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Effect of Cryoconcentration Assisted by Centrifugation-Filtration on Bioactive Compounds and Microbiological Quality of Aqueous Maqui (*Aristotelia chilensis* (Mol.) Stuntz) and Calafate (*Berberis microphylla* G. Forst) Extracts Pretreated with High-Pressure Homogenization

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Abstract: The objective of this study was to evaluate the effect of cryoconcentration assisted by centrifugation-filtration on the bioactive compounds and the microbiological quality of aqueous maqui (*Aristotelia chilensis* (Mol.) Stuntz) and calafate (*Berberis microphylla* G. Forst) extracts pretreated with high-pressure homogenization (HPH). Aqueous extracts were prepared from fresh fruits which were treated with HPH (predefined pressure and number of passes). The best pretreatment was determined by aerobic mesophilic, fungal, and yeast counts. Treated extracts were frozen at −30 °C in special tubes and centrifuged at 4000 rpm for 10 min to obtain the cryoconcentrated product. The optimal pretreatment conditions for HPH were 200 MPa and one pass in which the extracts exhibited no microorganism counts. Cryoconcentration by freezing and subsequent centrifugation-filtration in a single cycle showed high process efficiency (>95%) in both soluble solids and bioactive compounds (total polyphenols and anthocyanins) and antioxidant capacity of the fresh fruits and extracts. The HPH treatment and subsequent cryoconcentration assisted by centrifugation-filtration is an efficient technology to obtain concentrates with good microbiological quality and a high content of bioactive compounds.

Keywords: *Aristotelia chilensis*; *Berberis microphylla*; cryoconcentration; high-pressure homogenization; bioactive compounds; polyphenols

1. Introduction

Maqui (*Aristotelia chilensis* (Mol.) Stuntz) is one of the Chilean berries that is best known and most studied worldwide because of its high antioxidant capacity and high content of anthocyanins and phenolic compounds [1–4]. This growing interest has led to the commercialization of new maqui fruit-based products, thus increasing Chilean exports of this fruit and its products as a source of nutrients and bioactive compounds [5,6].

The calafate is another Chilean native berry, which belongs to the genus *Berberis microphylla* G. Forst [7]. It is found in the southernmost part of the country. It is a wild plant concentrated in small gardens located in the Aysén and the Magallanes Regions, although Hoffmann and Jullian [8] mentioned that calafate can be found from Curicó (central zone)

to Tierra del Fuego (southernmost tip). There are currently few reports that address the chemical characteristics of this fruit. However, Ruiz et al. [7] indicated high polyphenol content and antioxidant capacity in this type of native berry.

The bioactive compounds in these fruits, especially anthocyanins, are susceptible to degradation due to multiple factors such as environmental conditions, gastrointestinal tract (GI), changes in pH, temperature, and oxygen, and the presence of metal ions [9,10]. Therefore, the concentration of fruit juices has proven interesting to increase the content of these phytochemicals. Cryoconcentration has been one of the best processes used in the food industry because it minimizes the degradation of bioactive compounds and concentrates them with high separation efficiency [11–15].

Until now, cryoconcentration has focused on single-step systems because of its simple procedures to obtain the concentrated solute [11,16–18]. However, the separation efficiency of the concentrated solute from the ice matrix is low; therefore, assisted techniques have been sought to improve this deficiency in the process [19,20]. Centrifugation is one of the most promising methods [21]; it has been able to slightly increase the efficiency results, but it has not exceeded 60%. Therefore, research has been conducted to achieve the total separation between the concentrated solute and the ice in the cryoconcentration process.

Yang et al. [22] and Kranz et al. [23] used centrifuge tubes with filters inside for forensic applications and compound identification by high-performance liquid chromatography (HPLC), respectively. Although these tubes allowed separating the compounds in samples of human biological origin, the separation and concentration process of bioactive substances in fruit juices is more complex because of the size of the compounds and the cellulose filters available on the market. However, Bastías et al. [15] was able to cryoconcentrate aqueous maqui extract at laboratory scale using Amicon Ultra 15 tubes with high solute separation efficiency.

Pasteurization has been used as a thermal treatment to stabilize juices from the microbiological point of view [24–27]. However, anthocyanins and other bioactive compounds are degraded by this traditional process [28] and inevitably cause undesirable physical, chemical, and sensory changes.

As a result, non-thermal technologies have been proposed in addition to traditional thermal treatments, which reduce the microbial load [29] and minimize thermal damage on both the nutritional and the physicochemical quality of fruit juice [30–32]. Processing by high-pressure homogenization (HPH) has therefore been considered as a good option to commercialize fresh, healthy, safe, and premium quality juices [33,34].

This is exemplified in a study by Guan et al. [35] in which they evaluated the effect of HPH on microbial inactivation, quality attributes, bioactive components, and antioxidant capacity of mango juice, which resulted in the complete inactivation of mold and yeast microflora at 190 MPa with one and three passes and total plate count up to 2.0 $\log_{10}$ CFU/mL. It was also able to retain and increase carotenoids and phenols between 11.8% and 21.4% when compared with juice treated at high temperatures (90 °C for 5 min).

Stinco et al. [34] demonstrated that the physicochemical properties and the ascorbic acid content of orange juice remained unchanged at 150 MPa, even improving bioaccessibility, which is mainly induced by the size and the structure of the juice particles. It was also shown that this type of treatment improves the incorporation of bioactive nutrients from natural extracts.

It is important to note that this methodology has the advantage of being able to optimize temperature and pressure to achieve pasteurization or sterilization effects. Therefore, homogenization and preservation can occur in the same single operation [36].

No studies have been found yet that report the combination of non-thermal technologies, such as pretreatment with HPH followed by cryoconcentration, on the microbiological quality and the bioactive compounds in maqui and calafate extracts. Therefore, the objective of this study was to evaluate the effect of cryoconcentration assisted by centrifugation-filtration on the bioactive compounds and the microbiological quality in aqueous maqui and calafate extracts pretreated with HPH.

