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Physicochemical properties and bioactive compounds content in encapsulated freeze-dried powders obtained from blueberry, elderberry, blackcurrant and maqui berry

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Abstract.

BACKGROUND: Berry fruits stand out for their high contents of anthocyanins responsible for their red-purple color. The region of Patagonia, Argentina, is a significant cultivar region of these fruits. Due to berries' very short shelf-life, it is interesting the development of dehydrated products that can be stored at room temperature and still be a source of bioactive components and natural colorants.

OBJECTIVE: The objective of this work was to evaluate the effect of freeze-drying process and storage at 38°C on the levels of bioactive compounds and color, in lyophilized products based on blueberry, blackcurrant, elderberry and maqui berry pulps from the El Bolsón area, Río Negro, Argentina.

METHODS: Fruit pulps with addition of encapsulating agents (Maltodextrin/Arabic gum) were freeze-dried and evaluated for the content of monomeric anthocyanins, total polyphenols, antioxidant capacity, color (CIELab parameters) and physical characteristics.

RESULTS: The obtained freeze-dried powders showed high levels of retention of bioactive compounds, yielding between 68.2 and 99.6% of retention of polyphenols and anthocyanins depending on the fruit. Color parameters were also stable after freeze-drying and during 60 days of storage at 38°C.

CONCLUSIONS: The obtained stable powder ingredients based on berries from Patagonia can be used as a source of bioactive compounds and pigments.

Keywords: Anthocyanins, color, polyphenolics, berries, storage, freeze-dried powders

1. Introduction

The growing trend to consume healthy food generates a demand of functional foods containing bioactive compounds. Polyphenols present in fruits and vegetables, are included in this group mainly for their properties of free radical scavengers which confer antioxidant activity against cellular oxidation reactions [1–4]. In par-

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ticular, anthocyanins are compounds associated to health promoting properties such as anti-inflammatory and antimicrobial actions and also involved in reducing the risk of having heart diseases, neurodegenerative disorders and cancer [5–8].

Berry fruits such as blueberry, elderberry, cassis, cranberry, raspberry among others, have high polyphenol content compared to other foods [9]. In particular, their red-purple color is attributed to the monomeric anthocyanins present in these fruits.

In the last years in Patagonia, the southernmost region of Argentina, there has been a significant increase in the cultivars of berries, concentrating more than 70% of Argentina's total production. Given their very short shelf-life, berries' production is mainly destined to the commercialization of refrigerated or frozen fruit and the manufacturing of jam, jellies, marmalades and other products [10].

Due to the chemical nature of anthocyanins, they could present stability problems associated with factors like temperature, pH, presence of oxygen and light [11-12]. During processing, degradation and polymerization of monomeric anthocyanins could lead to discoloration and loss of attractive color [13]. For this reason, several technologies are applied to preserve the content of anthocyanins in fruits and to produce food products and ingredients with a longer shelf life. Preservation technologies by drying such as spray or freeze-drying, may be also used for encapsulation of bioactive compounds, thus obtaining a final product comprising highly concentrated and stable compounds with preserved characteristics such as color and chemical composition [14-15]. These dehydrated ingredients have a broad range of applications including their use as source of antioxidants and natural food colorants. In order to obtain dried foods from fruits or its juices, the proportion of the encapsulant agent to fruit solids used is relevant to obtain the dehydrated systems, since fruit juices have high concentration of sugars with low T_g (glass transition temperature) like sucrose and fructose.

Although spray-drying is one of the most common techniques used to microencapsulate components [16, 17], numerous studies have shown that the freeze-drying process achieves a better retention of bioactive compounds, especially in heat-sensitive components such as anthocyanins, and better conservation of antioxidant capacity [18–20]. Freeze-drying allows the long-term preservation of heat sensitive foods and other biological materials based on the phenomena of sublimation [21].

After the freeze-drying process and under storage conditions, the stability of the compounds of interest is relevant, and it is important to know their stability, and the identification of differences in the stability of the main anthocyanins [22]. Retentions of phenolic and anthocyanin contents higher than 80% after freeze-drying processes have been observed in such dissimilar sources as grape [23-24], black carrots [25] or star fruit [26].

The objective of this work was to evaluate the effect of freeze-drying process on the levels of bioactive compounds (total polyphenols, anthocyanin content), antioxidant capacity and color parameters (CIELab parameters), and study their stability during storage in powdered products obtained from berries cultivated in El Bolsón area, Río Negro, Argentina.

2. Materials and methods

2.1. Samples

Blueberry (*Vaccinium corymbosum*), elderberry (*Sambucus nigra*), blackcurrant (*Ribes nigrum*) and maqui berry (*Aristotelia chilensis*) pulps were provided by a producer from El Bolsón, Río Negro (Argentina), obtained from fruits of the same harvest year (2016), and harvested with a similar maturation degree. The pulps were obtained from fruit blanched in water (80° C, $3 \min$) and then crushed and peeled in an industrial pulper, packed, pasteurized (85° C, $15 \min$) for enzyme inactivation and microbial growth inhibition and frozen at -18° C.

