applied sciences

MDPI

Article

Influence of Cryoconcentration on Quality Attributes of Apple Juice (*Malus Domestica* cv. *Red Fuji*)

Patricio Orellana-Palma [1,*], Virgilio Lazo-Mercado [2], María Pía Gianelli [2], Eduard Hernández [3], Rommy N. Zúñiga [1,4] and Guillermo Petzold [2,*]

1 Department of Biotechnology, Universidad Tecnológica Metropolitana, Las Palmeras 3360, P.O. Box, 7800003 Ñuñoa, Santiago, Chile; rommy.zuniga@utem.cl
2 Department of Food Engineering, Universidad del Bío-Bío, Av. Andrés Bello 720, Casilla 447, 3780000 Chillán, Chile; vilo.lazo@gmail.com (V.L.-M.); mgianel@ubiobio.cl (M.P.G.)
3 Agri-Food Engineering and Biotechnology Department, Universitat Politècnica de Catalunya (UPC BarcelonaTech), Esteve Terradas, 8, 08860 Castelldefels, Barcelona, Spain; eduard.hernandez@upc.edu
4 Programa Institucional de Fomento a la I+D+i, Universidad Tecnológica Metropolitana, Ignacio Valdivieso 2409, San Joaquín, 8940577 Santiago, Chile
* Correspondence: p.orellanap@utem.cl (P.O.-P.); gpetzold@ubiobio.cl (G.P.)

Received: 30 December 2019; Accepted: 24 January 2020; Published: 2 February 2020

Abstract: Apple juice was subjected to centrifugal block cryoconcentration (CBCC) for three cycles and their effect on the physicochemical properties, bioactive compounds, antioxidant activity, volatile profile, and sensory analysis was investigated. In the final cycle, the solutes were approximately four-fold of the initial condition (≈14 °Brix) and the color (ΔE* ≈ 25.0) was darker than the fresh juice, with bioactive compound concentration values close to 819 mg GAE/100 g d.m., 248 and 345 mg CEQ/100 g d.m. for total polyphenol, flavonoid, and flavanol content, respectively, equivalent to a retention of over 60%. DPPH and FRAP assays presented high antioxidant activities, with values of approximately 1803 µmol TE/100 g d.m. and 2936 µmol TE/100 g d.m, respectively. The cryoconcentrate showed a similar aromatic profile to the fresh juice, with 29 and 28 volatile compounds identified, respectively. The centrifugal force allowed to obtain excellent process parameters, with 73%, 0.87 (kg/kg), and 85% for efficiency, solute yield, and percentage of concentrate, respectively. Sensory evaluation shows that the odor, aroma, and flavor of fresh sample were remained in the reconstituted cryoconcentrate sample, with good qualifications (four points in a five-score hedonic scale) by trained panelists. Therefore, CBCC can preserve important quality attributes from apple juice.

Keywords: cryoconcentration; apple juice; physicochemical properties; bioactive compounds; aromatic profile; process parameters; sensory analysis

1. Introduction

Apples have been widely recognized as a highly consumed fruit worldwide. Specifically, this fruit presented in 2019/2020 a world production of approximately 76 million tons [1]. Thus, the food industry has applied traditional technologies to keep this fruit available throughout the year through the development of numerous products from fresh apples, such as juice, jellies, jams, purees, wines, and sauces, among others [2]. However, concentration by evaporation, the most used technology in the food industry, exhibited disadvantages due to the high temperatures used, which accelerate the degradation of physicochemical properties and bioactive compounds, and affect the health benefits associated to fruit consumption [3]. Therefore, in the last decade, potential and alternative non-thermal technologies, such as pulsed UV-light (PL), ultrasound (US), irradiation (IR), pulsed electric field (PEF),

high-pressure processing (HPP), cold plasma (CP), and cryoconcentration (CC) have been studied in order to protect and preserve various heat-labile components, and so, retain important nutritional and sensory characteristics in the final product [4].

CC has been known as a novel, non-thermal and emerging technology, in which the temperature of an aqueous solution or dispersion is cooled below its freezing point until the complete solidification of the liquid sample, then the unfrozen liquid fraction (cryoconcentrate) is separated from the ice phase by natural thawing or by addiction of an external force (also called assisted technique) to the CC process [5]. Hence, CC can be used as a concentration technology to preserve heat-labile components, with a minimal loss of important components [6].

Actually, three CC procedures are available at industrial, semi-industrial and laboratory level: (i) suspension (SCC) [7], (ii) progressive (PCC) [8,9], and (iii) block cryoconcentration (BCC) [10]. In BCC, a liquid solution is completely freezing; then the block is thawed and, finally, the cryoconcentrate is separated from the ice fraction. Therefore, BCC utilizes three steps: complete freezing, thawing, and separating [11]. Precisely, the final stage in BCC has been carried out by passive thawing [12] or with the help of assisted techniques coupled to the BCC system to improve some process parameters such as efficiency, solute yield and/or percentage of concentrate [5].

Specifically, centrifugation has been applied as an assisted technique to BCC, with remarkable results, since it enhanced the solute extraction from the veins formed in the ice fraction and, thus, it increased the process parameters and final amount of cryoconcentrate solute after the separation step. Studies have reported that the efficiency process was improved with values between 60% and 75% at three centrifugal cycles [13]. Hence, centrifugal block cryoconcentration (CBCC) has been applied in different frozen liquid samples [14–20]. Although CBCC has been applied in fruit juices, there is very little scientific data reported in the literature on the retention of heat-labile components in various juices, e.g., fresh apple juice.

Therefore, the overall objective of the present study is to evaluate the effect of CBCC to protect valuable quality attributes, such as physicochemical parameters, bioactive compounds, antioxidant activity, volatile profile and sensory properties of apple juice (*Malus domestica* cv. *Red Fuji*).

2. Materials and Methods

2.1. Reagents and Standards

A general experimental procedure is schematized in Figure 1. The apple juice was axially frozen in Folin–Ciocalteu reagent, Na_2CO_3, Gallic acid, $NaNO_2$, $AlCl_3$, NaOH, Catequin, vanillin reagent, HCl, DPPH methanolic solution, FRAP reagent and the aroma standards were all purchased from rom Sigma-Aldrich (St. Louis, MO, USA).

2.2. General Experimental Procedure

A general experimental procedure is schematized in Figure 1. The apple juice was axially frozen in centrifugal tubes (45 mL of sample) at −20 °C for 12 h, and then the frozen samples were transferred to a centrifuge equipment operated at 20 °C for 15 min at 4000 rpm (1600 RCF) to force the separation of solutes from the ice fraction during thawing at three centrifugal cycles [16]. After each CBCC cycle, a portion of concentrated was collected, and thus, the quality attributes were determined.

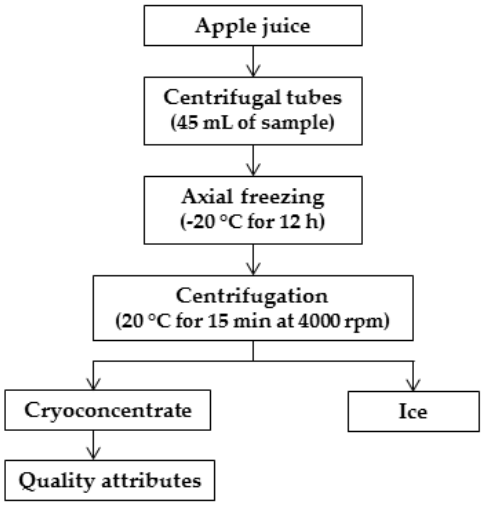


Figure 1. General centrifugal-assisted BCC experimental procedure.

2.3. Preparation of Apple Juice

Whole fresh apples (*Malus domestica* cv. *Red Fuji*) were acquired from a local market in Chillán (Región del Ñuble, south region of Chile). Specifically, the apples were manually collected, with commercial maturity (85%–100% mature stage), during the main harvest season (Summer 2018) from orchards in Villa Alegre (Región del Maule, south-central region of Chile). Región del Maule is characterized by a mid-latitude temperate climate with four seasons. In summer, the temperature in Maule ranges from 22 to 30 °C, and daylight hours from 60% to 70% of the day [16]. The fruits were stored under refrigeration at 4 °C until processing. The apples were washed, peeled, cut into half, and the seeds were removed with a knife. Later, the flesh was cut into small pieces and squeezed with a household juicer. The fruit juice was pressed with a fine-mesh nylon cloth (0.8 mm mesh) to avoid the presence of solids and seeds that might interfere with the CBCC process. The prepared juice was then kept at 4 °C until used.