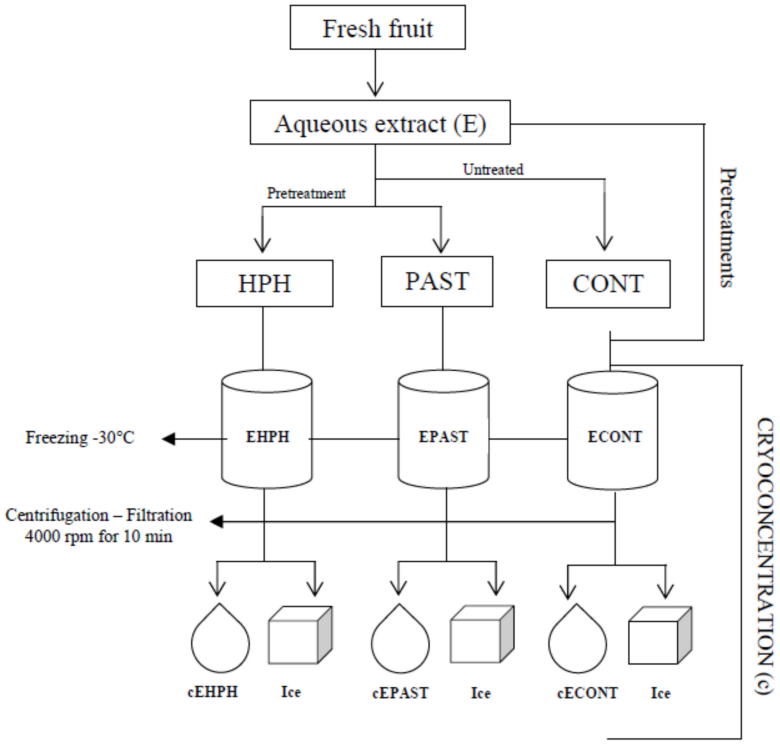
2. Materials and Methods

2.1. Raw Materials

Wild maqui (*Aristotelia chilensis* (Mol.) Stuntz) and calafate (*Berberis microphylla* G. Forst) fruits were collected in both the Ñuble and the Magallanes Regions in 2018. Fruits were washed, sanitized, conditioned, and refrigerated (4 °C) until processing.

2.2. General Experimental Procedure

Scheme 1 illustrates the experimental procedure to obtain maqui and calafate cryoconcentrates in which extracts were pretreated and later cryoconcentrated.



Scheme 1. General experimental procedure. Extracts were pretreated and later cryoconcentrated (c). HPH, high-pressure homogenization; PAST, pasteurization; CONT, untreated control sample; ECONT, untreated extract control; EHPH, extract treated by high-pressure homogenization: EPAST, extract treated by pasteurization; cECONT), untreated cryoconcentrated extract control; cEHPH, high-pressure cryoconcentrated extract; cEPAST, pasteurized cryoconcentrated extract.

The fresh fruits were pulped and subjected to the extraction of water-soluble compounds by mechanical agitation to obtain an aqueous extract. The extracts were pretreated by either HPH, pasteurization (PAST), or as an untreated control (CONT) and transferred to 700 mL centrifuge tubes with a filter separator according to patent INAPI-Chile N°2018–1410 [37]. These were frozen and transferred to a centrifuge to force the separation of the concentrated and frozen solution by cryoconcentration assisted by single-step centrifugation-filtration.

2.3. Production of Juice and Aqueous Extract

The fresh fruits were placed in a pulper (Phillips, HR-1832; Holland, Amsterdam) to separate the pulp from the seeds and the skin. The pulp was centrifuged at 4000 rpm for 10 min to obtain the juice, which was bottled in amber glass jars and stored at 4 °C.

The water-soluble compounds in the skin were extracted with distilled water (previously sterilized) at a ratio of 1:1.5 *w*/*v*; the mixture was shaken at 500 rpm (SCILOGEX, SK-018-Pro; London, United Kingdom) to obtain the aqueous E. Finally, the J and the E were mixed and vacuum filtered to produce the final maqui and calafate extracts, which

were poured into 100 mL amber glass bottles until further analysis. The extracts were free of antimicrobial preservatives, and the concentration of total soluble solids (TSS) depended on the initial fruit content.

Both HPH and PAST were used for microbial stabilization of the Es as described below.

2.4. High-Pressure Homogenization (HPH) and Pasteurization (PAST)

The extracts were separated into three batches of HPH, PAST, and CONT. The HPH treatments were performed at pressures of 100, 200, and 300 MPa and one and two passes in a continuous high-pressure system (Stanted 12500; Harlow, UK). The treated extracts were cooled in a water/ice mixture to 4 °C in order to apply the new HPH treatment; these were later stored in sterile plastic bottles to determine the microbiological quality parameters and the optimal conditions for the HPH treatment.

The PAST of the extracts was performed at 90 °C for 30 s according to Mena et al. [38]. The Es were placed in a temperature-controlled bath (JULABO ED 27; Seelbach, Germany), and the temperature at the geometric center of each sample was constantly monitored with a digital thermometer fitted with type K thermocouples (PCE T390; Meschede, Germany). The pasteurized maqui and calafate extracts (EPAST) were subsequently stored in sterile plastic bottles until further analysis.

Finally, the untreated extracts (ECONT) were used as controls (CONT).

2.5. Cryoconcentration by Centrifugation-Filtration

The maqui and calafate extracts treated by HPH, PAST, and CONT (EHPH, EPAST, ECONT) were cryoconcentrated according to the description in Section 2.2. The extracts were first transferred to tubes that were closed and frozen at −30 °C in a static freezer; the decrease in temperature was monitored by inserting type T thermocouples (copper-constantan) located in the geometric center of at least three tubes until completely frozen (−18 °C at the center).

The freezing rate (°C/min) was calculated as the temperature decreased from the freezing temperature to −10 °C [39]. Finally, the tubes were centrifuged (HETTICH ROTANTA 460 R) at 4000 rpm for 10 min to force the separation of the concentrated solute from the frozen fraction (ice) by simultaneously using centrifugation and filtration processes.

The maqui and calafate cryoconcentrates (c) (cEHPH, cEPAST, cCONT) were stored at 4 °C in plastic bottles until further analysis.

The concentration of soluble solids in c and the ice matrix was determined with a refractometer (ATAGO RX-5000 CX; Fukaya, Japan) at ambient temperature with a 0.1 °Bx precision.

