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Stability and bioaccessibility of anthocyanins from maqui (*Aristotelia chilensis* [Mol.] Stuntz) juice microparticles

Carolina Fredes[a,*], María Jesús Osorio[a], Javier Parada[b], Paz Robert[a]

[a] Departamento de Ciencia de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Sergio Livingstone Pohlhammer 1007, Independencia, Santiago, Chile

[b] Institute of Food Science and Technology, Faculty of Agricultural Sciences, Austral University of Chile, Valdivia, Chile

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ABSTRACT

The microencapsulation of maqui juice by spray-drying was studied as a strategy to protect anthocyanins in new value-added formulations. The objective of this research was to study the influence of each maqui-anthocyanin and encapsulating agent (inulin or sodium alginate) on the anthocyanin encapsulation efficiency, the stability during storage (160 days at 60 °C) and the bioaccessibility in *in vitro* digestion model. The highest encapsulation efficiency of anthocyanins (65.6–78.6%) was obtained with inulin. The chemical structure of anthocyanins also influenced the encapsulation efficiency; the highest was 78.6% for delphinidin-3-sambubioside-5-glucoside in maqui juice-inulin, and 51.2% for cyanidin-3-glucoside in maqui juice-alginate. For both maqui juice-inulin and maqui juice-alginate microparticles, the half-life values of delphinidin-3-sambubioside (198 days), delphinidin-3-glucoside (173–182 days) and cyanidin-3-glucoside (154-133 days) showed the lowest stability of 3-*O*-glycosylated anthocyanins. The bioaccessibility of anthocyanins of the maqui juice microparticles was 10% higher than maqui juice.

1. Introduction

Maqui (*Aristotelia chilensis* (Mol.) Stuntz, Elaeocarpaceae), also known as 'clon,' 'queldron,' and 'maquei,' is the most well-known Chilean fruit, studied worldwide for its phytochemicals and biological activities. New maqui fruit-based commodities are consistently appearing in the international market, correlating with an increase in Chilean exports of maqui fruit (ODEPA, 2017).

Traditional uses and current scientific evidences demonstrate the principal role of maqui fruit anthocyanins in potential antioxidant, anti-inflammatory, anti-diabetic, and hypoglycaemic activity (Fredes & Robert, 2014). The maqui fruit anthocyanin profile has been well-identified; eight anthocyanins have been reported (Céspedes et al., 2010; Escribano-Bailón, Alcalde-Eon, Muñoz, Rivas-Gonzalo, & Santos-Buelga, 2006; Fredes et al., 2014; Gironés-Vilaplana et al., 2014; Rojo et al., 2012). However, anthocyanins are susceptible to degradation against environmental, food, and gastrointestinal (GI) tract conditions, limiting their application as health food ingredients (Mahdavi, Jafari, Ghorbani, & Assadpoor, 2014). In this sense, the encapsulation technology may be used as a strategy to protect anthocyanins in new value-

Abbreviations: ALG, sodium alginate; aw, water activity; BA, bioaccessibility; cy-3-glu, cyanidin-3-glucoside; cy-3-sa, cyanidin-3-sambubioside; cy-3-sa-5-glu, cyanidin-3-sambubioside-5-glucoside; cy-3,5-diglu, cyanidin-3,5-diglucoside; del-3-sa-5-glu, delphinidin-3-sambubioside-5-glucoside; del-3,5-diglu, delphinidin-3,5-diglucoside; del-3-glu, delphinidin-3-glucoside; del-3-sa, delphinidin-3-sambubioside; EA, encapsulating agent; EE, encapsulation efficiency; GI, gastrointestinal; HPLC-DAD, high-performance liquid chromatography–photo diode array detector; IN, inulin; MJ, maqui juice; RSM, response surface methodology; TA, total anthocyanins; SEM, scanning electron microscopy

* Corresponding author.
E-mail address: carolina.fredes@postqyf.uchile.cl (C. Fredes).

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added formulations using maqui fruit.

The protection of target anthocyanins with specific structural features has special relevance when the primary bioactivity and/or specific health effect of a plant species is attributed to particular anthocyanins. For example, delphinidin-3-sambubioside-5-glucoside (del-3-sa-5-glu) from maqui has shown hypoglycaemic activity in *in vivo* models (Rojo et al., 2012). To the best of our knowledge, this is the first study in the literature focused on encapsulation of maqui anthocyanins.