2.4. CBCC Protocol

The cryoconcentrate juice was obtained using a method previously described by Orellana-Palma et al. [17], where fresh apple juice (45 mL) was placed in centrifugal plastic tubes (internal diameter, D_i = 22 mm). Later, the samples were isolated with foamed polystyrene (8 mm thickness, thermal conductivity K = 0.035 W/mK) in order to induce an axial freezing front propagation (from the top to bottom), and thus, the tubes were frozen at −20 °C (overnight) in a vertical static freezer (280, M and S Consul, Sao Paulo, Brazil). Then, the frozen samples were transported to a centrifuge with a 50 mL-tube rotor (Eppendorf 5430R, Hamburg, Germany) at 20 °C for 15 min at 4000 rpm (1600 RCF). Hence, the centrifugation was used as assisted technique in the BCC separation process. The cryoconcentrated solution after the first cycle was used as the new solution for the second cycle and so on, until obtaining three CBCC cycles as indicated in Figure 2. For CBCC protocol three replicates and the mean values were reported.

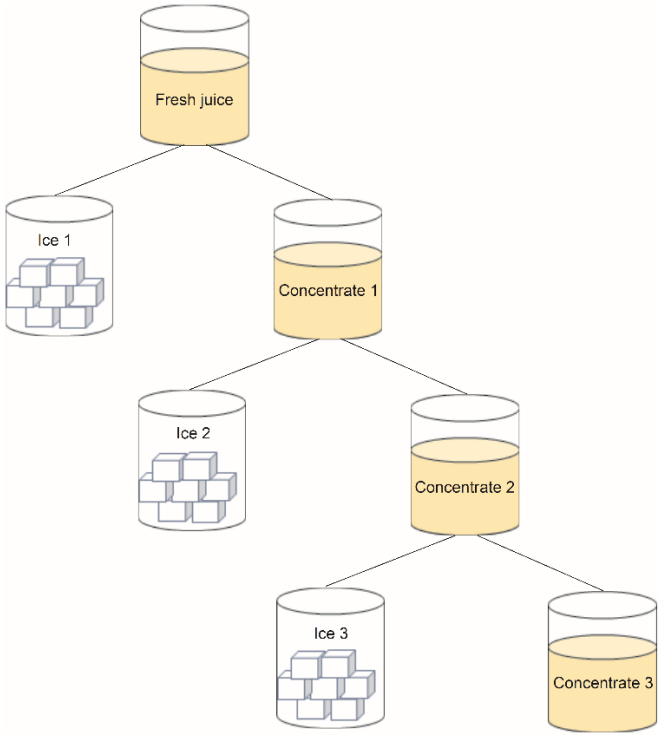


Figure 2. General centrifugal-assisted BCC protocol using three consecutive cycles.

2.5. Physicochemical Analysis

The total soluble solid content (TSSC, °Brix) was analyzed with a digital refractometer (PAL-1, Atago Inc., Tokyo, Japan). The pH was determined using a digital pH meter (Hanna model HI 2221, Woonsocket, RI, USA). The total titratable acidity (TTA) was estimated by titration with sodium hydroxide (0.1 N) and expressed in grams of malic acid per liter (g malic acid/L). The density (ϱ) was obtained by using the method previously proposed by Tansakul et al. [21]. The color values were measured with a spectrophotometer (Konica Minolta CM-5, Osaka, Japan) (D65 illuminant and 2° observer), in CIELab space terms, i.e., the L* (lightness), a* (ranging from green to red), and b* (ranging from blue to yellow) parameters were determined. From CIELab values, Hue angle (h^*_{ab}), chroma (C^*_{ab}), and total color difference (ΔE*, difference between the fresh juice and concentrated samples) were obtained using Equations (1), (2), and (3), respectively. Physicochemical analysis were performed for the initial solution (C_0) and each concentrated (C_s) fraction obtained along the cycles. All determinations were completed in triplicate at ambient temperature (≈22 °C).

$$h^*_{ab} = \text{arctg}\left(\frac{a}{b}\right) \quad (1)$$

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

$$\Delta E^* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3)$$

2.6. Bioactive Compounds (BC) Determination

The total polyphenol content (TPC), total flavonoid content (TFC) and total flavanols content (TFLC) of fresh juice and samples were measured in order to determine the changes on these through the CBCC cycles.

TPC was evaluated colorimetrically by the Folin–Ciocalteau assay according to the methodology proposed by Singleton et al. [22], with modifications. A total of 100 μL of sample was mixed with

100 µL of 10-fold diluted Folin–Ciocalteu reagent and 300 µL of 5% (*w/v*) Na_2CO_3. After 90 min in the dark at room temperature (incubation), the absorbance was measured at 760 nm. Gallic acid was used as standard and the results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry matter (mg GAE/100 g d.m.).

TFC was measured by the aluminum chloride colorimetric method described by Paz et al. [23], with modifications. 250 µL of sample, 1000 µL of distilled water and 75 µL of 5% (*w/v*) $NaNO_2$ were mixed. After 6 min (incubation), 75 µL of 10% (*w/v*) $AlCl_3$, 500 µL of NaOH (1 M), and 600 µL of distilled water were added and the absorbance was measured at 510 nm. Catequin was used as standard and TFC results were expressed as mg catechin equivalents (CEQ) per 100 g of dry matter (mg GAE/100 g d.m.).

TFLC was estimated according to the vanillin–HCl method proposed by Broadhurst and Jones [24], with some modifications. A total of 0.2 mL of sample was added to 1.5 mL of vanillin reagent and 0.8 mL of 1% (*w/v*) HCl, and the mixture was vortexed for 10 min. After 15 min (incubation), the absorbance was measured at 500 nm. Catequin was used as standard and TFLC results were expressed as mg catechin equivalents (CEQ) per 100 g of dry matter (mg GAE/100 g d.m.).

All bioactive compounds measurements were determined using a T70 UV–VIS spectrophotometer (Oasis Scientific Inc., USA) in triplicate at 25 °C.

2.7. Total Bioactive Compound (TBC) Retention

The TBC retention indicates the total bioactive compound percentage in the concentrated fraction respect to the initial sample (fresh apple juice). The retention was determined at each cycle using Equation (4) [17]:

$$\text{TBC retention } (\%) = \left(\frac{C_0}{C_s}\right)x\left(\frac{BC_s}{BC_0}\right)x100\% \quad (4)$$

where C_0 is the initial TSSC (°Brix), C_s is the concentration of TSSC (°Brix) at each cycle, BC_s is the TBC at each cycle, and BC_0 is the initial TBC.

2.8. Antioxidant Activity Determination

2.8.1. DPPH

A DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was performed as described by Thaipong et al. [25], with some modifications. 150 µL of sample was mixed with 2850 µL of 10 mg/L DPPH methanolic solution. After 35 min in the dark at room temperature (incubation), the absorbance was measured at 515 nm on a spectrophotometer (T70 UV–VIS spectrophotometer, Oasis Scientific Inc., Greenville, SC, USA). Trolox was used as standard, and the results were calculated and expressed as micromoles of Trolox equivalents (TE) per 100 g of dry matter (mg TE/100 g d.m.). All analyses were performed in triplicate at 25 °C.

2.8.2. FRAP

A FRAP (ferric reducing antioxidant power) assay was determined following the method reported by Benzie and Strain [26], with modifications. A total of 300 µL of sample was mixed with 2250 µL of FRAP reagent. Then, the mixture was kept in the dark at room temperature for 35 min (incubation). The absorbance was measured at 593 nm on a spectrophotometer (T70 UV–VIS spectrophotometer, Oasis Scientific Inc., USA). Trolox was used as standard, and the results were calculated and expressed as micromoles of Trolox equivalents (TE) per 100 g of dry matter (mg TE/100 g d.m.). All analyses were performed in triplicate at 25 °C.

2.9. Identification and Quantification of Volatile Compounds

Volatile profiles of each sample (fruit juice and cycles) were acquired using headspace vials. This determination was developed through the technique of solid phase microextraction (SPME), with a gas chromatograph-flame ionization detector (GC-FID).