2.2. Freeze-drying and encapsulation procedure

Pulps of blueberry (BB), blackcurrant (BC), elderberry (EB) and maqui berry (MB) were mixed with Maltodextrin DE_{10} and Arabic Gum (80:20) at a ratio of 20% of encapsulating agents, poured onto an aluminum tray and frozen at -20° C during 24 h. Then they were freeze-dried at room temperature ($22 \pm 3^{\circ}$ C) in a FIC-LI-I-E300-CRT freeze dryer (Buenos Aires, Argentina) operated with a freezing plate and condenser at -40° C and a vacuum of 100 μ m Hg during 40 h. The freez-dried amorphous carbohydrate matrix was milled to obtain a powder which was fractionated in sterile, hermetic flasks and stored at -18° C until use.

2.3. Storage conditions

Freeze-dried samples were stored in darkness at 38°C for 60 days and, at selected times (15 days intervals), 2 samples were removed to take measurements. A temperature of 38°C was selected because it is usually recommended for accelerated shelf life studies of foods to be marketed at ambient temperature [27].

2.4. Alcoholic extracts

Samples of powder were reconstituted with distilled water to obtain the same "Brix as the suspension of juice and carrier to be freeze dried. Reconstitution factors (g water/g powder) were: 2.68 for blueberry, 2.14 for blackcurrant, 2.40 for elderberry and 1.86 for maqui berry.

5 grams of pulps or freeze-dried reconstituted samples were extracted twice in 20 ml ethanol: HCl 0.1 N (85:15) [28]. The pellets with no detectable residual content of phenolics, were eliminated by centrifugation and the supernatants were mixed and utilized for measurements of total phenolics, total monomeric anthocyanins, polymeric color, total antioxidant capacity, color parameters and HPLC assay.

Results are expressed by gram of pulp or powder as indicated and taking into account their correspondent density.

2.5. Reagents

Maltodextrin Dextrose Equivalent 10 (MDE₁₀) from Productos de Maíz S.A., Buenos Aires, Argentina and Arabic Gum (AG) from Gelfix S.A., Buenos Aires, Argentina, were used for encapsulation of freeze-dried berry pulps. Ethanol and chlorhydric acid used as solvents for juice extraction were from Biopack, Buenos Aires, Argentina. Folin–Ciocalteu reagent was purchased from Merck KgaA Darmstadt, Germany. HPLC standard was obtained from Sigma-Aldrich, USA. Gallic acid used for phenolic standard curve was obtained from Anedra, Buenos Aires, Argentina. All chromatographic solvents were of HPLC grade and the purity of the reagents used was p.a. or similar.

2.6. Methods

2.6.1. Physicochemical properties

Total soluble solids content was evaluated in °Brix with a manual refractometer Atago N2 (Tokyo, Japan), pH was measured at 25°C using a Hanna HI 8424 instrument (Hanna Instruments Inc., Woonsocket, RI, USA).

Water activity (a_w) was determined using an electronic dew-point water activity meter Aqualab TE (Decagon Devices, Pullman, WA). The equipment was calibrated with saturated salt solutions in the water activity range of interest [29].

Viscosity was measured by means of a rotational viscometer (Brookfield DV-LVT; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) using S-34 and S-18 spindles. The sample chamber was placed in a water jacket connected to a bath to perform the determinations at a constant temperature of 25°C. Measurements were made at increasing rotational speeds (range 0.01–100 rpm) corresponding to a percentage torque ranging from 10 to 100. All determinations were made by duplicate for each sample before lyophilizing and with the reconstituted juices.

Shear rate profiles were fitted by the Power Law as:

$$\tau = K(D)^n \tag{1}$$

where by: τ = shear stress (mPa), D = shear rate (s⁻¹), K = consistency coefficient (mPa.sⁿ), n = flow index. Glass transition temperatures (Tg) of each freeze-dried system were determined by differential scanning calorimetry (DSC; onset values) using a TA Instrument Q2000 calorimeter (New Castle, England). The instrument was calibrated with indium (156.6°C). All measurements were performed at a heating rate of 10°C/min. Hermetically sealed 40 µL medium pressure pans were used, (an empty pan served as a reference). Thermograms were evaluated using Q2000 V24.11 program.

2.6.2. Total phenolics

Total phenolics (TP) were determined on the extracts using the Folin–Ciocalteu method according to Waterhouse [30]. Sample absorbance at 765 nm (PG Instruments T60U UV-Vis spectrophotometer, Leicestershire, United Kingdom) was measured, and phenolic concentrations were expressed as Gallic acid equivalent (GAE) in mg/l or g of product, calculated by means of a standard curve of Gallic acid. Percent of total polyphenolic retention (% TP) was relative to the initial polyphenol content considered as 100%.

2.6.3. Monomeric anthocyanin content, anthocyanins degradation index and polymeric color

Total monomeric anthocyanin content (TMA) and polymeric color (PC) of juices were estimated on alcoholic extracts based on a pH-differential spectrophotometric method and a bleaching reaction with bisulfite reported by Giusti and Wrolstad [31] respectively. The maximal λ considered was 510 nm for elderberry and 520 for the other berries studied. Their content was calculated as cyanidin-3-glucoside in mg/l or g of product (MW: 449.2 g mol⁻¹ and ε : 26 900 l cm⁻¹mol⁻¹).

The anthocyanin degradation index (DI) is the rate between total and monomeric anthocyanins. It was calculated on the basis of the absorbance of the samples diluted with pH 1.0 and 4.5 buffers (ApH1 and ApH4.5 respectively),

$$DI = \frac{ApH1}{(ApH1 - ApH4.5)} \tag{2}$$

Percent of total monomeric anthocyanin retention (% TMA) was relative to the initial content considered as 100%. PC was expressed in percent (%PC) [32].