The studies on the encapsulation of anthocyanins from other raw materials have been performed by the quantification of total anthocyanins (TA) by spectrometry (Robert, García, & Fredes, 2017) where the main goal has been provide protection to anthocyanins against environmental conditions during storage, extending shelf life (Mahdavi et al., 2014). Raw materials have been treated in different ways in order to obtain anthocyanin extracts, but encapsulation is a more sustainable process when pulps/juices are used, avoiding the use of organic solvents and the consequent step of solvent recovery.

Spray dryers are commonly available in the food industry, therefore spray-drying has been the most frequent method used to encapsulate anthocyanins (Mahdavi et al., 2014; Robert et al., 2017) allowing microparticles in powders to be obtained using a two steps process. In this context, the encapsulating agent (EA) plays an important role in proper encapsulation efficiency (EE), stability during storage, and release of active compounds in foods and the GI tract. Inulin (IN) and sodium alginate (ALG) are polysaccharides that are approved for use in foods which have not previously been assessed as anthocyanin EA or as biopolymers that release anthocyanins in the GI tract. In this work the maqui juice (MJ) was microencapsulated by spray-drying, in order to study the influence of each maqui-anthocyanin and EA (IN or ALG) on the anthocyanin encapsulation efficiency, the stability during storage (60 °C) and the bioaccessibility (BA) in *in vitro* digestion model.

2. Materials and methods

2.1. Raw material and EA

Organic concentrated (65 ºBrix) MJ (Patagonol™– LE, Bayas del Sur, Purranque, Chile) was microencapsulated with IN (Raftiline HP (DP > 23)) and (ALG) Alfa Chilena S.A., Santiago, Chile.

2.2. Maqui juice (MJ) analysis

The moisture content, soluble solids, pH, and titratable acidity were determined according to AOAC methods (1996) and total sugar was measured using the Antrona method (Osborne & Voogt, 1986).

2.3. Preparation of MJ microparticles

MJ microparticles with IN or ALG were prepared in 100 g solutions as follows: IN (2.8–10 g) was dissolved in distilled water at 70 °C, cooled to 25 °C, and then MJ (2.33 g) was added. Sodium alginate (0.47–3 g) was stirred in distilled water (15 h), and then MJ (1.4 g) was added. Each preparation was homogenized at 11,000 rpm for 5 min using a Polytron PT-2100 (Kinematica A.G, Switzerland). The resulting solutions were fed into a mini spray-dryer B290 (Buchi, Flawil, Switzerland). The spray-dryer was operated at an inlet air temperature of 150–190 ± 1 °C. Air flow, rate of feeding, and atomization pressure were 600 L/h, 1 mL/min and 0.14 MPa, respectively. The resulting powders were stored absent of light at −20 °C for further analysis.

The experiments were performed in a Central Composite experimental design with 12 runs for each EA system (MJ-IN and MJ-ALG). The independent variables were the MJ/EA ratio (1:1.5–1:4.0 for MJ-IN and 1:0.5–1:2.0 for MJ-ALG) and inlet air temperature (150–190 °C). The dependent variables were the EE of anthocyanins and the yield. Response surface methodology (RSM) (Software Statgraphics Centurion XV, Statpoint Inc., VA, USA) was applied to maximize the dependent variables and a multiple response optimization using the desirability function (DF) was used to determine the optimal conditions, considering all of the response variables. The data were fitted to a second-order regression model, considering linear, quadratic, and cross-product forms for each independent variables at a confidence level of 95%. All the experiments were conducted randomly to avoid systematic bias.

2.4. Determination of anthocyanin profiles by HPLC-DAD

The MJ and MJ microparticles were redissolved in LiChrosolv® water for chromatography (Merck-Millipore) at 2.8 mg cy-3-glu/g (in triplicate), filtered through 0.22 µm PTFE membrane filters (VWR International, Atlanta, GA, USA), and then injected into the HPLC. HPLC was coupled with a DAD (Flexar, Perkin Elmer, England) detector using a C18 column (5 µm × 4.6 mm i.d. × 25 cm, Symmetry, Waters, Ireland). Formic acid (5%) in H_2O (A) and 100% methanol (B) were used for the mobile phase, as described by Fredes et al. (2014) and Rojo et al. (2012). Detection was at 520 nm and the anthocyanin compound identities were verified as was reported by Fredes et al. (2014). Individual anthocyanins were quantified using a cy-3-glu (Sigma-Aldrich, USA) calibration curve (0.16–24.8 µg/mL; $r^2 = 0.9998$). Total anthocyanins content (TA) corresponded to the sum of all of the anthocyanins peaks.