A total of 8 mL of sample were inserted into headspace vials and sealed with a PTFE-faced silicone septum (Supelco, Bellefonte, PA, USA). Afterwards, the samples were warmed up for 30 min at 60 °C in a thermoblock (2050-ICE, Paris, France). These conditions allow to equilibrate the headspace and SPME fiber. A fiber carboxen/polydimethylsiloxane (85 µm, Car/PDMS, Supelco, Bellefonte, PA, USA) adsorbed the volatile compounds, which were injected in the GC (Perkin-Elmer, Clarus 680, Shelton, CT, USA). A DB-624 capillary column (Length: 60 m, id: 0.25 mm, film thickness: 1.8 µm, J&W Scientific, Folsom, CA, USA) was used to separate the compounds. The carrier gas employed was nitrogen at a 1.2 mL/min speed rate.

The samples in the SPME fiber were inserted in the port, with the purge valve off (splitless mode). Thus, in the first 5 min, the temperature was maintained at 50 °C. Later, the temperature was increased by 4 °C/min until reaching 98 °C. Next, three gradients at 4 °C/min were applied, until reaching 130 °C, 150 °C, and 230 °C, respectively. The time of the temperature process was equivalent to 50 min. The retention time, authentic standards and the Kováts Index (KI) allowed to identify each volatile compounds.

Three replicates of each sample were reported, with the mean and standard deviation values, and the percentage of area.

2.10. Process Parameters

2.10.1. Efficiency Process (Eff)

Eff is defined as the increase in the concentration of the solution relative to the quantity of solids remaining in the frozen fraction and it was calculated according to Equation (5) [27]:

$$\text{Eff}(\%) = \left(\frac{C_s - C_i}{C_s}\right)x100\% \quad (5)$$

where C_s and C_i are the concentration (TSSC, °Brix) in the cryoconcentrate and ice fractions, respectively.

2.10.2. Percentage of Concentrate (PC)

PC represents the evolution of the solution removal from the frozen phase and it was calculated according to Equation (6) [13]:

$$PC = \frac{W_0 - W_i}{W_0}x100\% \quad (6)$$

where W_0 and W_i are the initial and final weight of the frozen fraction, respectively.

2.10.3. Solute Yield (Y)

Y represents the relationship between the mass of solute in the concentrated fraction and in the initial sample. Y was determined with Equation (7) [17]:

$$Y = \frac{m_s}{m_0} \quad (7)$$

where m_s and m_0 are the solute mass in the concentrated solution and the initial solute mass, respectively.

2.11. Sensory Analysis

A sensory evaluation was performed by 21-member trained panelists to measure the degree of acceptance or rejection of samples. Specifically, the odor, aroma, flavor, and global assessment were

evaluated according to a five-score hedonic scale: 5 = Like very much; 4 = Like moderately; 3 = Indifferent; 2 = Dislike moderately; 1 = Dislike very much. Thus, this sensory evaluation was used to verify if the reconstituted cryoconcentrate sample (3rd cycle) has significant differences with the fresh juice, in hedonic scale terms. After each CBCC cycle, the cryoconcentrate samples were reconstituted with the addition of distilled water until to obtain a similar TSSC value than the fresh apple juice (≈14 °Brix). The samples were evaluated at room temperature and numbered with three digits at random.

2.12. Stadistical Analysis

Data were analyzed using analysis of variance (ANOVA) with Statgraphics Centurion software (Version XVI, StatPoint Technologies Inc., Warrenton, VA, USA). Differences between the mean values were established by Student's t-test and the least significant difference (LSD) at 5%.

3. Results and Discussion

3.1. Physicochemical Analysis

The results from the physicochemical analysis are summarized in Table 1. Firstly, the TSSC increased gradually compared with the fresh sample (≈14 °Brix), with 31, 45, and 55 °Brix along the cycles. The TSSC at each cycle were superior to those obtained in previous studies under comparable conditions in our laboratory with fruit juices such as orange juice [14], pineapple juice [20], and blueberry juice [28], in which the final concentration values were approximately 40, 36, and 33 °Brix at the third cycle, respectively. Similarly, the TSSC results were higher than those reported by Sánchez et al. [29], Moreno et al. [30], Zielinski et al. [31] and Ding et al. [32], in which orange juice, coffee extract, and apple juice were cryoconcentrated by falling-film (FFCC), BCC, and SCC, respectively. Despite the values in our study, SCC, FFCC, and PCC have more advanced technological development than BCC. So, future studies could focus on block cryoconcentration at semi-industrial scale or pilot-plant scale.

Table 1. Physicochemical properties of apple fresh juice and cryoconcentrated samples at each cycle[1].

	Fresh Juice	CBCC Cycle 1	CBCC Cycle 2	CBCC Cycle 3
TSSC (°Brix)	13.9 ± 1.0^{d}	31.4 ± 1.9^{c}	44.7 ± 1.7^{b}	54.9 ± 0.7^{a}
pH	3.5 ± 0.0^{a}	3.4 ± 0.0^{b}	3.3 ± 0.0^{c}	3.1 ± 0.0^{d}
TTA (g malic acid/L)	2.3 ± 0.0^{d}	2.5 ± 0.0^{c}	2.8 ± 0.0^{b}	3.0 ± 0.1^{a}
ρ (g/mL)	1.1 ± 0.0^{d}	1.2 ± 0.0^{c}	1.4 ± 0.0^{b}	1.5 ± 0.0^{a}
Color				
L*	78.1 ± 1.3^{a}	76.9 ± 3.0^{a}	68.1 ± 1.9^{b}	68.4 ± 0.4^{b}
a*	3.9 ± 0.2^{c}	6.1 ± 1.6^{b}	10.9 ± 0.9^{a}	11.0 ± 0.6^{a}
b*	27.7 ± 0.4^{c}	38.9 ± 4.7^{b}	41.1 ± 2.2^{a}	43.5 ± 3.7^{a}
ΔE*	-	11.6 ± 0.2^{c}	17.2 ± 0.1^{b}	25.0 ± 0.0^{a}

[1] Within each row, a, b, c, d when there are no significant differences ($p \leq 0.05$), are identified by the same superscript letter, according to a LSD test.

In addition, the results reflected an increase over 2.3, 3.2, and 4.0 times, in concentration index (CI, ratio C_s/C_0) terms, from the first to the third cycle, respectively. Therefore, the difference in the TSSC results could be assigned to the sized tubes that contained the liquid samples. Concretely, in our studies, we used a 50 mL-tube (45 mL sample). Besides, the temperature used, i.e., −20 °C allows a moderate freezing propagation on the samples. Thus, these three conditions promote a better movement of the solids in the freezing step and favors the separation of solutes from the ice fraction during thawing [17]. The solutes occluded in the ice phase (C_i) presented values close to 6, 10, and 15 °Brix as the CBCC cycles progressed (data not shown). Thus, in CI terms, the C_s presented an increase of 0.4, 0.7, and 1.1 times with regard to the first, second, and third cycle, respectively. This behavior could be comparable with CBCC studies applied to orange juice [19] and blueberry juice [28]. However, the C_i results were better (purest ice samples) than those reported by Petzold et al. [28] in the cryoconcentration of

commercial juice due to that natural apple juice has no added additional components (preservatives, stabilizers, and dyes) and/or any previous treatment carried out, such as in the commercial juice produced. An important point is that the ice fraction can be frozen again, and then centrifugation or other external force applied to separate the C_s from the C_i, as recently reported by Orellana-Palma et al. [19] to recover as much cryoconcentrate as possible.

The pH and TTA were changed significantly ($p \leq 0.05$) along CBCC cycles compared to the fresh sample. Specifically, an opposite effect was observed for pH and TTA. This inverse behavior could be attributed to the high content of organic acids in the samples, mainly malic acid. A comparable phenomenon was described by Khajehei et al. [33] in pomegranate juice.

The density (ρ) increased as the solutes increased at each cycle. Thus, an increment close to 36.4% in relation to fresh apple juice was reached in the last cycle. These experimental behaviors were in concordance with previous data for orange juice [19] and pineapple juice [20] under cryoconcentration.

The CIELab colorimetric space was used to characterize the change in color during the CBCC cycles between the fresh juice and cryoconcentrates. The samples showed significant changes in the L*, a*, and b* values after the applied CBCC technique. In particular, the initial L* value decreased at each cycle, which indicated that concentrate samples were darker than the initial juice. The darkening is due to the increase in TSSC cycle by cycle, which also generates the increase of bioactive compounds [15]. Furthermore, these results are in agreement with different cryoconcentrated liquid samples [19,28,34]. For a* and b*, the values increased as the number of cycles increased. This trend indicates a slight deviation towards the red color (a*) and a large increase in the yellow color (b*), which allow for a clear difference with the natural color of the fresh juice (Figure 3). The total color difference (ΔE*) between fresh juice with each cryoconcentrated samples can be estimated as not noticeable ($\Delta E^* \leq 3$) and well visible ($\Delta E^* \geq 3$) according to the human visual discrimination threshold [35].

