity in concentrated maqui products have not yet been conducted. However, Miranda-Rottman et al. [52] compared the content of polyphenols and anthocyanins in different concentrated juices, such as blueberry, raspberry, cranberry, blackberry, and maqui, as well as red wine. The results showed that maqui exhibited the highest values compared with the other fruits, thus corroborating the results obtained in the present study [60]. Among the commonly consumed fruit in Latin America, Speisky et al. [61] reported that maqui and calafate (*Berberis microphylla*) have the highest antioxidant capacity (ORAC) compared with 27 other fruits; this is due to the high anthocyanin content in purple fruits. González et al. [62] indicated that the highest antioxidant potential of maqui is mainly found in the fleshy parts of the fruit (2.3 times higher than in the seeds). They also reported that the antioxidant capacity does not vary in the fruit maturation process and values fluctuated between 71.3 and 88.6 μmol ET/g fresh fruit. However, they did find a variation among clones (in San Fernando, San Clemente, Entre Lagos, and Puerto Montt), which ranged from 18.3 to 138.2 μmol ET/g fresh fruit, NS values were higher in Entre Lagos. Gironés-Vilaplana et al. [63] showed results similar to those already reported in our research of freeze-dried maqui samples. Studies related to concentrates explaining the antioxidant potential through these methods are scarce.

The present study did not perform studies of cell lines to evaluate the effect of the antioxidant activity of maqui on human health. However, current interest in understanding its effects is an important area of research because the human body possesses endogenous or antioxidant mechanisms that function to protect the development of oxidative stress and maintain the chemical balance in body cells.

For this reason, numerous methods were developed to quantify antioxidants in the human diet and discover how they can influence health. Depending on the reactions involved, these methods can be classified as hydrogen atom transfer (HAT) and electron transfer (ET). The advantage of these assays is that they can quantify the effect of processing and the stability of the bioactive compounds undergoing redox reactions in the final product. In particular, the ORAC assay has emerged as a test of choice to measure peroxyl radical uptake in foods and other matrices [62]. Epidemiological studies have shown that there is a direct relationship between the consumption of antioxidant-rich foods and the effects on health. This is how Gifkins et al. [64] found that there was a decreasing trend of endometrial cancer in a population that consumed more than 16.3 μmol Trolox equivalent of ORAC per day, suggesting that a higher antioxidant intake can decrease the risk of endometrial cancer. Other studies evaluating the effect of consuming different fruits, seeds, and legumes have shown that high antioxidant consumption in the diet was associated with lower risk of non-Hodgkin lymphoma [65], lower risk of hypertension in type 2 diabetes patients [66], and favorable effects in metabolic disorders, preventing subsequent weight and abdominal fat gain [67]. Therefore, by determining the antioxidant capacity in maqui extract and cryoconcentrates, it is possible to have some idea of the effect that its consumption would have compared with other research that correlated antioxidant activity with epidemiological studies in humans.

3.5. Determination of Color

Color data are displayed in Table 3. The aqueous maqui extract exhibited higher L* compared with concentrates by freezing or evaporation, which is due to the extraction process used to obtain maqui extract from the fresh fruit.

Table 3. Color in concentrated products.

Coordinates/Products	Extract	C	R50	R70	R80
L*	0.14 ± 0.04^{a}	0.023 ± 0.01^{b}	0.023 ± 0.02^{b}	0.017 ± 0.01^{b}	0.013 ± 0.01^{b}
a*	0.11 ± 0.03^{a}	-0.08 ± 0.02^{b}	-0.023 ± 0.01^{c}	-0.08 ± 0.02^{b}	0.01 ± 0.00^{d}
b*	-0.10 ± 0.01^{a}	0.03 ± 0.01^{b}	-0.017 ± 0.01^{c}	-0.03 ± 0.01^{c}	0.02 ± 0.01^{b}
Hab*	318.60 ± 4.58^{a}	160.90 ± 1.22^{b}	233.90 ± 15.34^{c}	248.60 ± 5.32^{c}	59.99 ± 13.61^{d}
Cab*	0.15 ± 0.00^{a}	0.081 ± 0.02^{b}	0.029 ± 0.01^{c}	0.083 ± 0.02^{b}	0.023 ± 0.02^{c}
ΔE	-	0.252 ± 0.04^{a}	0.107 ± 0.06^{b}	0.241 ± 0.02^{a}	0.204 ± 0.02^{a}

Extract—maqui extract; C—cryoconcentrate. Concentrates by evaporation: R50 at 50 °C, R70 at 70 °C, and R80 at 80 °C. L*—luminosity; a*— red to green colorimetric coordinate; b*—yellow to blue colorimetric coordinate; h^*_{ab}—angle tone; C^*_{ab}—chroma; ΔE—color difference. $^{(a–d)}$ Superscripts with different lowercase letters in the same row indicate significant differences among treatment means according to Tukey's test ($p \leq 0.05$).