2.6. Microbiological Parameters

The microorganism count was performed in the treated (EHPH, EPAST) and untreated (ECONT) maqui and calafate extracts and in the cryoconcentrates (cEHPH, cPAST, cECONT) by the total plate count (TPC) and the mold and yeast (M & Y) count methods [40].

Decimal dilution of the samples was prepared with a sterile NaCl solution, and 1.0 mL of each dilution was seeded on duplicate plates in plate count agar for TPC and 0.1 mL on dichloran-rose bengal chloramphenicol (DRBC) agar for the M & Y count. The plates were incubated at 30 ± 1 °C for 48 ± 2 h for TPC and 25 ± 1 °C for 5 d for M & Y.

Log N was calculated to determine the inactivation effect where N (CFU/mL) was the number of viable microorganisms after each treatment.

2.7. Analysis of Total Soluble Solids (TSS)

The TSS content was determined in the treated (EHPH, EPAST) and the untreated (ECONT) maqui and calafate extracts, in the cs (cEHPH, cPAST, cECONT), and in the frozen fraction (ice). Results were expressed as °Bx using a digital refractometer ((ATAGO RX-5000 CX; Fukaya, Japan)) at 25 ± 1 °C with a ± 0.1 °Bx precision. All the assays were performed in triplicate.

2.8. Process Parameter Calculations

The cryoconcentration process calculations were determined after concentrating the maqui and calafate extracts pretreated by HPH and PAST or as an untreated CONT.

2.8.1. Concentration Efficiency

Efficiency (η) was calculated according to the methodology described by Orellana et al. [14] and using Equation (1).

$$\eta\ (\%) = \frac{Cs - Cf}{Cs} \times 100 \quad (1)$$

where Cs and Cf are soluble solids (°Bx) in the concentrated solution and the ice fraction, respectively.

2.8.2. Solute Yield

Solute yield (Y) was calculated according to the methodology described by Moreno et al. [41] and using Equation (2).

$$Y = \frac{m_s}{m_o} \quad (2)$$

where Y is the solute yield, m_s is the solute mass in the concentrate, and m_o is the initial solute mass. The results were expressed as kg (mass) concentrate/kg (mass) extract.

2.8.3. Percentage of Impurities (I)

The percentage of impurities (I) was defined as the ratio of soluble solids that remain in the frozen fraction after the cryoconcentration process. These were calculated according to the description by Sánchez et al. [11] using Equation (3).

$$I\ (\%) = \frac{C_H}{C_{FS}} \times 100 \quad (3)$$

where C_H is the ice concentration expressed as °Bx, and C_{FS} is the final concentration of the concentrated solution.

2.8.4. Validation of Results

The experimental results were validated with a mass balance after the cryoconcentration process. These were compared with the predicted value according to the information provided by Petzold et al. [42] in Equation (4).

$$Wp = \frac{Cs - Co}{Cs - Cf} \quad (4)$$

where Wp is the predicted value of the ice mass ratio (kg ice/kg initial sample), Cs is the initial concentration of soluble solids (°Bx) in the concentrate, Co is the initial concentration of soluble solids of the aqueous extract (°Bx), and Cf is the concentration of soluble solids (°Bx) in the ice.

The theoretical value (We) of the ice mass ratio (kg ice/kg initial sample) was then calculated according to the description by Petzold et al. [42] using Equation (5).

$$We = \frac{Mf}{Mf + Mc} \quad (5)$$

where We is the theoretical value of the ice mass ratio (kg ice/kg initial sample), Mf is the ice mass (g), and Mc is the mass (g) of the concentrate after the cryoconcentration process, respectively.

Finally, the root mean square (RMS) was calculated by Equation (6) to determine the fit between the experimental and the predicted values of the ice mass ratio (We and Wp, respectively) for N experimental points of each extract subjected to cryoconcentration.

$$\mathrm{RMS}(\%) = 100\sqrt{\frac{\Sigma\left(\frac{\mathrm{We}-\mathrm{Wp}}{\mathrm{We}}\right)^2}{\mathrm{N}}} \quad (6)$$

2.9. Quantification of Bioactive Compounds

Total polyphenol content (TPC), total anthocyanin content (TAC), total flavonoid content (TFC), and antioxidant capacity (AOX) were determined in maqui and calafate aqueous extracts before and after the HPH and PAST treatments. In addition, these were determined for the products obtained by cryoconcentration.

2.9.1. Total Polyphenol Content (TPC)

The TPC was determined by the Folin–Ciocalteu method [43] in which a sample of 250 µL was mixed with 1250 µL Folin–Ciocalteu reagent. Subsequently, 2500 µL sodium carbonate (Na_2CO_3) previously diluted at 20% (*w*/*v*) was added. Finally, the mixture was shaken, the reaction occurred at ambient temperature (20 °C) in complete darkness for 30 min, and the absorbance was measured at 765 nm wavelength with a UV-Visible (UV-VIS, Spectrometer PG T-70; Greenville, SC, USA) spectrophotometer. Gallic acid (GA) was used to construct the standard curve pattern, and TPC results were expressed as mg gallic acid equivalents (GAE) for each 100 mL sample (mg GAE/100 mL).

2.9.2. Total Anthocyanin Content (TAC)

The TAC was quantified by the pH differential method [44]. A sample of 400 µL was added to 3600 µL potassium chloride (pH 1.0, 0.025 M, KCl) and 3600 µL sodium acetate (pH 4.5, 0.4 M, CH_3COONa). Absorbance was measured at 510 and 700 nm wavelengths with a UV-Visible (UV-VIS, Spectrometer PG T-70; Greenville, SC, USA) spectrophotometer. Differential absorbance was calculated by Equation (7).

$$\Delta A = [(A_{510} - A_{700})_{\mathrm{pH\ 1.0}} - (A_{510} - A_{700})_{\mathrm{pH\ 4.5}}] \quad (7)$$

Finally, TAC was determined according to Equation (8).

$$\mathrm{Anthocyanins}\left(\frac{\mathrm{mg}}{\mathrm{mL}}\right) = \frac{\Delta A \times \mathrm{MW} \times \mathrm{FD} \times 1000}{\varepsilon \times 1} \quad (8)$$

where ΔA is the absorbance differential at pH 1 and 4.5, FD is the dilution factor, ε is the molar extinction coefficient (29,000) of delphinidin-3-glucoside, and MW is the molecular weight of delphinidin-3-glucoside (462.5 g/mol).2.9.3. Antioxidant Capacity (AOX)

The AOX was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay based on the description by Brand-Williams et al. [45]. A sample of 100 µL was added to a 2900 µL DPPH (0.1 mM) solution. The mixture was homogenized and kept in the dark at ambient temperature (20 °C) for 40 min until a reaction occurred. Sample readings were taken with a UV-Visible (UV-VIS, Spectrometer PG T-70; Greenville, SC, USA) spectrophotometer at 515 nm wavelength. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard sample pattern, and results were expressed as mg trolox equivalents for mL of sample (mg TE/mL).