(**a**) (**b**)

Figure 3. Samples of apple juice: (**a**) fresh juice; (**b**) cryoconcentrated sample (3rd cycle).

For the first cycle, the ΔE* values were over eleven CIELab units, and in the final cycle, ΔE* reached a value of twenty-five CIELab units. Therefore, all cryoconcentrate samples showed visual differences with fresh juice. These values confirm that CBCC process intensifies the natural color of fresh juice, in CIELab values terms.

3.2. Bioactive Compound Content and Antioxidant Activity Determinations

The fresh apple juice had TPC, TFC and TFLC values (Table 2) of approximately 244 mg GAE/100 g d.m., 82 mg CEQ/100 g d.m., and 124 mg CEQ/100 g d.m., respectively, which were significantly higher than those previously informed by Sun et al. [36]. The difference in results could be explained by factors, such as type of harvesting, ripening stage, climatic conditions in the fresh fruits, and/or specific methods used during juice preparation [37].

Table 2. Bioactive compounds content and TBC retention (%) of fresh juice and cryoconcentrate samples at each cycle[1].

	Fresh Apple Juice	Cryoconcentration		
		Cycle 1	Cycle 2	Cycle 3
Bioactive compound content				
TPC (mg GAE/100 g d.m.)	244.3 ± 17.0^{d}	364.8 ± 29.0^{c}	606.3 ± 41.9^{b}	818.9 ± 33.0^{a}
TPC retention, %	-	66.1	77.2	84.9
TFC (mg CEQ/100 g d.m.)	81.5 ± 12.2^{d}	115.6 ± 4.5^{c}	185.5 ± 13.1^{b}	247.8 ± 17.2^{a}
TFC retention, %	-	62.8	70.8	77.0
TFLC (mg CEQ/100 g d.m.)	123.8 ± 6.1^{d}	169.8 ± 10.0^{c}	255.1 ± 16.3^{b}	344.9 ± 20.0^{a}
TFLC retention, %	-	60.7	64.1	70.5
Antioxidant activity				
DPPH (μmol TE/100 g d.m.)	522.5 ± 44.9^{d}	1039.6 ± 43.4^{c}	1315.7 ± 14.5^{b}	1803.2 ± 25.5^{a}
FRAP (μmol TE/100 g d.m.)	467.1 ± 27.2^{d}	1277.4 ± 121.5^{c}	1635.8 ± 78.4^{b}	2935.5 ± 198.3^{a}

[1] Within each row, a, b, c, dwhen there are no significant differences ($p \leq 0.05$), are identified by the same superscript letter, according to a LSD test".

At each CBCC cycle, the results increased significantly ($p \leq 0.05$) when the bioactive compounds content were compared to the fresh apple juice, and in the final cycle, the concentrates samples exhibited values close to 819 mg GAE/100 g d.m., 248 mg CEQ/100 g d.m., and 345 mg CEQ/100 g d.m. for TPC, TFC, and TFLC, respectively. Hence, the cryoconcentrated samples presented an increase up to 3.4 (TPC), 3.0 (TFC), and 2.8 (TFLC) times compared to the initial value. This tendency has been observed with liquid samples such as orange juice [14], strawberry juice [15], blueberry juice [17], pineapple juice [19], yerba mate [38], and coffee extract [39] obtained by the various CC techniques.

Similarly, from the TBC results, the retention was calculated at each cycle. From the first cycle, the TBC retention was more than 60%, which allows highlighting the potential advantage, in TBC retention terms, of cryoconcentration as non-thermal concentration technology. Specifically, the TBC retention was approximately 66%, 77%, and 85% for TPC, 63%, 71%, and 77% for TFC, and 61%, 64%, and 71% for TFLC along the cycles. Orellana-Palma et al. [17,19] and Correa et al. [39] presented comparable values for fruit juices with CBCC and aqueous coffee extract with FFCC. Specifically, the studies informed TBC retention values from 70% to 95% and 90%, respectively. Thus, TBC retention shows the beneficial CBCC effects to obtain a liquid fraction with high TSSC values and attractive color and, in addition, this emerging technology allows the preservation of important thermolabile bioactive compounds in the final cryoconcentrate.

In antioxidant activity terms, the DPPH and FRAP assays showed a value close to 523 μmol TE/100 g d.m. and 467 μmol TE/100 g d.m for the fresh apple juice, respectively, which was higher than previously reported by Silva et al. [40]. The variation could be related to the growth conditions of apple fruits and the methodology used to obtain the fresh juice, which, in turn, influences the anthocyanins content (main contributors in antioxidant capacity) [41]. Thus, the values exhibited an increasing with significant differences ($p \leq 0.05$) as the cycles progressed. CBCC presented values of approximately 1040 μmol TE/100 g d.m., and 1277 μmol TE/100 g d.m. (1st cycle) to 1803 μmol TE/100 g d.m. and 2936 μmol TE/100 g d.m. (3rd cycle) for DPPH and FRAP, respectively. As mentioned above, this behavior could be associated with the use of low temperatures in CBCC to concentrate bioactive compounds, since the damage to the sensitive components is minimal and, therefore, this process allows a higher anthocyanin concentration than other concentration technologies. A similar trend was observed by Correa et al. [39] and Silva et al. [40] during the freeze concentration of coffee extract and apple juice, respectively.

3.3. Profile of Volatile Compounds

A total of twenty-eight volatile molecules were identified in fresh apple juice (Table 3). These compounds recognized were similar to those found in other studies for different apple juice

varieties [42–44], which indicates that several factors affect the production and amount of volatile compounds in a fruit juice, such as harvest, juice extraction, and/or industrial steps, among others.

The fresh juice presented fourteen esters, eight alcohols, four aldehydes and two ketones. Specifically, four esters (propanoate, 3-methylbutyl acetate, 2-methyl butyl acetate, ethyl pentanoate) exhibited higher concentration than other compounds (Figure 4). These results corroborate that esters are the main volatile compounds in apple that contribute to its characteristic fruity aroma [45]. In addition, in both fresh juice and cryoconcentrated samples, sixteen volatile molecules are unknown since no reliable matching was performed with previous studies.

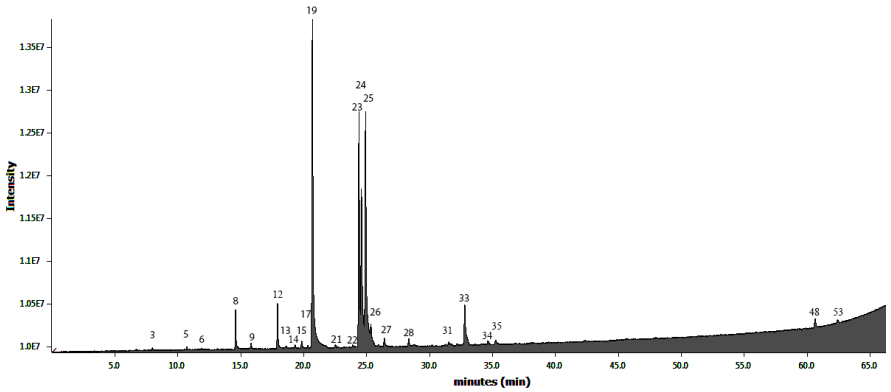


Figure 4. Volatile compounds profile of fresh apple juice.

A lower amount of volatile compounds in the all cycles than fresh juice was detected after CBCC, which can be attributed to the volatilization of components through repeated freezing and thawing operations (Figure S1, see Supplemental Materials). However, as cycles progressed, the aromatic profile was increased, with 18, 22, and 29 compounds in the first, second, and third cycle, respectively. The increase in volatile components could be due to the high concentration of each component (reflected in TSSC) cycle-by-cycle, which can be detected by the chromatograph. This effect is consistent with observed by Piccone et al. [46] in coffee beverages, indicating that the concentration of sugars (such as sucrose, glucose, and fructose) positively affects the release of volatiles in the headspace. The volatile compounds concentrated by BCC are showed in the chromatograms. These results were consistent with those found by Moreno et al. [27], Bonilla-Zavaleta et al. [47], Gunathilake et al. [48], and Miyawaki et al. [49] for coffee extract, pineapple juice, pear juice, and apple juice obtained by different CC techniques, respectively.