The maqui extract has a less intense purple coloring because of dilution, compared with the color of the concentrates in which part of the added water was eliminated in the extraction process of soluble compounds in the fruit, thus producing a darker and more intense purple coloring. For the a*, b*, Hab*, and Cab* parameters, it is evident that there were significant differences between the color of C and concentrates by evaporation. In the latter, as concentration temperature increased, coloring tended to be reddish-yellow, while C maintained the intense purple tones of the fruit (Table 3). Research related to the determination of color in maqui fruit and its derived products are still limited. Girones-Vilaplana et al. [63] conducted a study of color in isotonic beverages prepared from lemon juice and citric acid with added freeze-dried maqui, acai, and blackthorn. They found that the mixture with maqui provided a darker purple color compared with the mixture prepared with the other two berries, whose color is similar to maqui. In a study with fresh blueberries (*Vaccinium corymbosum* L., cv. Bluecrop), it was found that the L* coordinate was low (36.2) [68]. In the juice of different blueberry varieties, the CIELAB coordinates ranged from 21.70 to 23.8 for L*, 0.06 to 6.22 for a*, and 0.14 to 1.98 for b*, while Hab* varied between 0.19 and 1.17 and Cab* between 0.17 and 6.53; variability mainly depended on the evaluated blueberry genotype [69]. Tiwari et al. [70] found similar results in a study of blackberry (*Rubus laciniatus*) juice in which a* and b* had values of 4.24 and −3.14, respectively; it is evident that the a* and b* parameters in juices prepared from dark-purple-colored fruit tend toward the value 0 and can be negative. Regarding concentrated juice color, Khajehei et al. [71] evaluated pomegranate juice treated by freezing (cryoconcentration) and high temperature (60 °C). They observed that L* decreased in all the assayed concentration treatments, while a* increased approximately from 29 to 36 and b* decreased from 13 to 10; this change produced a red color that was darker than the initial raw material. In contrast, when comparing the control sample with samples treated with high temperature, both parameters (a* and b*) significantly decreased to 8.66 and −100, respectively, and coloring acquired more yellow tones. The temperature treatment produced brown coloring compared with the coloring obtained by the freezing treatment, which was similar for maqui concentrates (Table 3). Finally, for the color difference (ΔE) between maqui concentrates, low values were detected. According to the description by Martínez et al. [72], ΔE cannot be perceived by the human eye because it can only be perceived when the value is >40 CIELAB units.

4. Conclusions

Cryoconcentration by centrifugation–filtration is an innovative process for separating soluble solids from aqueous maqui extract, and its separation efficiency was greater than 95% in the second cycle (C) of the process. In addition, the contents of total polyphenols, total anthocyanins, and antioxidant capacity of the aqueous maqui extract increased by 280%, 573%, and 226%, respectively. Eight anthocyanins were identified in C (delphinidin-3-sambubioside-5-glucoside, delphinidin-3,5-diglucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside-5-glucoside, delphinidin-3-sambubioside, delphinidin-3-glucoside,

cyanidin 3-sambubioside, and cyanidin 3-glucoside) in which the delphinidin derivatives prevailed over the cyanidin derivatives.

In the concentrates obtained by evaporation at 70 and 80 °C, the processing temperature significantly affected the contents of polyphenols and anthocyanins and antioxidant capacity of the aqueous extract, causing the loss of cyanidin 3,5-diglucoside.

Therefore, cryoconcentration by centrifugation–filtration is an effective and efficient technology to obtain maqui concentrates with the characteristic color of fresh fruit (intense purple) with a high antioxidant potential, maintain the anthocyanin profile, and significantly increase the percentage of bioactive compounds of the fruit for future applications, such as microencapsulation in the nutraceutical industry.

Author Contributions: Conceptualization, J.M.B.-M. and X.L.; Formal analysis, C.V.-S.-M., O.G.-F., R.Q.-L. and C.L.C.-A.; Investigation, Y.T.-P.; Methodology, C.V.-S.-M., O.M.-F. and O.G.-F.; Project administration, J.M.B.-M.; Software, R.Q.-L. and Z.-J.W.; Visualization, Z.-J.W.; Writing—original draft, C.V.-S.-M.; Writing—review & editing, Y.T.-P., O.M.-F., X.L. and C.L.C.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research and Development Agency 563 (Agencia Nacional de Investigación y Desarrollo, ANID), Chile, Grant FONDECYT N° 564 1191127.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors gratefully acknowledge by Suzanne Theberge Suzanne Theberge, who is a language teacher and translator.

Conflicts of Interest: The authors have no conflict of interest to report.

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