2.10. Statistical Analysis

The results were expressed as mean ± standard deviation. The statistical analysis was evaluated by the analysis of variance (ANOVA), and the treatment means were compared via least significant diference (LSD) or Student's t-test at level of significance $p \leq 0.05$. The Statgraphics Centurion XVI 4 (StatPoint Technologies Inc., Warrenton, VA, USA) version 16.2.04 software was used to analyze the data.

3. Results and Discussion

3.1. Optimization of Process Parameters of High-Pressure Homogenization (HPH) in Maqui and Calafate Extracts

Results of the counts for aerobic mesophilic microorganisms (AMM) and M & Y in HPH-pretreated maqui and calafate extracts are shown in Table 1. Specifically, the count of microbial aerobic mesophiles in maqui decreased from 2.92 $\log_{10}$ CFU/mL in the control (ECONT) to 1.76 $\log_{10}$ CFU/mL at 100 MPa with a single pass. A second pass at the same pressure decreased the count to 0.76 $\log_{10}$ CFU/mL. At $\geq$ 200 MPa, the count of microbial aerobic mesophiles and M & L were below the detection limit (ND, not detected). It should be noted that the initial AMM load in the extracts was low, which indicates their good microbiological quality.

Table 1. Counts of aerobic mesophilic microorganism (AMM) and mold and yeast (M & Y) in maqui and calafate extracts subjected to high-pressure homogenization (HPH) and controls.

Sample/ Treatment	Maqui		Calafate	
	AMM (Log_{10} CFU/mL)	M & Y (Log_{10} CFU/mL)	AMM (Log_{10} CFU/mL)	M & Y (Log_{10} CFU/mL)
Control	2.92 ± 1.98 [a]	3.69 ± 1.23 [a]	1.94 ± 0.07 [a]	4.14 ± 0.002 [a]
Control HPH W/P	2.71 ± 1.68 [a]	3.83 ± 1.00 [a]	1.98 ± 0.55 [a]	1.12 ± 0.01 [b]
100 MPa/1 pass	1.76 ± 2.49 [a]	ND	1.26 ± 0.30 [a]	ND
100 MPa/2 passes	0.76 ± 1.08 [a]	ND	1.21 ± 0.06 [a]	ND
200 MPa/1 pass	ND	ND	ND	ND
200 MPa/2 passes	ND	ND	ND	ND
300 MPa/1 pass	ND	ND	ND	ND
300 MPa/2 passes	ND	ND	ND	ND

Control, untreated extract; Control HPH W/P, extract subjected to HPH without applying pressure; ND, not detected. Different letters ([a,b]) in the same column indicate significant differences between the means of the different treatments ($p \leq 0.05$).

The highest count for M & Y was found in the untreated E (3.69 $\log_{10}$ CFU/mL). It was possible to reduce these microorganisms to undetectable levels at 100 MPa pressure and a single pass.

The AMM for the calafate E showed no significant difference ($p < 0.05$) between the controls and the 100 MPa treatments with one and two passes. However, microorganisms were reduced to undetectable values at $\geq$200 MPa with one pass. The M & Y showed no microorganism count at $\geq$100 MPa, which was the same result obtained for the maqui extracts (Table 1).

The behavior observed in the present study was similar to reports by several authors for the reduction of the microbial load (aerobic mesophilic bacteria, molds, and yeasts) in fruit juices. Geciova et al. [46] reported that the disruption of M & Y growth is more feasible than bacteria because of their larger size and different cell wall structures.

In the present study, the population of M & Y was completely eliminated at 100 MPa with one pass; this concurs with results reported by Bevilacqua et al. [47], Tribst et al. [48], Suárez-Jacobo et al. [25], and Guan et al. [35].

Welti-Chanes et al. [49] studied the effect of the number of passes on the microbial load in orange juice homogenized at 100 and 250 MPa and 22 °C, which decreased by approximately one algorithmic cycle when the number of passes increased (total of five passes). These findings concur with the results of the present study.

According to the results for the microbial count of AMM and M & Y in the aqueous maqui and calafate extracts treated by HPH, it was concluded that the most suitable treatment to continue with the subsequent cryoconcentration process was 200 MPa with one pass for both extracts. The content of bioactive compounds (total polyphenols, total anthocyanins, and antioxidant capacity) were not affected by the HPH treatment (data not shown).

3.2. Parameters of Cryoconcentration Process of Maqui and Calafate Cryoconcentrated Products

3.2.1. Soluble Solids Content in Maqui and Calafate Extracts and Concentrates

Table 2 shows the soluble solids content in the maqui and the calafate extracts and cryoconcentrates after the HPH process, PAST, and in the untreated control sample.

Table 2. Soluble solids content in maqui and calafate extracts and concentrates obtained by cryoconcentration after high-pressure homogenization, pasteurization, and an untreated control sample.

Sample	Maqui		Calafate	
Soluble Solids/ Treatment	**Sample (°Bx)**	**Ice (°Bx)**	**Sample (°Bx)**	**Ice (°Bx)**
Extract	10.54 ± 0.42 [a]	-	6.82 ± 0.19 [a]	-
cCONT	53.41 ± 2.99 [b]	2.11 ± 0.07 [a]	39.84 ± 6.39 [b]	1.57 ± 0.52 [a]
cHPH	49.89 ± 0.19 [b]	1.95 ± 0.06 [a]	43.48 ± 6.17 [b,c]	1.68 ± 0.57 [a]
cPAST	49.72 ± 2.76 [b]	2.01 ± 0. 35 [a]	49.46 ± 3.50 [c]	2.10 ± 0.40 [a]

cCONT, cryoconcentrate control; cHPH, cryoconcentrates previously treated by high–pressure homogenization; cPAST, cryoconcentrates previously treated by pasteurization. Different letters ([a–c]) in the same column indicate significant differences between the means of the different treatments ($p \leq 0.05$).