2.6.4. Analysis of anthocyanin content by HPLC

HPLC analyses were performed on elderberry extracts with an Agilent 1260 series HPLC system according to the method reported by Hager et al. [33]. The injection volume was 20 μ l of juice extract. Separation was achieved on a reverse phase C18 Gemini® 150 × 4,6 mm; 5 μ column. Detection was performed using a diode array detector. Two solvents were used during the analysis. Solvent A: distilled water/formic acid (95/5) and solvent B: methanol. A constant flow of 1 ml/min was applied with a linear gradient elution profile. An external calibration curve of cyanidin-3-glucoside (cy-3G) was used for quantification, in the range 10–100 μ g/ml. The content of total anthocyanins (TA) and the major compound (cy-3G) were analyzed by absorption at 515 nm and the results expressed in mg of cyanidin equivalents per liter of berries' pulp (mg cy-3G/l). The sample was prepared in duplicate and then analyzed.

2.6.5. Color measurements

Juices color was analyzed using a Minolta Spectrophotometer CM-600d (Konica Minolta Observer), with D65 illuminant and an observer angle of 2° . The calibration was done against standard white and black tiles. Color measurements were performed by applying samples of juices, extracts or powders in plastic white containers. CIELab parameters (CIE 1976 L* a* b*) were L* for lightness, a* for redness and b* for yellowness. Calculations of h°

(arctan b*/a*) for hue angle were made. Total color difference was calculated as $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ and expressed the magnitude of color difference between 0 and 30 days of juice storage.

2.6.6. Antioxidant activity

The total antioxidant activity (TAC) was evaluated using two *in vitro* assay: DPPH radical scavenging activity reported as mmol Trolox/ml [34] and inhibition of lipid peroxidation in brain homogenates with or without (control) the addition of berry extract. The results are expressed as TBARS pmol/mg protein and % TBARS inhibition relative to control [35].

2.7. Data analysis

Replicate samples of each berry product were analyzed. All the physicochemical parameters studied were determined at least by triplicate (duplicate only for viscosity) and the average of the measurements was reported. The total phenolics, total monomeric anthocyanins from pulps and freeze-dried powders, and color parameters during storage were analyzed by one-way analysis of variance (ANOVA) test using Infostat v.2017 (Universidad Nacional de Córdoba, Argentina) [36]. Means comparisons were carried out by Student Newman-Keuls (SNK) test at P < 0.05. Pearson's correlations between anthocyanins content and color parameters were performed (n = 15).

3. Results and discussion

3.1. Physico-chemical and sensorial characteristics of the different berry pulps

Table 1 shows the physico-chemical characteristics of blueberry, elderberry, blackcurrant and maqui berry pulps used to compare with freeze-dried products and raw material to produce freeze-dried products. Soluble solids range between 9.0 and 14.8 °Brix and pH between 3.42 and 4.17, which helps to prevent microbial growth in this kind of products, and water activity ranged among 0.983 and 0.989. These parameters are in concordance with literature data [37–40], Skupien and others [41] reported in the physicochemical characterization of four highblush blueberry cultivars that soluble solids were influenced by environmental factors. The results showed that there is a concordance between higher values of soluble solids with the decrease of water activity for the different juices. Besides, is important to mention, according to bibliography references, that the characteristics of maqui berry cultivar [42].

Color parameters are also associated to the sensory quality of juices, the values presented in (Table 2) describe the typical dark colors of the berries and their alcoholic extracts. Maqui berry shows the highest value of L^{*} ("lightness") in both products (14.43 ± 0.72 ; 31.83 ± 0.20) while elderberry and maqui berry have the highest a^{*} ("redness") parameters in natural juices (14.72 ± 1.29) though blackcurrant presents the highest value of a^{*} in the alcoholic extract (47.91 ± 0.50), b^{*} parameters are similar in both samples of blueberry. The tone values (h°

| | Table 1 | | | |
|-----------------|---------------|-------------|-------|--------|
| Physicochemical | properties of | f different | berry | juices |

| | Blueberry | Elderberry | Blackcurrant | Maqui berry |
|------------------------|-------------------|-------------------|-------------------|-------------------|
| Soluble solids (°Brix) | 9.0 ± 0.1 | 11.8 ± 0.1 | 14.8 ± 0.1 | 13.0 ± 0.1 |
| pH | 3.54 ± 0.01 | 3.63 ± 0.01 | 3.42 ± 0.01 | 4.17 ± 0.01 |
| a _w | 0.989 ± 0.001 | 0.987 ± 0.001 | 0.983 ± 0.001 | 0.988 ± 0.001 |

Values are mean \pm standard error (n = 6).

hue angle) are in the same range for all juices. These low values for both h° and Chroma parameters represent a purplish red color, that is a typical characteristic of different berries with high anthocyanins content [43–45]. Also, in Table 2, it can be seen that differences in color parameters are more evident in alcoholic extracts because the samples are clearer and translucent (higher values of L^{*}) and only the compounds of interest for the development of colors are present due to the extraction process [46].