2.5. Total anthocyanin determination in MJ microparticles

For total anthocyanin determination is necessary to destroy the microparticles before extracting the anthocyanins with solvents. MJ-IN microparticles (200 mg) were dispersed in 1 mL of methanol: acetic acid: water (50:8:42 v/v/v), vortexed (1 min), ultrasonicated (20 min) and centrifuged at 112,000 g (20 min). MJ-ALG microparticles (100 mg) were dispersed in LiChrosolv® water (3 mL) for chromatography (pH 6.0), vortexed (3 min) and ultrasonicated (10 min). Then, ethanol (3 mL) was added, ultrasonicated again (10 min) and centrifuged at 112,000 g (8 min). The corresponding supernatants were filtered (0.22 µm PTFE filters) before HPLC analysis.

2.6. Surface anthocyanin determination in MJ microparticles

The surface anthocyanins were determined using a mixture of solvents, where the polymers used as EA are insoluble. MJ-IN microparticles (200 mg) were dispersed in 2 mL of methanol:acetic acid (99:1 v/v) and softly vortexed (1 min). MJ-ALG microparticles (200 mg) were dispersed in 2 mL of ethanol:acetic acid (99:1 v/v), softly vortexed (1 min) and then centrifuged twice at 1000 rpm (4 min). The corresponding supernatants were filtered (0.22 µm PTFE filters) before HPLC analysis.

The yield, recovery, and EE for anthocyanins were calculated following the methodology described by Robert et al. (2010).

2.7. Analysis of MJ microparticles obtained under optimal conditions

The moisture content and water activity (a_w) of MJ microparticles obtained under optimal conditions were measured according to AOAC method (1996). Hygroscopicity was determined according to the procedure described by Cai and Corke (2000). Total, surface, and individual anthocyanin content were determined as was described previously.

2.7.1. Scanning electron microscopy (SEM)

Samples of microparticles obtained under optimal conditions were coated with a 10 nm film of gold using a Sputter Coater 108auto with a thickness Controller MTM-20 (Cressington Scientific Instruments, Watford, UK) and analyzed with a High Resolution Scanning Electron Microscope (HR-SEM) with a secondary electron detector (SED) (model INSPECT-F50, FEI, Thermo Fisher Scientific, Hillsboro, Oregon, USA)