The initial soluble solids content of the fruits directly influenced the increased soluble solids in the cryoconcentrate. It was possible to increase the soluble solids content of maqui and calafate by 4 and 4.8 times, respectively. The residual content in the ice matrix was significantly low with values not exceeding 2.2 °Bx.

Studies conducted by different authors mentioned that the significant increase of the soluble solids content occurred after at least three cryoconcentration cycles. Petzold et al. [42] studied blueberry and pineapple juice using the cryoconcentration technique by freezing with three cryoconcentration cycles at a centrifugation speed of 4600 rpm for 10 min in each cycle. Results showed that soluble solids in blueberry juice increased from 13 to 20 °Bx in the first cycle, 26 °Brix in the second, and 33 °Bx in the third cycle. A similar behavior occurred in pineapple juice in which the initial solute increased from 14 to 32 °Bx in the third cryoconcentration cycle.

In another study with maqui, Bastías et al. [15] indicated higher values in three cycles of the process, which reached 34.95, 48.10, and 54.20 °Bx in the first, the second, and the third cycles, respectively, and at the freezing temperature of −20 °C. The large increase in soluble solids content resulted from the centrifugation process using cryoconcentration tubes with a filter inside, which allowed the complete separation of the concentrated liquid and the ice fraction.

In the present study, significantly higher values were obtained in a single cycle because the cryoconcentration tubes, along with having a filter to separate the concentrated solute and ice, had a greater sample capacity (approximately 350 mL extract), resulting in a larger amount of cryoconcentrated sample with high soluble solids content. This demonstrates the efficiency of the design of the filter separator tubes used in the cryoconcentration process.

3.2.2. Freezing Curves for Aqueous Maqui and Calafate Extracts

Figure 1 shows typical subcooling freezing curves for aqueous maqui and calafate extracts.

To begin freezing, the temperature had to be lowered to −0.95 °C for maqui E and −1.5 °C for calafate E. The sample began to freeze at a rate of 0.17 and 0.18 mm min^{-1} for maqui and calafate, respectively; complete freezing of 300 mL of the extract required 170 and 168 min, respectively.

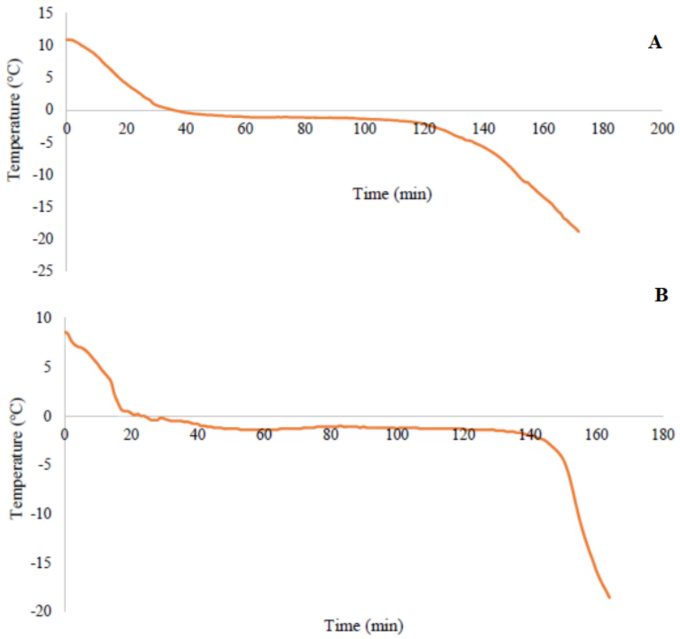


Figure 1. Freezing curves for the aqueous maqui (**A**) and calafate (**B**) extracts.

Estimation of the freezing point was previously reported by Raventós et al. [50], who designed a model to predict freezing points in orange juice at different concentrations of soluble solids. They mentioned that, when the juice reached 10 °Bx, the freezing point was −1.07 °C, and the freezing point was −4 °C for the same juice at 30 °Bx. Therefore, when the initial soluble solids content of the juice was higher, a lower temperature was required to begin the freezing process.

Petzold et al. [42] indicated that the soluble solids content in blueberry and pineapple juice produced a relevant difference in the freezing point, which was initially −3 °C at 13 °Bx and then decreased to −8 °C at 33 °Bx. Bastías et al. [15] mentioned that the freezing point for maqui extract with an initial 16.2 °Bx was −5.9 °C.

The difference between the freezing points can be due to the difference in the concentration and the type of soluble solids in the juice. Sánchez et al. [11] reported that, when solutes increased, the values of the freezing point decreased. This would explain the difference in freezing point obtained in the present study with other juices and maqui extract [15].

3.2.3. Cryoconcentration Parameters (Separation Efficiency, Recovered Solute Yield, Impurities, and Validation of Results) in Maqui and Calafate Cryoconcentrates

Table 3 shows the cryoconcentration parameters (separation efficiency, recovered solute yield, and impurities) after concentrating the aqueous maqui and calafate extracts, respectively, in a single cycle of the cryoconcentration process.

Table 3. Cryoconcentration parameters (separation efficiency, recovered solute yield, and impurities) in aqueous maqui and calafate extracts.

Sample	Maqui			Calafate		
Parameters/ Treatments	**Efficiency (%)**	**Solute Yield ***	**Impurities (%)**	**Efficiency (%)**	**Solute Yield ***	**Impurities (%)**
cCONT	96.08 ± 0.32 ^{a}	0.166 ± 0.01 ^{a}	3.92 ± 0.32 ^{a}	96.15 ± 0.68 ^{a}	0.150 ± 0.03 ^{a}	3.85 ± 0.68 ^{a}
cHPH	96.11 ± 0.12 ^{a}	0.192 ± 0.01 ^{a}	3.89 ± 0.12 ^{a}	96.23 ± 1.04 ^{a}	0.137 ± 0.04 ^{a}	3.77 ± 1.04 ^{a}
cPAST	95.97 ± 0.48 ^{a}	0.193 ± 0.02 ^{a}	4.03 ± 0.48 ^{a}	95.76 ± 0.71 ^{b}	0.107 ± 0.02 ^{b}	4.24 ± 0.71 ^{b}

cCONT, cryoconcentrate control; cHPH, cryoconcentrates previously treated by high-pressure homogenization; cPAST, cryoconcentrates previously treated by pasteurization; * kg (mass) concentrate/kg (mass) extract. Different letters (^{a,b}) in the same column indicate significant differences between the means of the different treatments ($p \leq 0.05$).