Table 3, shows total phenolics, total monomeric anthocyanins content and total antioxidant capacity measured by two *in vitro* assays: DPPH and TBARS, from all these berries. The blackcurrant stands out above other fruits for its content in TP and maqui berry for its TMA. In comparison, blueberry presented the lowest value of TP and the content of TMA was between 3 and 6 times lower than the other ones.

A variable fraction of TMA relative to TP content was obtained for each berry, being the corresponding value 30.2%, 16.5%, 11.8% and 9.7% for MB, EB, BC and BB respectively. The highest proportion in TMA was coincident with the highest values of red color parameter a^{*} of the juice.

TAC of these berries varied from 24.5 (BC) to 5.5 (BB) mmol Trolox/ml. Kevers et al. [47] showed, that the difference of both TP contents and TAC between several cultivars of blackcurrant and blueberry could be up to 2.4 times. A general good correlation between TAC and TP was observed (R = 0.98, P < 0.05).

In a previous study including elderberry, blackcurrant, strawberry and blackberry, Jakobek and others [48] found that the highest TP contents were observed for elderberry and blackcurrant with 6361.9 and 2770.9 GAE mg/l respectively. Both fruits also showed the highest TAC values 30.15 (elderberry) and 62.14 (blackcurrant) Trolox μ mol/ml. The relative content of TMA to TP had a broad variation ranging from 66% to 16% among all the fruits studied here.

| | Blueberry | Elderberry | Blackcurrant | Maqui berry |
|----------------------------|------------------|------------------|------------------|----------------|
| L_{J}^{*} | 1.67 ± 0.14 | 2.06 ± 0.20 | 1.50 ± 0.01 | 14.43 ± 0.72 |
| L^{*}_{JE} | 30.13 ± 1.11 | 22.34 ± 0.48 | 16.96 ± 0.44 | 31.83 ± 0.20 |
| a* J | 11.66 ± 0.89 | 14.72 ± 1.29 | 11.15 ± 0.06 | 14.63 ± 0.60 |
| a^*_{JE} | 39.20 ± 1.04 | 42.82 ± 1.14 | 47.91 ± 0.50 | 28.92 ± 0.18 |
| $b^*{}_J$ | 2.87 ± 0.24 | 3.55 ± 0.35 | 2.59 ± 0.02 | 4.07 ± 0.33 |
| $b^{\ast}{}_{J\mathrm{E}}$ | 2.66 ± 0.19 | 11.98 ± 0.49 | 13.60 ± 2.43 | 10.06 ± 0.16 |
| C^{*}_{J} | 12.01 ± 0.92 | 15.15 ± 1.34 | 11.44 ± 0.06 | 15.19 ± 0.65 |
| $C^{\ast}{}_{JE}$ | 39.29 ± 1.03 | 44.47 ± 1.18 | 49.88 ± 0.94 | 30.62 ± 0.48 |
| $h^\circ J$ | 13.82 ± 0.01 | 13.53 ± 0.12 | 13.00 ± 0.01 | 15.32 ± 0.72 |
| h°_{JE} | 3.90 ± 0.32 | 15.65 ± 0.50 | 15.79 ± 2.67 | 19.18 ± 0.24 |

| Table 2 | |
|---|------------|
| Color parameters of juice (J) and juice ext | tracs (JE) |

Values are mean \pm standard error (n = 8).

Table 3 Content of bioactive compounds of different berries juices

| | | Blueberry | Elderberry | Blackcurrant | Maqui berry |
|---|--------------------------|---|------------------------|----------------------------|----------------------------|
| Total phenolics (mg Ga | AE/l) | 2970.4 ± 178.2^{a} | 5584.6 ± 668.7^{b} | $9340.3 \pm 757.3^{\circ}$ | 5944.9 ± 793.8^{b} |
| Total monomeric anthocyanins (mg Cy-3G/l) | | 288.5 ± 19.7^{a} | $922.8\pm49.2^{\rm b}$ | $1222.0\pm50.0^{\rm c}$ | $1795.4 \pm 186.9^{\rm d}$ |
| Antioxidant capacity | TAC (mmol Trolox/ml) | 5.5 ± 0.5 14.5 ± 0.3 24.5 ± 1.0 | | 11.8 ± 0.2 | |
| | TBARS* (pmol/mg protein) | $1113\pm46^*$ | $983 \pm 18^*$ | $950\pm171^*$ | $978\pm35^*$ |
| | %TBARS inhibition | 86.7 | 88.3 | 88.7 | 88.3 |

Values are means \pm standard error, n = 6. For each bioactive compound, means with different letters within the same row are significantly different (P < 0.05), according to SNK test.

*reference control: the content was 8395 ± 259 (pmol/mg protein); P < 0.001 vs control.

| | | Blueberry | Elderberry | Blackcurrant | Maqui berry |
|---|----------------------|-------------------|------------------------|------------------------|--------------------------|
| | | 0.251 ± 0.001 | 0.145 ± 0.001 | 0.090 ± 0.001 | 0.096 ± 0.001 |
| $T_g^{\circ}C$ | T _{g onset} | 40.0 ± 0.14 | 38.0 ± 0.20 | 37.8 ± 0.74 | 36.0 ± 0.21 |
| c | Tg midpoint | 44.9 ± 0.44 | 44.9 ± 0.22 | 47.4 ± 0.11 | 43.1 ± 0.15 |
| Total phenolic (mg GAE/g) | • | 7.69 ± 0.43^a | $12.42 \pm 1.06^{b,c}$ | $14.72\pm0.72^{\rm c}$ | 11.21 ± 0.89^{b} |
| Total monomeric anthocyanins (mg 3G-Cy/g) | | 0.743 ± 0.05^a | $1.718\pm0.05^{\rm b}$ | $2.546\pm0.08^{\rm b}$ | $4.218 \pm 0.53^{\circ}$ |
| TP Retention (%) | | 99.64 | 84.35 | 68.23 | 95.10 |
| TMA Retention (%) | | 99.61 | 71.69 | 90.14 | 94.65 |