In general, the CBCC effect on volatile compounds was varied, since some components increasing and others decreasing in each cycle. Mao et al. [43] mentioned that the volatile compounds variation in a concentrate juice regard to the fresh juice is due to a synergistic effect between the esters (high concentration) with other compounds, resulting in an increases on other volatile compounds concentration, and thus, these can be identifiable by the chromatograph. This coincided with our results, since among for all the samples there was a decrease or increase in components, such as the appearance components, that are not identified in other cycles or fresh juice. Therefore, CBCC allows recovering certain components that are in low concentration in the fresh juice. Furthermore, this technique demonstrated that some volatile compounds were remained in the cryoconcentrated sample. According to the results, future studies could be focused on the particular identification of certain volatile components in different cryoconcentrated food liquids.

Table 3. Volatile profile of fresh juice and cryoconcentrated samples at each cycle[1].

No[2]	Compound[3]	RT[4] (min)	KI[5]	Fresh Juice		CBCC Cycle 1		CBCC Cycle 2		CBCC Cycle 3	
				Area ± DS[6] (μV·s)	Area[7] (%)	Area ± DS[6] (μV·s)	Area[7] (%)	Area ± DS[6] (μV·s)	Area[7] (%)	Area ± DS[6] (μV·s)	Area[7] (%)
1	NI	6.724		-		282830.0 ± 102706.3	0.01	318078.0 ± 127061.1	0.02	343100.0 ± 202337.3	0.03
2	NI	7.401		79843.7 ± 14999.8	0.01	-		-		-	
3	2-Propanol	7.970	541	297065.3 ± 72216.7	0.04	26522226.0 ± 243313.7	0.88	44308240.0 ± 2419159.8	1.11	2795932.0 ± 778665.7	0.27
4	NI	9.285	568	86606.7 ± 4348.5	0.01	-		-		-	
5	Butanal	10.721	603	899295.3 ± 467383.0	0.10	4323310.0 ± 580702.2	0.14	12829114.0 ± 673907.7	0.33	2938169.0 ± 690048.5	0.28
6	Ethyl acetate	11.876	637	217697.0 ± 107843.9	0.03	-		3638095.0 ± 356798.3	0.09	-	
7	2 Methyl-1- propanol	12.853	664	51488.3 ± 5197.4	0.01	665740.0 ± 126926.5	0.02	1830674.0 ± 183780.9	0.05	524879.3 ± 47988.2	0.05
8	1-Butanol	14.588	709	20481655.7 ± 361528.7	2.43	96660253.0 ± 1745979.4	3.40	195310887.0 ± 1996063.3	4.98	46142108.0 ± 4665844.3	4.39
9	Propyl acetate	15.673	734	1399590.0 ± 764702.1	0.17	-		-		2622423.7 ± 1295829.1	0.25
10	2-Pentanone	15.816	746	671524.0 ± 14587.0	0.01	-		-		354600.3 ± 304093.3	0.19
11	NI	16.361	758	57146.0 ± 11278.2	0.01	-		-		-	
12	Ethyl 2-methyl propanoate	17.925	784	25673504.7 ± 919970.3	3.17	135434152.0 ± 7823690.3	4.68	275036482.0 ± 2859035.3	7.01	61557574.3 ± 4399046.7	5.87
13	2-Methyl butanol	18.611	799	293726.3 ± 226346.6	0.04	-		109469714.0 ± 30464924.4	2.79	3655384.7 ± 1424984.6	0.35
14	1-Pentanol	19.312	812	946200.3 ± 393295.3	0.12	5607103.0 ± 959253.5	0.19	15885328.0 ± 315675.8	0.50	3155739.3 ± 785815.3	0.30
15	2-Penten-1-ol	19.841	822	1307300.6 ± 152564.3	0.16	-		-		769415.8 ± 58147.2	0.07
16	NI	19.915	826	-		-		-		61358.5 ± 8789.1	0.01
17	Methyl isopentanoate	20.003	829	-		-		-		84779.2 ± 13559.9	0.01
18	Ethyl butanoate	20.306	831	-		188154.0 ± 33997.0	0.01	-		-	
19	Propyl propanoate	20.701	838	265527334.3 ± 2211731.2	32.78	744949465.0 ± 11043397.5	24.56	927660144.0 ± 36874740.6	23.73	271190131.0 ± 10810605.6	25.74
20	Ethyl 3-methyl butanoate	22.509	872	-		-		858797.0 ± 318410.1	0.02	387819.0 ± 141958.1	0.03
21	Ethyl 2-methyl butanoate	22.832	879	115971.0 ± 47112.6	0.01	-		-		74396.0 ± 3335.7	0.01
22	Propyl isobutyrate	23.885	897	363829.0 ± 461296.3	0.03	6516629.0 ± 1550179.1	0.22	8901868.0 ± 1242428.9	0.23	1072184.0 ± 588990.5	0.10
23	3-Methylbutyl acetate	24.390	906	161453176.3 ± 7205273.7	19.93	299040619.0 ± 7574801.7	9.90	419808246.0 ± 28706962.8	10.70	150049001.3 ± 31966969.9	14.30
24	2-Methyl butyl acetate	24.594	909	113265346.3 ± 4841724.61	13.98	761197445.0 ± 19537256.3	25.20	754701538.0 ± 51802781.9	19.23	177007026.3 ± 56927390.8	16.77
25	Ethyl pentanoate	24.915	919	165161225.33 ± 3722692.8	20.53	809534184.0 ± 25344664.5	26.79	1046607079.0 ± 54059121.1	26.66	278107449.7 ± 33341609.1	26.44
26	1-Hexanol	25.325	921	10568683.0 ± 2696706.6	1.30	1015759.0 ± 159355.2	0.03	30848006.0 ± 4908217.2	0.79	14051512.3 ± 7343551.9	1.34
27	2-Heptanone	26.401	939	201229.0 ± 98152.8	0.28	5335595.0 ± 219869.2	0.18	5669031.0 ± 1660335.6	0.14	3932498.0 ± 2801013.5	0.38
28	2,4-Hexadienal	28.333	970	2981978.0 ± 934205.0	0.37	3575161.0 ± 250404.8	0.14	1074023.0 ± 353934.4	0.03	1974035.7 ± 1925044.1	0.19
29	1-Heptanol	28.649	975	-		-		-		89140.0 ± 17472.9	0.05
30	NI	30.152	1.001	-		-		-		-	
31	Ethyl hexanoate	31.498	1.024	281486.0 ± 57977.3	0.04	-		-		-	
32	NI	32.117	1.030	-		-		-		-	
33	Octanal	32.791	1.049	31600971.7 ± 3572723.2	3.70	80883130.0 ± 709157.4	2.67	32316356.0 ± 4685213.8	0.91	18271366.7 ± 10206857.7	1.75
34	2-Ethyl-1-hexanol	34.606	1.083	580902.7 ± 277644.3	0.07	-		-		-	
35	Pentyl butanoate	35.360	1.097	598638.7 ± 461256.6	0.07	-		3189589.0 ± 758448.3	0.08	431032.3 ± 361305.8	0.06
36	Nonanal	39.545	1.150	60112.6 ± 9852.2	0.01	-		-		81955.3 ± 9122.6	0.01
37	Heptanoic acid	41.207	1.170	-		195623.1 ± 175114.1	0.01	-		-	
38	Benzyl acetate	44.348	1.213	80022.0 ± 11152.2	0.01	-		-		85308.7 ± 16928.7	0.01
39	Methyl nonanoate	48.928	1.256	-		-		-		70145.7 ± 8071.2	0.01
40	Octanoic acid	50.263	1.263	-		-		264806.0 ± 18648.2	0.01	-	