The separation efficiency of the solute for both maqui and calafate was greater than 95% for a single centrifugation-filtration cycle at 4000 rpm for 10 min, regardless of the applied microbiological stabilization treatment. Impurities, which are directly related to the separation level of the solute from the ice matrix, were maintained at less than 4.3% for both maqui and calafate.

Finally, the recovered solute yield showed that, for every 1000 g of extract subjected to the cryoconcentration process, a mean of 184 g and 131 g of maqui and calafate concentrates, respectively, can be recovered. In addition, a mass balance was performed for each of the cryoconcentrated extracts to validate the results. This balance relates the soluble solids content and mass of each extract pre- and post-treatment by comparing the predicted and the theoretical values. The mass balance provides the validation of results by determining the value of RMS, indicating the fit between We and Wp, as shown in Figure 2.

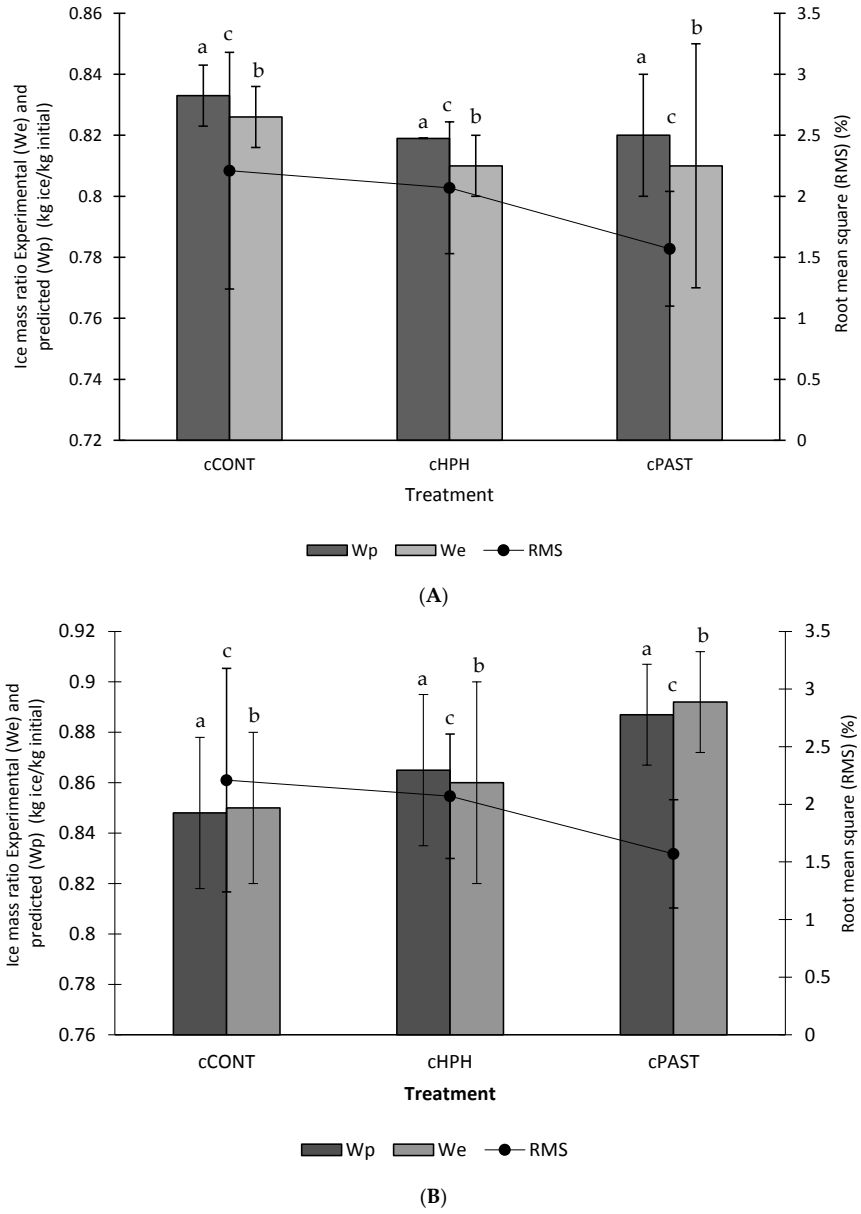


Figure 2. Validation of results obtained in the cryoconcentration process of aqueous maqui (**A**) and calafate (**B**) extracts. cCONT, cryoconcentrate control; cHPH cryoconcentrate previously treated by high–pressure homogenization; cPAST, cryoconcentrate previously treated by pasteurization. Different letters (a–c) for the same variable indicate significant differences between the means of the different treatments ($p \leq 0.05$).

Figure 2A,B illustrate the results for Wp and We of the maqui and the calafate cryoconcentrates, respectively, after the microbial stabilization treatments.

There was no significant difference ($p \leq 0.05$) between the experimental (We) and the predicted (Wp) values in both Figure 2A,B. The same occurred for RMS whose values were less than 2.2% for maqui and 1.6 % for calafate.

Results for process efficiency and impurities were significantly higher than those indicated by other authors. When Petzold et al. [42] studied centrifugation-assisted cryoconcentration in blueberry and pineapple juice, they found that the juice separation efficiency values were 66.08% and 58.72%, respectively. These values are approximately 30% lower than those of the present work.

The reported values for recovered solute yield were 0.67 kg solute/kg initial solute for blueberry juice and 0.48 kg solute/kg initial solute for pineapple juice; the validated results were approximately 0.6 for Wp and We. Petzold et al. [51] studied vacuum-assisted cryoconcentration for wine, and they mentioned that the amount of solute increased exponentially over time. They revealed that values of 0.5 kg solute/kg initial solute were reached in 10 min with 87% efficiency and 0.82 kg solute/kg initial solute with 90% efficiency when time was increased to 21 min.