Table 4 Physicochemical properties and bioactive compounds content of different freeze-dried powders

Values are means \pm standard error, n = 6. For each bioactive compound, means with different letters within the same row are significantly different (P < 0.05), according to SNK test.

Regarding TAC, blackcurrant is the one with the highest value, probably due to its high content of ascorbic acid [49]. Different authors reported that ascorbic acid in blackcurrants berries stimulates the antioxidant activity of polyphenolic compounds and, taking into account that anthocyanins represent for about two-thirds of antioxidant potency for these fruits, it would indicate that other very potent antioxidants were present in phenolic fraction of this berry juice [47, 50, 51].

In recent works maqui berry showed the highest TP content (14.6 g GAE kg⁻¹ fresh weight) in comparison to blueberry, blackberry, and strawberry (with nearly 6 g GAE kg⁻¹ fresh weight), without the interference of vitamin C which has not been detected in this fruit [52]. In those works substantially higher levels of TMA in maqui berries (from 6 to 12.6 g kg⁻¹ fresh weight) were also reported, including blackcurrant and elderberry in the comparison [53]. High correlations between TP and TAC were obtained [54]. The contribution to antioxidant capacity may vary depending on the fruit, in maqui berry the anthocyanin rich fraction has been considered as the main source, however in blueberry total phenolic content and ascorbic acid were considered the main responsible of TAC [53, 55].

Although blueberry pulp showed the lowest bioactive compounds values between the berries considered in present study, it showed higher levels than many other fruits reported in literature and presents an interesting anthocyanin profile, which includes it among the fruits with richest levels in these components [54].

The inhibition of lipid peroxidation using TBARS in brain homogenates (Table 3) shows values near the 90% for all the fruits. These results show that the systems studied behave as efficient antioxidants in comparison with standard polyphenolic substances and suggest that the samples analyzed not only showed a significant antioxidant capacity *in vitro* but also can efficiently act as chain breaking antioxidants and be involved in final process of lipid peroxidation [56].

3.2. Physicochemical and sensorial characteristics of freeze – dried encapsulated pulps

Table 4 presents physico-chemical properties analyzed to characterize the freeze dried powders obtained from berry pulps. The resultant freeze-dried powders showed an a_w value in a range of 0.090–0.251 which could contribute to physico-chemical and microbiological stability of these products. The differences obtained could be due to the different composition (soluble solids and sugar profile) and viscosity of studied fruits, that can affect the process of mass transfer during dehydration.

In all cases, dehydration allowed to obtain systems rich in bioactive compounds, up to 2.5 times higher than in liquid systems, due to the concentration of these ingredients. Freeze-dried blueberry and maqui berry powders had the highest TP retention (%TP) after treatment, 99.6 and 95.1% respectively, followed by elderberry 84.3% and blackcurrant that only retained 68.2% of bioactive compounds. About TMA retention (%TMA), in BB, BC and MB was higher than 90%, only in the case of EB it was observed a decrease of up to 71.6%. These

results are in concordance with literature data in freeze-dried formulated samples of pomegranate that showed a %TP retention of 90–95% [24] or a reduction of 6.4%–18.6% of TP and a %TMA retention of 99.5–80.75% in freeze-dried grapes [23]. Cheng et al [57] indicated in the production of freeze-dried bayberry powder that the freeze-drying method is more beneficial for the retention of anthocyanins compared to the spray-drying method.

Other authors [32] reported that total polyphenols and monomeric anthocyanin composition in freeze-dried systems based on berries could show little differences compared to the fresh pulp depending on the type of fruit involved. These slight differences could be associated to a better extraction of the bioactive compounds in the freeze-dried matrix and with material grinding after freeze drying, that could strengthen the differences observed in BC.

The DI (degradation index) of the powders showed little variation (1.38 BB; 1.21 BC; 1.65 EB; 1.13 MB) compared with DI index of natural pulps (1.29 BB; 1.25 BC; 1.29 EB; 1.15 MB) and affirms the concept that freeze-drying process is one of the best treatments to improve the retention of bioactive compounds (anthocyanins and polyphenols) compared to air-drying or spray-drying [19, 58].