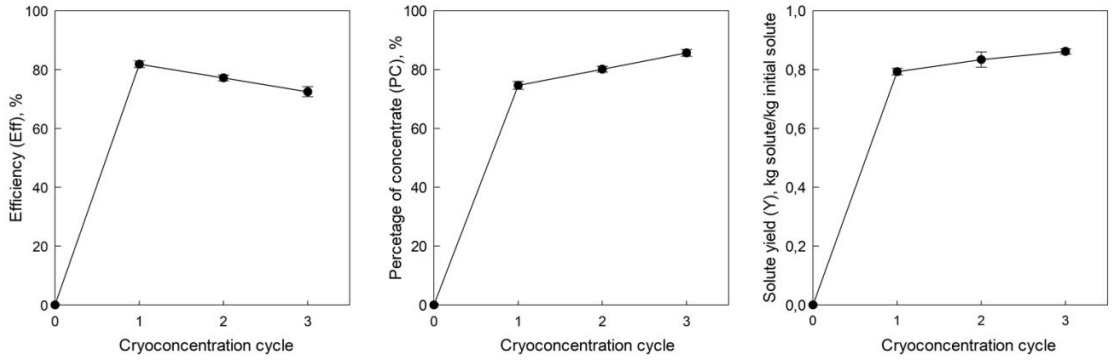
Table 3. *Cont.*

No[2]	Compound[3]	RT[4] (min)	KI[5]	Fresh Juice		CBCC Cycle 1		CBCC Cycle 2		CBCC Cycle 3	
				Area ± DS[6] (μV·s)	Area[7] (%)	Area ± DS[6] (μV·s)	Area[7] (%)	Area ± DS[6] (μV·s)	Area[7] (%)	Area ± DS[6] (μV·s)	Area[7] (%)
41	NI	51.915	1.284	-		-		-		89854.3 ± 40343.6	0.01
42	NI	54.328	1.300	-		-		-		59558.8 ± 7154.4	0.01
43	2,4-Decadienal	56.586	1.392	-		-		152347.5 ± 33258.4	0.01	-	
44	NI	57.536	1.412	-		-		-		110933.3 ± 32305.7	0.01
45	NI	58.701	1.419	-		-		-		-	
46	NI	59.187	1.425	-		-		-		-	
47	NI	59.543	1.437	-		-		-		-	
48	Ethyl decanoate	60.595	1.442	3909910.0 ± 2829238.5	0.49	25309997.0 ± 1427639.1	0.78	17520119.0 ± 1762721.8	0.50	6322302.5 ± 427412.8	0.60
49	NI	62.819	1.501	-		-		-		98941.0 ± 23746.7	0.01
50	Tetradecanoic acid	64.699	1.539	-		-		-		215383.0 ± 232813.3	0.02
51	NI	65.074	1.543	-		-		-		-	
52	NI	65.690	1.559	87066.3 ± 11890.7	0.01	-		-		94033.0 ± 20933.8	0.01
53	Geranyl butyrate	67.627	1.598	294987.0 ± 33403.3	0.02	-		-		-	
	Total			**809595513.2**	**100.0**	**3007237375.1**	**100.0**	**3908198561.5**	**100.0**	**1048871472.1**	**100.0**

[1] Within each row, different letters are significantly different ($p \leq 0.05$). [2] Number of peaks. [3] Already reported in Kheshti et al. [42], Mao et al. [43], Medina et al. [44], Perestrelo et al. [50], López-Fructuoso and Echeverría-Cortada [51], and Zhu et al. [52]. [4] Retention time. [5] Kováts index calculated for DB-624 capillary column. [6] Mean of three replicates of the total ion current (TIC) area of the gas chromatography-flame ionization detector (GC-FID) ± standard deviation. [7] Percentage of the TIC area. NI: unidentified.

3.4. Process Parameters

As mentioned above, the centrifugal force is an excellent option as an assisted technique in the BCC technology, since it improves the separation process. Figure 5 shows the efficiency (Eff), solute yield (Y), and percentage of concentrate (PC) at each CBCC cycle.



(**a**) (**b**) (**c**)

Figure 5. Process parameters: (**a**) efficiency; (**b**) percentage of concentrate; (**c**) solute yield.

Initially, the Eff presented an 82%, 77%, and 73% from the first to the third CBCC cycle, respectively. The results were better, in each cycle, than previous studies in our laboratory (with same conditions), with values close to 58%, 70%, 72%, and 79% in the first cycle to pineapple juice [28], sucrose solution [13], blueberry juice [17], and orange juice [19], respectively. The decrease in Eff is due to the increase in solutes (C_s), that increase the viscosity and prevents a better extraction of solutes from the ice phase [53].

The PC increased significantly ($p \leq 0.05$) at each cycle, with values of 74% (1st cycle), 80% (2nd cycle) and 85% (3rd cycle). The results were significantly higher than the PC values obtained in our laboratory with different liquid samples such as blueberry juice [17], orange juice [19], and pineapple juice [28]. The different values are due to the apple has fewer components such as wastes, shells and/or seeds in the juice than other juices, which facilitates the separation of fractions.

The solute recovery was calculated as solute yield (Y), and in the first cycle, it reached a value close to 0.79 kg solute per 1 kg initial (kg/kg). Later, Y showed a linear and significant increase, with values of ≈0.83 kg/kg and ≈0.86 kg/kg for the second and third CBCC cycle, respectively. The results had a similar trend in previous investigations in liquid samples [13,14,40]. This behavior could be associated with the solute mass (m_s) at each centrifugation step, since the solid-liquid interface accumulates more mass due to the increase in the solute content [54].

3.5. Sensorial Analysis

Figure 6 shows the sensory evaluation of fresh apple juice and reconstituted cryoconcentrate sample (3rd cycle). Results showed that cryoconcentration did not affect significantly ($p \geq 0.05$) the odor, aroma, flavor, and global assessment of the reconstituted sample, in comparison to the fresh apple juice, although there is an apparent lower score for the cryoconcentrated samples. These results were similar to studies on cryoconcentration applied to black currant juice [55], since the cryoconcentrated juice was equivalent to the fresh sample for the same qualities analyzed in the present study. This advantage in the original characteristics preservation from the fresh sample has also been ratified by sensory evaluation on color, aroma, taste, and total quality of cryoconcentrated Andes berry pulp obtained by PCC [56].

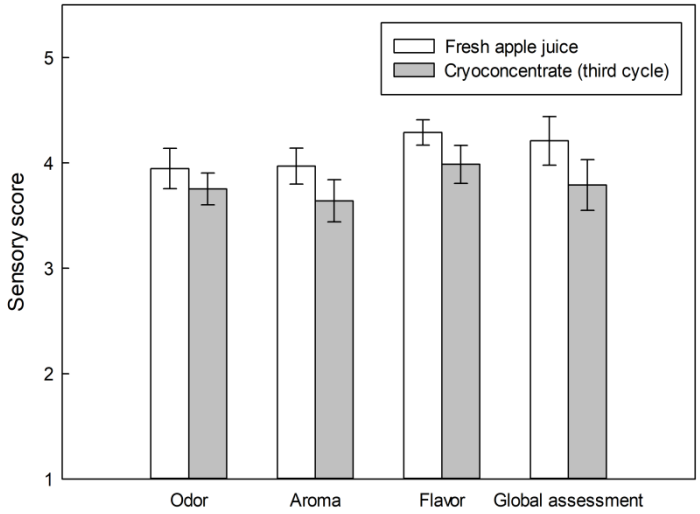


Figure 6. Sensory evaluation of fresh juice and cryoconcentrated sample (3rd cycle).

In the same way, the use of this concentration technology is advantageous from the sensory and organoleptic viewpoint, not only in juices and fruit pulps, but also in infusions as popular as coffee, since Moreno et al. [30] demonstrated a high retention in sensory qualities of coffee extract samples subjected to PCC and FFCC. These results confirm the advantage of CBCC technique to concentrate fruit juices, in particular for the retention of the fresh sensory characteristics [6].

Generally, the sensory evaluation results showed that samples treated with CBCC were less appreciated than fresh apple juice, but there was no significant difference ($p \geq 0.05$) between the characteristics evaluated. Furthermore, the CBCC treatment had no negative effect on the odor, aroma, flavor, and global assessment. Therefore, the reconstituted cryoconcentrate is an excellent option for studies on commercialization.

4. Conclusions

CBCC is an effective combination to concentrate and extract solids from an ice matrix. Precisely, in the third cycle, the cryoconcentrate samples showed a high final solute concentration (≈55 °Brix) and lower luminosity (L* ≈ 68) than fresh juice, and total color difference (ΔE*) showed CIELab values close to 25 units. Similarly, a high bioactive compound retention was achieved with 85% for TPC, 77% for TFC and 71% for TFLC. In addition, the DPPH and FRAP assays showed high levels of antioxidant activity (523 µmol TE/100 g d.m. to 1803 µmol TE/100 g d.m., and 467 µmol TE/100 g d.m to 2936 µmol TE/100 g d.m.). The cryoconcentrated sample (3rd cycle) showed a similar number of volatile compounds related to the fresh juice, with 29 and 28 volatile compounds, respectively. In process parameter terms, the centrifugal force allowed excellent process parameters, with 73%, 0.87 (kg/kg), and 85% for efficiency, solute yield and percentage of concentrate, respectively, and the sensory evaluation shows that the odor, aroma and flavor of fresh sample were remained in the cryoconcentrated samples, with good qualifications by the panelists. Therefore, CBCC is an emerging and effective technology to preserve and enhanced important quality attributes, such as physicochemical properties, bioactive compounds, aromatic profile, antioxidant activity, and sensory properties from fresh apple juice.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/3/959/s1, **Figure S1.** Aromatic profile at each CBCC cycle. (**a**) Cycle 1; (**b**) cycle 2; (**c**) cycle 3.