Bastías et al. [15] studied centrifugation-assisted cryoconcentration (under the same speed and time conditions as in the present work) in maqui; their results indicated that separation efficiency, recovered solute, and impurities were 99.28%, 0.43 kg solute/kg initial solute, and 0.72%, respectively, in a cryoconcentration cycle. As for the mass balance, these authors mention values of 0.54 and 0.57 kg ice/kg initial mass for We and Wp, respectively, and 8.76% for RMS in the first cryoconcentration cycle.

Orellana-Palma et al. [17] studied centrifugation-assisted cryoconcentration at different freezing temperatures (−20 °C and −80 °C) and the heat transfer direction (radial and unidirectional). They obtained higher values at −20 °C unidirectional freezing for separation efficiency and obtained a recovered solute yield of 72% and 0.78 kg solute/kg initial solute, respectively. They also observed, as did the aforementioned authors, a good fit between the experimental (We) and the predicted (Wp) ice mass ratios during the cycles and obtained RMS values between 2.6% and 8.5% at the end of the third cycle.

Compared with the previously mentioned scientific evidence, the present study showed a higher separation efficiency and lower impurity levels. However, the amount of recovered solutes was smaller because of the high concentration of soluble solids obtained in a single cycle of the cryoconcentration process by centrifugation-filtration. This increased the soluble solids content by four times in maqui and five times in calafate.

There were no significant differences between Wp and We values in the present study and results were similar. This demonstrates that the fit of the calculated values for soluble solids and mass were highly correlated. Finally, for the validation of results, the RMS value did not exceed 2% in the cryoconcentration process of both aqueous maqui and calafate extracts. This is an acceptable value according to Lewicki [52] because it is less than 25%.

3.3. Bioactive Compounds (Total Polyphenols, Total Anthocyanins, and Antioxidant Capacity) Pre- and Post-Treatment by High–Pressure Homogenization (HPH) and Cryoconcentration of Aqueous Maqui and Calafate Extracts

Figure 3A,B show the content of the bioactive compounds in maqui and calafate extracts and cryoconcentrates pre- and post-treatment by HPH and PAST. These results indicate that maqui had a higher content of bioactive compounds and antioxidant capacity than calafate.

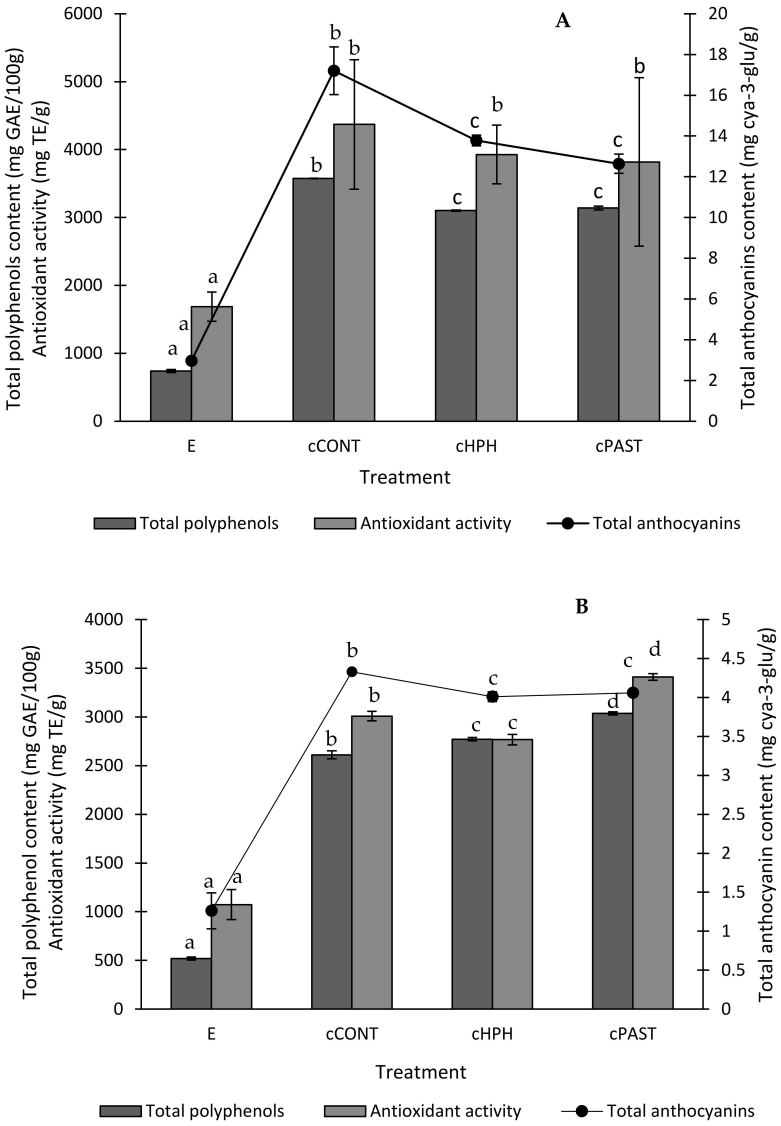


Figure 3. Contents of bioactive compounds (total polyphenols, total anthocyanins, and antioxidant capacity) in maqui (**A**) and calafate (**B**) extracts and cryoconcentrates. E, extract; cCONT, cryoconcentrate control; cHPH cryoconcentrate previously treated by high–pressure homogenization; cPAST, cryoconcentrate previously treated by pasteurization. Different letters (a–d) for the same variable indicate significant differences between the means of the different treatments ($p \leq 0.05$).

Single-step cryoconcentration by centrifugation-filtration significantly increased the content of bioactive compounds in aqueous maqui and calafate extracts. Total polyphenols, total anthocyanins, and antioxidant capacity of maqui increased by 4.8, 5.8, and 2.6 times, respectively. Meanwhile, cryoconcentration increased the initial content of total polyphenols, total anthocyanins, and antioxidant capacity by 5.0, 3.4, and 2.8 times, respectively, in the aqueous calafate extract.

However, comparing the cryoconcentrates in the different microbial stabilization treatments applied to maqui (Figure 3A), such as HPH and PAST, with the untreated control cryoconcentrate, there was a 10% to 25% decrease. Meanwhile, in calafate, there was a slight increase of these compounds, approximately 6% to 16% in total polyphenols and up to 13% in antioxidant capacity in the PAST sample.