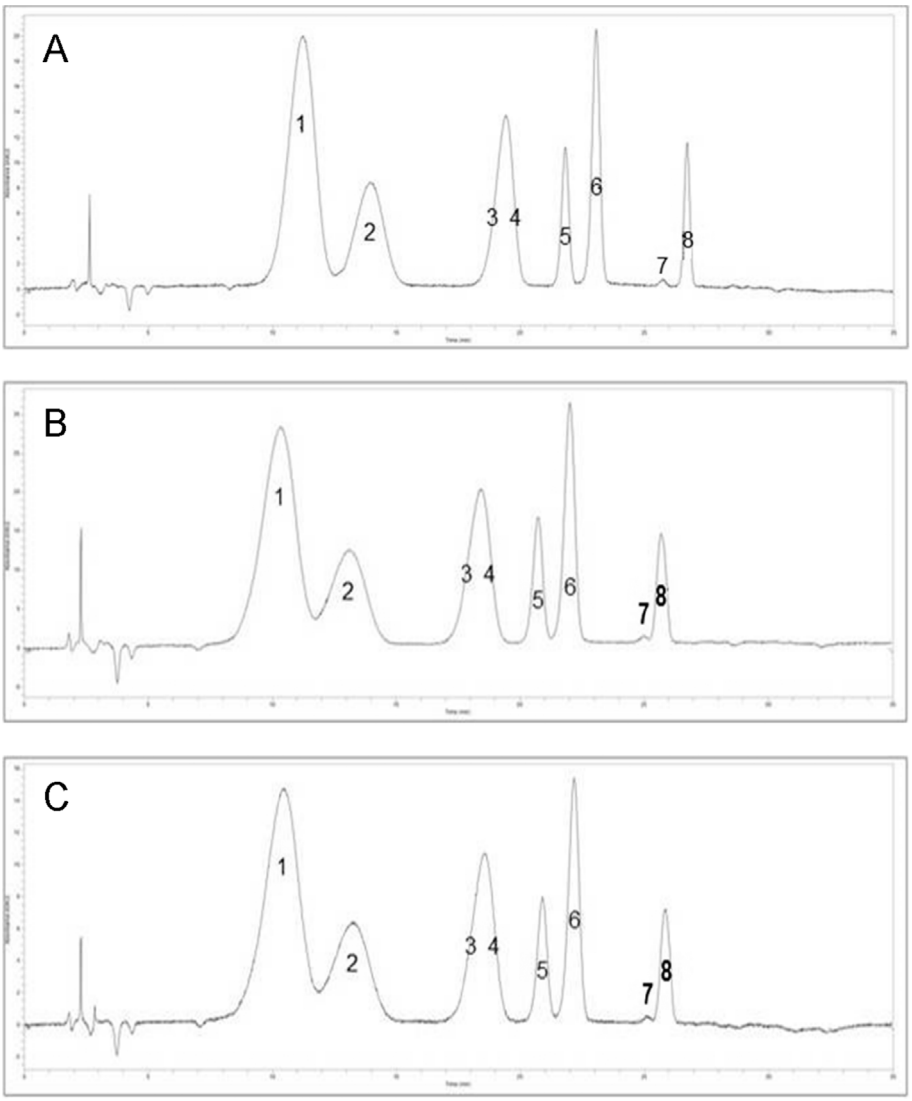

Fig. 1. Representative HPLC-DAD chromatograms of anthocyanin compounds in MJ (A), MJ microparticles with IN (B) or ALG (C) at 520 nm. Peaks: 1 = delphinidin-3-sambubioside-5-glucoside (del-3-sa-5-glu); 2 = delphinidin-3,5-diglucoside (del-3,5-diglu); 3–4 = cyanidin-3-sambubioside-5-glucoside + cyanidin-3,5-diglucoside (cy-3-sa-5-glu + cy-3,5-diglu); 5 = delphinidin-3-sambubioside (del-3-sa); 6 = delphinidin-3-glucoside (del-3-glu); 7 = cyanidin-3-sambubioside (cy-3-sa); 8 = cyanidin-3-glucoside (cy-3-glu).

operated at 5.00 KV.

2.7.2. Accelerated storage conditions

MJ microparticles (0.2 g for MJ-IN and 0.1 g for MJ-ALG) obtained under optimal conditions were transferred to clear glass vials (450 × 250 mm) and stored at 60 ± 1 °C (isothermal method) in a forced-air oven (Memmert, model BE 500, Schwabach, Germany) in the absence of light for 6 months (Harbourne, Jacquier, Morgan, & Lyng, 2008). The vials were withdrawn at specific times to determine TA. Individual anthocyanin content data were best fit a first-order kinetic model, $\ln C = \ln C_0 - k(t)$. Degradation rate constants (k_{obs}) were obtained from the slope of a plot of the natural log of the individual anthocyanin retention vs. time. Retention (%) = $(C_t/C_o)*100$, where C_t is the anthocyanin concentration at "t" time, and C_o is the initial individual anthocyanin concentration (Brauch, Kroner, Schweiggert, & Carle, 2015; Harbourne et al., 2008; Robert et al., 2010). Half-life values of individual anthocyanins were calculated from: $t_{1/2} = \mathrm{Ln}2/k_{obs}$

2.8. Stability of anthocyanins in in vitro digestion model

Samples of 0.5 g (in triplicate) of MJ microparticles obtained under optimal conditions and MJ were processed following Aravena, García, Muñoz, Pérez-Correa, and Parada (2016).

2.8.1. Mouth digestion

Artificial saliva (9 mL) was added to each flask with sample. This solution was comprised of 14.4 mM sodium bicarbonate, 21.1 mM potassium chloride, 1.59 mM calcium chloride, and 0.2 mM magnesium chloride. The pH was adjusted to 7 with HCl (1 M). Sixty α-amylase units per milliliter of buffer were incorporated the same day the test was performed. Samples were incubated in a thermostatic bath (Zhicheng ZHWY-110X30); 37 °C for 5 min, at a shaking speed of 185 rpm.

2.8.2. Stomach digestion

The pH of the samples was adjusted to 2.0 using HCl (1 M), then 36 mL of a pepsin solution (25 mg/mL in 0.02 M HCl) was added. Therefore, each sample (containing 9 mL of artificial saliva) was diluted 5-fold with artificial gastric juice, as it occurs in the stomach. Samples were incubated for 2 h at 37 °C with a stirring speed of 130 rpm.