Glass transition temperature (Tg) of the freeze-dried systems was determined. Tg onset and middle point values are shown in Table 4 for each fruit system, and Figure 1 shows the thermograms obtained for the different berries. The T_g midpoint obtained for the freeze-dried powders varies between 43.1°C and 44.9°C. The differences up to 10% in juice solids among the berries freeze-dried powders, could contribute to the variations in the Tg values obtained, and also the aw obtained for the dehydrated system. Can Karaca et al [59], observed that the increase of cherry solids from 25% to 35% respect to total solids in spray-dried sour cherry powders produced with MDE₁₂, caused the drop of T_g value from 68°C to 40°C. In another work, a T_g value of 35.9°C was obtained for freeze-dried powder of cherry juice with 14% of cherry solids and 25% w/w of MDE₁₀: AG mix added (80:20) and aw = 0.10 [60]. In freeze-dried powders obtained from bayberry juice and grape skin phenolic extract with the addition of 20% MDE₁₂ and 10% of AG respectively, Tg values between 40°C and 50°C were obtained, and a a_w range between 0.10-0.30) [15, 23]. The juice of berry fruits can contain high proportion of low molecular weight sugars and organic acids with low Tg values and high hygroscopicity that affect the free-flow properties of the dehydrated powder. Fang et al [61] reported that the addition of a MDE₁₀ fraction relative to total solids higher than 0.3 in bayberry juice, was necessary to produce a compatible matrix with bayberry solids raising the overall T_g and obtaining values for the spray dried powder of up to 30°C. T_g is the transition temperature resultant of the interaction among juice encapsulated compounds and the carrying materials like maltodextrin and arabic gum. High molecular weight of both polysaccharides increases the Tg but the intensity of the interactions may vary together with the components in each juice and thus produce the slight variations observed in Tg values [62].

Table 5 shows the color parameters determined for freeze-dried powders, its reconstituted products and alcoholic extracts. As the matrix formulations have carrier materials (MDE_{10} and AG) which are white in the dry state, it is natural that the powders have higher L* values than fresh juices (which corresponds to the brightening of product color). The highest a* correspond to BC and MB which are jointly related to their superior fraction of juice solids included in the matrix, with more TMA content and high % retention compared to BB and EB. MB had the lowest value of h° thus indicating the deepest red tone powder.

In most of the reconstituted products the a* parameter tends to increase, meaning that they became visually more "red" than the fresh juice probably by the interaction with other components, because reconstituted powders have the addition of encapsulant agents. Similar trend was observed in freeze-dried chokeberrys powder [19], the value of blue-yellow parameter b* changed, which represented the intensity of yellow color, the greatest increase in the value of b* parameter was in BB, which means that more yellow component in this color spectrum, also shown in high hue angle h° values (variations in color tone), a similar behavior was reported in freeze-dried blackberries powder [15]. Besides that, the BB extract shows the lowest values for a* and b* parameters respect to the others extracts, with a negative and small h° angle reflexing a bluish red color that can be associated with lower TMA content. It is important to remark that in maqui powder and juice the aleatory presence of seeds could cause interference in their extraction results, for this reason it can be considered that the seeds may affect the



Fig. 1. DSC thermograms for freeze-dried berry powders of elderberry (EB), blackcurrant (BC), blueberry (BB) and maqui berry (MB). The value of $T_{g \text{ midpoint}}$ is indicated in thermograms.

| Table 5 |
|--|
| Color parameters of freeze - dried powders (P), reconstituted freeze-dried powders (R) |
| and its alcoholic extract (Re) |

| | | Blueberry | Elderberry | Blackcurrant | Maqui berry |
|--|-------------------------------|------------------|----------------|----------------|------------------|
| | L*P | 12.92 ± 0.14 | 10.29 ± 0.01 | 14.76 ± 0.03 | 11.96 ± 0.04 |
| | a* _P | 12.18 ± 0.11 | 11.68 ± 0.04 | 21.71 ± 0.03 | 17.84 ± 0.02 |
| | b*P | 0.97 ± 0.02 | 2.35 ± 0.03 | 3.81 ± 0.01 | 0.26 ± 0.03 |
| | $\mathbf{h^{o}}_{\mathbf{P}}$ | 4.58 ± 0.15 | 11.34 ± 0.15 | 9.95 ± 0.05 | 0.83 ± 0.11 |
| | L_{R}^{*} | 16.81 ± 0.44 | 2.55 ± 0.13 | 2.34 ± 0.04 | 19.05 ± 0.29 |
| | a* _R | 23.92 ± 0.35 | 16.90 ± 0.65 | 16.44 ± 0.19 | 10.26 ± 0.14 |
| | b* _R | 6.85 ± 0.20 | 4.39 ± 0.22 | 4.03 ± 0.06 | 2.44 ± 0.04 |
| | $h^{\circ}{}_{R}$ | 15.75 ± 0.26 | 14.42 ± 0.15 | 13.76 ± 0.05 | 13.37 ± 0.26 |
| | L^*_{Re} | 33.90 ± 0.17 | 24.25 ± 0.07 | 19.05 ± 0.13 | 27.27 ± 0.13 |
| | a* _{Re} | 35.89 ± 0.17 | 46.50 ± 0.05 | 50.25 ± 0.08 | 43.92 ± 0.18 |
| | b* _{Re} | -3.87 ± 0.01 | 9.52 ± 0.04 | 8.48 ± 0.51 | 4.13 ± 0.07 |
| | h°_{Re} | -6.15 ± 0.03 | 11.55 ± 0.15 | 9.58 ± 0.15 | 5.37 ± 0.07 |

Values are mean \pm standard error (n = 8).

color changes in its alcoholic extract, showing that the major differences are in the a* increment and b* reduction in comparison with powder and juice extract.