The content of bioactive compounds in the aqueous maqui and calafate extracts represented approximately 20% of the content of the fresh fruit of both fruits (data not shown). This was mainly due to the dilution process in the extraction of water-soluble compounds to which the fruits were subjected and their large number of seeds and reduced amount of pulp. This treatment consisted of diluting the content of these compounds in the

fruit with water at a 1:1.5 w/v ratio. However, total polyphenol content, total anthocyanin content, and antioxidant capacity were in line with findings obtained in other studies using maqui and calafate fruits [15,53,54].

The slight decrease in the total polyphenol content of the maqui E treated by HPH and subsequently cryoconcentrated was reported by Velázquez-Estrada et al. [31] for orange juice; the control sample containing 77.10 mg GAE/100 mL was reduced to contents of 76.5, 75.9, and 72 mg EAG/100mL when treated by HPH at 100, 200, and 300 MPa, respectively. These results showed that using higher process pressure leads to a greater reduction in the content of these compounds from 2% to 7%.

However, Suárez-Jacobo et al. [55] reported the opposite when studying the influence of HPH at 200 MPa with an inlet temperature at 4 °C on the total polyphenol content of apple juice. They found an increase from 11.69 mg EAG/L in fresh juice to 12.89 in HPH and 14.50 mg EAG/L in pasteurized juice (90 °C for 4 min). These same authors indicated that the low total polyphenol content in the control sample compared with the HPH-treated and thermally pasteurized sample can be attributed to enzymatic oxidation, which produced high molecular weight compounds that were unmeasurable [56].

Velázquez-Estrada et al. [31] studied the effect of HPH at 200 MPa in orange juice, and they observed that this treatment decreased the total polyphenol content. However, it also significantly decreased in pasteurized juice (90 °C for 1 min) compared with the untreated juice. Guan et al. [35] studied the effect of HPH in mango juice at 190 MPa (one pass) and compared it with the thermal pasteurization process at 90 °C for 5 min; they reported that the juice had 154.53 and 148.31 mg EAG/L total polyphenols in each treatment, respectively. These results are comparable with the findings of the present study of maqui and calafate, whose loss can be attributed to polymerization and oxidation, because polyphenols reduce the number of hydroxyl groups measured by the Folin–Ciocalteu method [57].

Previous research of anthocyanins has mentioned that the processing of berries usually causes anthocyanin degradation, which arises as a result of indirect oxidation by phenolic quinones produced by polyphenoloxidase and peroxidase [58–60]. This can occur to a greater or lesser degree depending on the process being applied.

Frank et al. [61] studied anthocyanin stability directly using HPH from 0 to 150 MPa in blueberry extract solutions; they detected no degradation of these compounds due to mechanical stress. Specifically, high shear rates and local cavitation did not significantly affect the content of these compounds at these treatment pressures.

Yu et al. [26] studied the anthocyanins of blackberry juice subjected to HPH at 200 MPa and compared this with the PAST process at 95 °C for 1 min. They found that fresh juice had 0.565 g/L equivalent to cyanidin 3-glucoside, which was treated by HPH at 200 MPa with one pass, and the content decreased by 38.8%, while it decreased by 10.8% using PAST. We observed that the content of bioactive compounds decreased when comparing cHPH and cPAST for maqui in contrast with cCONT. However, calafate was not affected in cHPH, while total polyphenols and anthocyanins in cPAST increased as compared with cCONT (Figure 3).

This was likely due to the presence of fruit enzymes also found in unpasteurized juices, such as polyphenoloxidase, peroxidase, and β-glucosidases, which can cause rapid anthocyanin oxidation [62,63]. It is also well known that anthocyanin stability can be affected during storage by enzymatic degradation, temperature, light, pH, ascorbic acid, sugars, oxygen, metal ions, and pigments [64].

The differences between the two fruits for anthocyanin content after subjecting them to different treatments (HPH and PAST) could be due to the type of anthocyanin compounds of each fruit. These compounds have a greater or lesser capacity to form copigmentation with metal ions and phenolic compounds or to bind with one or more sugars to form lateral glyco-linkages, which increase stability and prevent the degradation of their structures [28].

This co-pigmentation is a very important mechanism because it stabilizes the color in plants [65] and products made from fruits containing high anthocyanin contents, such as maqui and, to a lesser extent, calafate.

Finally, according to studies referring to antioxidant capacity, the HPH treatment most often increased the availability of antioxidant compounds because cells and particles were broken into smaller fragments [66]. Kubo et al. [67] studied the use of HPH in tomato juice and reported that the decrease in particle size by pressure and number of passes led to the release of cellular material and thus improved the antioxidant content because of particle extraction or depolymerization of complexes induced by the treatment [68,69].

However, the opposite occurred in the present study in which the untreated cryoconcentrate exhibited higher antioxidant capacity. Given the small amount of pulp and the high number of seeds, this likely occurred because the fruits were subjected to a grinding process in which they were ground and subjected to the aqueous extraction of water-soluble compounds. The antioxidant compounds were therefore released and did not increase after HPH and PAST treatments but rather slightly decreased.

4. Conclusions

The high-pressure homogenization (HPH) pretreatment at 200 MPa (one pass) resulted in maqui (*Aristotelia chilensis* (Mol.) Stuntz) and calafate (*Berberis microphylla* G. Forst) extracts with a microbial load below the detection limit for aerobic mesophilic microorganisms (AMM) and molds and yeast (M & Y). In addition, the applied cryoconcentration process achieved solute separation efficiency from the frozen matrix greater than 95%, increasing soluble solids from the aqueous maqui and calafate extracts by more than four times. Total polyphenols were concentrated 4.8 and 5.0 times and total anthocyanins 5.8 and 3.4 times for maqui and calafate, respectively. The antioxidant capacity of the cryoconcentrates increased 2.6 times more than the content found in the maqui and the calafate extracts. Accordingly, HPH and subsequent cryoconcentration by centrifugation-filtration is a potential technology to obtain fruit concentrates with good microbiological quality and high contents of bioactive compounds (total polyphenols, total anthocyanins, and antioxidant capacity) in a single cycle and high process efficiency using the new design of cryoconcentrate tubes.

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