Author Contributions: Conceptualization: P.O.-P., E.H., and G.P.; methodology: P.O.-P., V.L.-M., and M.P.G.; software: M.P.G., E.H., R.N.Z., and G.P.; validation: P.O.-P., V.L.-M., and M.P.G.; formal analysis: P.O.-P., V.L.-M., and M.P.G.; investigation: P.O.-P., R.N.Z., E.H., and G.P.; resources: M.P.G., E.H., R.N.Z., and G.P.; data curation: P.O.-P., V.L.-M., M.P.G., and G.P.; writing—original draft preparation: P.O.-P., E.H., and G.P.; writing—review and editing: E.H., R.N.Z., and G.P.; visualization: P.O.-P., E.H., and G.P.; supervision: M.P.G., E.H., R.N.Z., and G.P.;

project administration: P.O.-P. and G.P.; funding acquisition: P.O.-P. and G.P. All authors have read and agreed to the published version of the manuscript.

Funding: Patricio Orellana-Palma acknowledge the financial support of CONICYT-Chile (Comisión Nacional de Investigación Científica y Tecnológica) through the FONDECYT Postdoctoral Grant 2019 (Folio 3190420).

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

References

1. USDA. Fresh deciduous fruit: World markets and trade (apples, grapes, and pears). In *Foreign Agricultural Service (FDA)*; Department of Agriculture: Washington, DC, USA, 2019. Available online: https://apps.fas.usda.gov/psdonline/circulars/fruit.pdf (accessed on 15 December 2019).
2. Phaiphan, A.; Panichakool, P.; Jinawan, S.; Penjumras, P. Effects of heat and shallot (*Allium ascalonicum* L.) supplementation on nutritional quality and enzymatic browning of apple juice. *J. Food Sci. Technol.* **2019**, *56*, 4121–4128. [CrossRef] [PubMed]
3. Zorenc, Z.; Veberic, R.; Stampar, F.; Koron, D.; Mikulic-Petkovsek, M. Thermal stability of primary and secondary metabolites in highbush blueberry (*Vaccinium corymbosum* L.) purees. *LWT Food Sci. Technol.* **2017**, *76*, 79–86. [CrossRef]
4. Zhang, Z.H.; Wang, L.H.; Zeng, X.A.; Han, Z.; Brennan, C.S. Non-thermal technologies and its current and future application in the food industry: A review. *Int. J. Food Sci. Techol.* **2019**, *54*, 1–13. [CrossRef]
5. Amran, N.A.; Samsuri, S.; Safiei, N.Z.; Zakaria, Z.Y.; Jusoh, M. Review: Parametric study on the performance of progressive cryoconcentration system. *Chem. Eng. Commun.* **2016**, *203*, 957–975. [CrossRef]
6. Petzold, G.; Orellana, P.; Moreno, J.; Junod, J.; Bugueño, G. Freeze concentration as a technique to protect valuable heat-labile components of foods. In *Innovative Processing Technologies for Foods with Bioactive Compounds*, 1st ed.; Moreno, J.J., Ed.; CRC Press: Boca Raton, FA, USA, 2016; pp. 184–190.
7. Yin, Y.; Yang, Y.; de Lourdes, M.; Zhai, S.; Feng, W.; Wang, Y.; Gu, M.; Cai, L.; Zhang, L. Progressive freezing and suspension crystallization methods for tetrahydrofuran recovery from Grignard reagent wastewater. *J. Clean. Prod.* **2017**, *144*, 180–186. [CrossRef]
8. Muñoz, I.D.B.; Rubio, A.; Blanco, M.; Raventós, M.; Hernández, E.; Prudêncio, E.S. Progressive freeze concentration of skimmed milk in an agitated vessel: Effect of the coolant temperature and stirring rate on process performance. *Food Sci. Technol. Int.* **2019**, *25*, 150–159. [CrossRef]
9. Zambrano, A.; Ruiz, Y.; Hernández, E.; Raventós, M.; Moreno, F.L. Freeze desalination by the integration of falling film and block freeze-concentration techniques. *Desalination* **2018**, *436*, 56–62. [CrossRef]
10. Moreno, F.L.; Raventós, M.; Hernández, E.; Ruiz, Y. Block freeze-concentration of coffee extract: Effect of freezing and thawing stages on solute recovery and bioactive compounds. *J. Food Eng.* **2014**, *120*, 158–166. [CrossRef]
11. Orellana-Palma, P. External Forces Assisted Cryoconcentration to Improve the Concentration Process and the Quality of Fruit Juices. Ph.D. Thesis, Universidad del Bío-Bío, Chillán, Chile, April 2018.
12. Aider, M.; de Halleux, D.J. Passive and microwave-assisted thawing in maple sap cryoconcentration technology. *J. Food Eng.* **2008**, *85*, 65–72. [CrossRef]
13. Petzold, G.; Aguilera, J.M. Centrifugal freeze concentration. *Innov. Food Sci. Emerg. Technol.* **2013**, *20*, 253–258. [CrossRef]
14. Orellana-Palma, P.; Petzold, G.; Andana, I.; Torres, N.; Cuevas, C. Retention of ascorbic acid and solid concentration via centrifugal freeze concentration of orange juice. *J. Food Quality* **2017**, *2017*, 5214909. [CrossRef]
15. Adorno, W.T.; Rezzadori, K.; Arend, G.D.; Chaves, V.C.; Reginatto, F.H.; di Luccio, M.; Petrus, J.C. Enhancement of phenolic compounds content and antioxidant activity of strawberry (*Fragaria×ananassa*) juice by block freeze concentration technology. *J. Food Sci. Technol.* **2017**, *52*, 781–787. [CrossRef]
16. ODEPA. Región del Maule, Información Regional 2019, Ministerio de Agricultura, Gobierno de Chile, Chile, 2019. Available online: https://www.odepa.gob.cl/wp-content/uploads/2019/03/Maule.pdf (accessed on 13 January 2020).
17. Orellana-Palma, P.; Petzold, G.; Guerra-Valle, M.; Astudillo-Lagos, M. Impact of block cryoconcentration on polyphenol retention in blueberry juice. *Food Biosci.* **2017**, *20*, 149–158. [CrossRef]