The most remarkable differences described above are highlighted in (Fig. 2) that represents the total color difference (ΔE^*) between juices pre and post freeze-drying, and also between their correspondent alcoholic extracts, where BB and MB again stand out from the others. Global color of BB juice results the most affected by the process, and it is probably related to its lowest TMA content, on the other hand in MB, the highest value calculated on its alcoholic extracts could be influenced by the removal of seeds, EB and BC have similar values in concordance with the description above.

Also, the viscosity of samples could have an influence on the brightness, L* parameter. Table 6 shows the values of the consistency index (K) and the flow behavior index (n) for samples of fresh pulps and reconstituted powders, which contain encapsulating agents in a proportion of 20%. The shear stress profiles of juices (J) and reconstituted freeze-dried berries powders (RJ) were well fitted to the power law and the parameters obtained were significant (R² > 0.98; P < 0.05). In all cases it was observed that natural pulps have higher values of K than reconstituted juices. Comparing with fresh pulps, it was observed a decrease in K value in about 98% for BB and 90% for EB in reconstituted powders. In the case of BC and MB, K values were 56% and 64% lower, respectively. Although the mixtures have 20% of added solids before drying, which could lead to an increase in the viscosity of the system, the final result after the freeze drying and grinding of the powder samples was a system with reduced consistency, probably due to the rupture of structures present in the fresh fruit that confer its



Fig. 2. Comparison of total color difference ΔE between natural juice and reconstituted freeze-dried powder (IIII) and between their alcoholic extracts (IIII) from blueberry (BB), elderberry (EB), blackcurrant (BC) and maqui berry (MB).

| | J | RJ | J | RJ |
|-----------------|--------------------------|-------------------------|-------------------------|---------------|
| | K (mPa | s ⁿ) | | n |
| | freeze-dr | ied berries powders (R | J) | |
| Rheological par | ameters: consistency ind | ex (K) and flow index | (n) of juices (J) and r | reconstituted |
| | | Table 6 | | |

| Blueberry | 12852.6 ± 257.0 | 276.6 ± 5.6 | 0.41 ± 0.01 | 0.64 ± 0.04 |
|--------------|---------------------|-------------------|---------------|---------------|
| Elderberry | 3438.2 ± 69.4 | 354.5 ± 6.5 | 0.30 ± 0.01 | 0.67 ± 0.01 |
| Blackcurrant | 13921.6 ± 180.6 | 6100.3 ± 48.8 | 0.40 ± 0.02 | 0.26 ± 0.02 |
| Maqui berry | 13429.4 ± 201.4 | 4815.0 ± 38.1 | 0.14 ± 0.01 | 0.35 ± 0.01 |

Values are mean \pm standard error (n = 4).

high viscosity on the pulp [63]. In the case of flow behavior index n, both the juices and the reconstituted powders have a pseudoplastic behavior, with values of n between 0.14 and 0.41 for the natural juices, and between 0.26 and 0.64 for the reconstituted powders. In general, it was observed an increase in n for reconstituted samples, except in the case of BC, where n value is lower than in the natural pulp. These results show that, in general, after the dehydration and grinding process, a lower consistency and a fluidization of the samples is observed compared to the natural juices. This aspect could have relevance when comparing the consistency between natural juices and reconstituted powders for example for industrial applications where these powder ingredients can be used in replacement of natural pulps.

3.3. Storage effect on physico-chemical and sensorial characteristics of freeze-dried encapsulated pulps at 38°C.

Figure 3a shows the evolution of TMA% retention throughout 60 days of storage at 38°C for the four berry powder systems. Considering the initial and final content after storage, the percent of retention was similar in all cases: 84.67 in MB, 83.43 in EB, 84.02 in BC and 75.79 in BB. The stability of anthocyanins was checked by HPLC for elderberry, where the major anthocyanin, cy-3G, that represented 42% of total anthocyanins was monitored at the beginning and at 60 days of storage of the powder. The percent of retention after storage was 85.9%, and the major peak in HPLC profiles corresponding to cy-3G, remained stable. Taking into account that at zero time TA content was 931 mg/l, at 60 days it maintained a value of 834.74 mg/l. Sanchez et al, [60] reported TMA stability values of 90% for freeze-dried cherry juice in a MDE₁₀/AG matrix stored at similar conditions. Others authors [32] found little variation on DI during storage for various freeze dried berries. Garzón and Worlstad [64] indicated that chemical structure of anthocyanin (glicosidic and acilation structure) were the main factor that affected their stability and found a direct relationship between water activity increase and DI. Besides, the stability of anthocyanins and polyphenols encapsulated is dependent on physical changes of the amorphous glassy matrix. As Tg values of the powders determined (Table 4) are slightly higher than the storage temperature of 38°C, and collapse or caking were not observed during storage time, it would be expected that systems maintained a glassy state and therefore high stability of their biocompounds.

The evolution of the color parameters was also studied during storage, in particular the red color that plays a vital role on the consumer election. Color parameter a^* is also shown on (Fig. 3a), considering the close relation existing between the TMA content and the redness color [49–65, 66]. Taking into account the particular differences of each fruit, a^* values remained quite constant during the 60 days, following the same tendency as the degradation of TMA, behaving both parameters similarly in these freeze-dried products. Considering the corresponding data of BB, EB and BC a high global correlation between parameter a^* and TMA content during storage was obtained, being R = 0.95 (P < 0.001, n = 15).