18. Bastías-Montes, J.M.; Martín, V.S.; Muñoz-Fariña, O.; Petzold-Maldonado, G.; Quevedo-León, R.; Wang, H.; Yang, Y.; Céspedes-Acuña, C.L. Cryoconcentration procedure for aqueous extracts of maqui fruits prepared by centrifugation and filtration from fruits harvested in different years from the same localities. *J. Berry Res.* **2019**, *9*, 377–394. [CrossRef]
19. Orellana-Palma, P.; González, Y.; Petzold, G. Improvement of centrifugal cryoconcentration by ice recovery applied to orange juice. *Chem. Eng. Technol.* **2019**, *42*, 925–931. [CrossRef]
20. Orellana-Palma, P.; Zuñiga, R.N.; Takhar, P.S.; Gianelli, M.P.; Petzold, G. Effects of centrifugal block freeze crystallization on quality properties in pineapple juice. *Chem. Eng. Technol.* **2020**, *43*, 355–364. [CrossRef]
21. Tansakul, A.; Kantrong, H.; Saengrayup, R.; Sura, P. Thermophysical properties of papaya puree. *Int. J. Food Prop.* **2012**, *15*, 1086–1100. [CrossRef]
22. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.R. Analysis of total phenols and other oxidation substrates and oxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
23. Paz, M.; Guillon, P.; Barroso, M.F.; Carvalho, A.P.; Domingues, V.F.; Gomes, A.M.; Becker, H.; Longhinotti, E.; Delerue-Matos, C. Brazilian fruit pulps as functional foods and additives: Evaluation of bioactive compounds. *Food Chem.* **2014**, *172*, 462–468. [CrossRef]
24. Broadhurst, R.B.; Jones, W.T. Analysis of condensed tannins usingacidified vanillin. *J. Sci. Food Agric.* **1978**, *29*, 788–794. [CrossRef]
25. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D.H. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* **2006**, *19*, 669–675. [CrossRef]
26. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]
27. Orellana-Palma, P.; Takhar, P.S.; Petzold, G. Increasing the separation of block cryoconcentration through a novel centrifugal filter-based method. *Sep. Sci. Technol.* **2019**, *54*, 149–158. [CrossRef]
28. Petzold, G.; Moreno, J.; Lastra, P.; Rojas, K.; Orellana, P. Block freeze concentration assisted by centrifugation applied to blueberry and pineapple juices. *Innov. Food Sci. Emerg. Technol.* **2015**, *30*, 192–197. [CrossRef]
29. Sánchez, J.; Ruiz, Y.; Raventós, M.; Auleda, J.M.; Hernández, E. Progressive freeze concentration of orange juice in a pilot plant falling film. *Innov. Food Sci. Emerg.* **2010**, *11*, 644–651. [CrossRef]
30. Moreno, F.L.; Quintanilla-Carvajal, M.X.; Sotelo, L.I.; Osorio, C.; Raventós, M.; Hernández, E.; Ruiz, Y. Volatile compounds, sensory quality and ice morphology in falling-film and block freeze concentration of coffee extract. *J. Food Eng.* **2015**, *166*, 64–71. [CrossRef]
31. Zielinski, A.A.; Zardo, D.M.; Alberti, A.; Bortolini, D.G.; Benvenutti, L.; Demiate, I.M.; Nogueira, A. Effect of cryoconcentration process on phenolic compounds and antioxidant activity in apple juice. *J. Sci. Food Agric.* **2019**, *99*, 2786–2792. [CrossRef]
32. Ding, Z.; Qin, F.G.; Yuan, J.; Huang, S.; Jiang, R.; Shao, Y. Concentration of apple juice with an intelligent freeze concentrator. *J. Food Eng.* **2019**, *256*, 61–72. [CrossRef]
33. Khajehei, F.; Niakousari, M.; Eskandari, M.H.; Sarshar, M. Production of pomegranate juice concentrate by complete block cryoconcentration process. *J. Food Process Eng.* **2015**, *38*, 488–498. [CrossRef]
34. Petzold, G.; Orellana, P.; Moreno, J.; Cerda, E.; Parra, P. Vacuum-assisted block freeze concentration applied to wine. *Innov. Food Sci. Emerg.* **2016**, *36*, 330–335. [CrossRef]
35. Melgosa, M.; Hita, E.; Poza, A.J.; Alman, D.H.; Berns, R.S. Suprathreshold color-difference ellipsoids for surface colors. *Color Res. Appl.* **1997**, *22*, 148–155. [CrossRef]
36. Sun, Y.; Zhong, L.; Cao, L.; Lin, W.; Ye, X. Sonication inhibited browning but decreased polyphenols contents and antioxidant activity of fresh apple (*Malus pumila mill*, cv. *Red Fuji*) juice. *J. Food Sci. Technol.* **2015**, *52*, 8336–8342. [CrossRef] [PubMed]
37. Laaksonen, O.; Kuldjärv, R.; Paalme, T.; Virkki, M.; Yang, B. Impact of apple cultivar, ripening stage, fermentation type and yeast strain on phenolic composition of apple ciders. *Food Chem.* **2017**, *233*, 29–37. [CrossRef] [PubMed]
38. Nunes, G.L.; Boaventura, B.C.B.; Pinto, S.S.; Verruck, S.; Murakami, F.S.; Prudêncio, E.S.; Amboni, R.D.D.M.C. Microencapsulation of freeze concentrated *Ilex paraguariensis* extract by spray drying. *J. Food Eng.* **2015**, *151*, 60–68. [CrossRef]
39. Correa, L.J.; Ruiz, R.Y.; Moreno, F.L. Effect of falling-film freeze concentration on bioactive compounds in aqueous coffee extract. *J. Food Process Eng.* **2018**, *41*, e12606. [CrossRef]

40. Silva, K.M.; Zielinski, A.A.F.; Benvenutti, L.; Bortolini, D.G.; Zardo, D.M.; Beltrame, F.L.; Nogueira, A.; Alberti, A. Effect of fruit ripening on bioactive compounds and antioxidant capacity of apple beverages. *Food Sci. Technol.* **2019**, *39*, 294–300. [CrossRef]
41. Skrovankova, S.; Sumczynski, D.; Mlcek, J.; Jurikova, T.; Sochor, J. Bioactive compounds and antioxidant activity in different types of berries. *Int. J. Mol. Sci.* **2015**, *16*, 24673–24706. [CrossRef]
42. Kheshti, N.; Melo, A.A.M.; Cedeno, A.B.; Obenland, D.; Prakash, A. Physiological response of 'Fuji'apples to irradiation and the effect on quality. *Radiat. Phys. Chem.* **2019**, *165*, 108389. [CrossRef]
43. Mao, D.; Liu, H.; Li, Z.; Niu, Y.; Xiao, Z.; Zhang, F.; Zhu, J. Impact of sensory interactions among volatile compounds of juice of Red Delicious apples. *Hortic. Environ. Biotechnol.* **2019**. [CrossRef]
44. Medina, S.; Perestrelo, R.; Santos, R.; Pereira, R.; Câmara, J.S. Differential volatile organic compounds signatures of apple juices from Madeira Island according to variety and geographical origin. *Microchem. J.* **2019**, *150*, 104094. [CrossRef]
45. Espino-Díaz, M.; Sepúlveda, D.R.; González-Aguilar, G.; Olivas, G.I. Biochemistry of apple aroma: A review. *Food Technol. Biotechnol.* **2016**, *54*, 375–394. [CrossRef] [PubMed]
46. Piccone, P.; Lonzarich, V.; Navarini, L.; Fusella, G.; Pittia, P. Effect of sugars on liquid–vapour partition of volatile compounds in ready-to-drink coffee beverages. *Int. J. Mass Spectrom.* **2012**, *47*, 1120–1131. [CrossRef] [PubMed]
47. Bonilla-Zavaleta, E.; Vernon-Carter, E.J.; Beristain, C.I. Thermophysical properties of freeze-concentrated pineapple juice. *Ital. J. Food Sci.* **2006**, *18*, 367–376.
48. Gunathilake, M.; Shimmura, K.; Dozen, M.; Miyawaki, O. Flavor retention in progressive freeze-concentration of coffee extract and pear (La France) juice flavor condensate. *Food Sci. Technol. Res.* **2014**, *20*, 547–554. [CrossRef]
49. Miyawaki, O.; Gunathilake, M.; Omote, C.; Koyanagi, T.; Sasaki, T.; Take, H.; Matsuda, A.; Ishisaki, K.; Miwa, S.; Kitano, S. Progressive freeze-concentration of apple juice and its application to produce a new type apple wine. *J. Food Eng.* **2016**, *171*, 153–158. [CrossRef]
50. Perestrelo, R.; Silva, C.; Silva, P.; Medina, S.; Câmara, J.S. Differentiation of fresh and processed fruit juices using volatile composition. *Molecules* **2019**, *24*, 974. [CrossRef]
51. López-Fructuoso, M.L.; Echeverría-Cortada, G. Apple (*Malus x domestica* Borkh.). In *Handbook of Fruit and Vegetable Flavors*, 1st ed.; Hui, Y.H., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2010; pp. 247–264.
52. Zhu, D.; Ren, X.; Wei, L.; Cao, X.; Ge, Y.; Liu, H.; Li, J. Collaborative analysis on difference of apple fruits flavour using electronic nose and electronic tongue. *Sci. Hortic.* **2020**, *260*, 108879. [CrossRef]
53. Welti-Chanes, J.; Bermúdez, D.; Valdez-Fragoso, A.; Mújica-Paz, H.; Alzamora, S.M. Principles and applications of freeze-concentration and freeze-drying. In *Handbook of Food Science, Technology, and Engineering*, 1st ed.; Hui, Y.H., Ed.; Marcel Dekker: New York, NY, USA, 2004; pp. 13–17.
54. Gu, X.; Watanabe, M.; Suzuki, T.; Miyawaki, O. Limiting partition coefficient in a tubular ice system for progressive freeze-concentration. *Food Sci. Technol. Res.* **2008**, *14*, 249–252. [CrossRef]
55. Dette, S.S.; Jansen, H. Freeze concentration of black currant juice. *Chem. Eng. Technol.* **2010**, *33*, 762–766. [CrossRef]
56. Ramos, F.A.; Delgado, J.L.; Bautista, E.; Morales, A.L.; Duque, C. Changes in volatiles with the application of progressive freeze-concentration to Andes berry (*Rubus glaucus* Benth). *J. Food Eng.* **2005**, *69*, 291–297. [CrossRef]