As seen in (Fig. 3b) and considering the close relationship between the degradation of anthocyanins and color changes [67] the polymeric color (% PC) was measured during storage, because its increase is usually associated with brownish tones. Graphically, the evolution of %PC remains near to the initial value: in EB and BC it increases 11 and 19.5% and decreases in BB and MB 5.54% and 19% respectively. These results would be aligned with the little variations in the parameter a* observed during storage of freeze – dried powders. The initial values (BC 30% PC, EB 42.81% PC, BB 45.33% PC and MB 51% PC) are in the range reported in freeze dried berry products [68] and were lower than values obtained during storage of pasteurized berry juices found in previous studies [46].

The stability of TP was calculated after 60 days of storage, only in the blueberry there was a slight decrease of 19% with respect to the initial value (TP_i: 2367.88 – TP_f: 1911.75 GAE mg/l reconstituted juice) and in the rest of the fruits the TP content increased up to 1.48 times, being their values expressed as GAE mg /l reconstituted juice: 1,48 (TP_i: 4523.02 – TP_f: 6676.12) for MB, 1.43 (TP_i: 3768.33 – TP_f: 5387.46) for EB and 1.35 (TP_i: 5098.50 – TP_f: 6898.09) for BC.



Fig. 3. (a) Comparison of total monomeric anthocyanins % retention (%TMA \Box) and a* (redness Δ) color parameter of reconstituted freezedried powder of blueberry (\blacksquare), elderberry (\Box), blackcurrant (\blacksquare) and maqui berry (\boxtimes) stored at 38°C. Values are mean \pm standard error. (b) Comparison of percent polymeric color (%PC \bigcirc) of reconstituted freeze-dried powder of blueberry (\blacksquare), elderberry (\Box), blackcurrant (\blacksquare) and maqui berry (\boxtimes) stored at 38°C. Values are mean \pm standard error.

Following the same tendency shown by anthocyanin content, antioxidant activity remained almost unchanged in the four powder systems, confirming the relationship existing between anthocyanin content and antioxidant activity in foods that contain these pigments [69]. The correspondent values, expressed as % inhibition were: TAC_i: 13.89% – TAC_f: 12.24% in BB; TAC_i: 19.82% – TAC_f: 17.43% in EB; TAC_i: 25.6% – TAC_f: 25.15% in MB and TAC_i: 31.83% – TAC_f: 34.01% in BC.

Flores and others [70] observed for spray-dried blueberry powder stored at 37°C during 35 days, that TP and TAC increased between 2 and 2.5. Moser et al [71] informed a small decrease in antioxidant activity,



Fig. 4. Comparison of color parameters (L*, a*, b* h°) for extracts of reconstituted freeze-dried powder of elderberry (EB), blackcurrant (BC), blueberry (BB), and maqui berry (MB) at 0 and 30 days stored at 38°C. Different letters above the data bars indicate that color parameter differed between storage time, P < 0.05. Values are mean \pm standard error, n = 8.

between 6%–14% at the end of storage for grape microencapsulated powder with whey protein concentrate and maltodextrin. Rocha and team [72] reported that the antioxidant activity of freeze dried wine powder showed a good stability during 145 days at 38°C of accelerated storage, suggesting that despite having detected losses of certain phenolic compounds studied, they could be involved in the formation of new antioxidant compounds. Furthermore, the stability of the anthocyanin pigments extracted from acid cherries and stored with dextrins has been attributed to the complex formed between the flavylium cation of the anthocyanins and the dextrins that avoided their transformation into less stable structures [73].

Figure 4 shows the changes in CIELab parameters a^* , b^* , L^* and h° in the alcoholic extracts of reconstituted powder, at initial time and after 30 days of storage at 38°C. It should be noted that after 30 days no significant changes were observed in the color parameters. Samples based on elderberry and blueberry showed slight significant differences in all the parameters (P < 0.05), that were manifested in highest values obtained of the total color difference (ΔE^*): 7.73 ± 0.39 for EB; 6.23 ± 0.31 for BB; 4.76 ± 0.24 for BC; and 3.61 ± 0.18 for MB. A minor proportion of juice solids encapsulated in EB and BB and their particular phenolic composition could contribute to produce more notorious changes during storage. Color and polyphenols stability are concordant with data found in literature for cherry, raspberry and Andes berry freeze-dried juices with addition of MD [60, 74, 75].

4. Conclusions

The results obtained in present study allow to properly describe each powder system obtained from berry pulps and confirms their high levels of bioactive compounds, providing an important contribution to the knowledge of anthocyanin and polyphenols content and stability in fruits form Patagonian region of Argentina.

The powder systems obtained by freeze drying using 20% of encapsulating agents showed a high stability, associated with relatively high values of T_g (range between 43–45°C) and low values of a_w . The powders stored at 38°C showed very good retention of TMA (higher than 75%) and for TP content only a 15% of decrease was seen in the case of blueberry meanwhile an increase was found for the rest. Stability was also observed in TAC. The high content of bioactive compounds in the powder samples makes them interesting for various applications as a functional ingredient for the development of food with benefits for human health industries and are the basis for future studies on this subject associated to the fruits of the region of Argentine Patagonia. Also, for the high color stability observed after freeze drying and storage, these dehydrated systems can be used as potential natural colorants in food or cosmetics industries, as ingredients of easy addition which can be stored at room temperature.

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Conflict of interest

The authors have no conflict of interest to declare.

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