2.8.3. Gut digestion

The pH of the samples was adjusted to 6.0 with $NaHCO_3$ (1 M). Then, 0.25 mL per mL of sample of an artificial gut solution, containing

pancreatin (2 g/L) and bile salts (12 g/L) dissolved in aqueous $NaHCO_3$ (0.1 M) was added. Incubation was carried out for 2 h at 37 °C and a shaking speed of 45 rpm.

Each digestion product was transferred to 50 mL Falcon tubes and the pH was adjusted (pH 3) (Oidtmann et al., 2012). It was then centrifuged for 10 min at 5000 rpm to recover the liquid fraction. Next, the liquid digestion product was centrifuged at 12,000 rpm before anthocyanin analysis. The measured amount of individual anthocyanins was the bioaccessible portion, which was calculated as follows:

$$\text{Bioaccessibility } \% = \frac{\text{mg anthocyanin}_i \text{ on digest product}}{\text{mg anthocyanin}_i \text{ in MJ or MJ microparticles}} \text{ x } 100$$

where i corresponds to each anthocyanin quantified in the MJ and the microparticles.

2.9. Statistical analysis

The differences between both MJ microparticles for each parameter and among individual anthocyanins were analyzed using a one-way ANOVA test. When significant differences were found, the Tukey HSD (honest significant differences) multiple-comparison test ($P \leq 0.05$) was applied. Analyses were performed with SAS 9.2 for Microsoft Windows (2009; SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Maqui juice (MJ) characterization

The MJ had a moisture content (41.0 ± 0.03 g/100 g), total soluble solids (66.0 ± 0.1 g/100 g), pH (3.8 ± 0.03), titratable acidity (5.8 ± 0.1 g citric acid/100 g) and total sugars (61.0 ± 0.8 g/100 g) that showed that the high soluble solid content was attributed to a high sugar content. The anthocyanin profile of the MJ (Fig. 1A) was similar to those described previously (Céspedes et al., 2010; Escribano-Bailón et al., 2006; Flores, Singh, Kerr, Pegg, & Kong, 2014; Gironés-Vilaplana et al., 2014; Rojo et al., 2012) showing eight anthocyanins, even though cyanidin-3-sambubioside (cy-3-sa) was found under the limit of quantification (0.16 μg/mL). According to the anthocyanin profile of MJ, 3,5-*O*-diglycosylated anthocyanins (70.7%) such as del-3-sa-5-glu (5.7 mg/g, 35.7% w/w), delphinidin-3,5-diglucoside (del-3,5-diglu) (2.7 mg/g, 16.9%) and cyanidin-3-sa-5-glu + cyanidin-3,5-diglucoside (cy-3-sa-5-glu + cy-3,5-diglu) (2.9 mg/g, 18.1%) predominated over 3-*O*-glycosylated anthocyanins (29.3%) such as delphinidin-3-sambubioside (del-3-sa) (1.3 mg/g, 8.4%), delphinidin-3-glucoside (del-3-glu) (2.1 mg/g, 13.5%) and cyanidin-3-glucoside (cy-3-glu) (1.2 mg/g, 7.3%).

3.2. The encapsulation of anthocyanins from MJ

In order to evaluate the effects of the MJ/EA ratio and the inlet air temperature on the EE of anthocyanins and the yield and to find the optimal conditions for encapsulation by spray-drying, a RSM was carried out. For MJ-IN system, the experimental data showed that EE of anthocyanins ranged from 41.3 to 68.6% (del-3-sa-5-gu), 36.3–66.1% (del-3,5-diglu), 36.8–64.7% (cy-3-sa-5-glu + cy-3,5-diglu), 32.5–61.8% (del-3-sa), 26.7–61.4% (del-3-glu), and 31.0–59.7% (cy-3-glu). The higher the EA content, the greater the EE of anthocyanins. The lineal form of MJ/IN ratio (A) was significant ($p < 0.05$) for the EE of all of the anthocyanins, whereas the quadratic form (A^2) was only significant ($p < 0.05$) for the EE of del-3-sa. The lineal form of the inlet air temperature (B) was significant ($p < 0.05$) for the EE of all anthocyanins except del-3-glu and cy-3-glu, whereas the quadratic form (B^2) was not significant ($p < 0.05$) for the EE of any anthocyanins. The cross-product form (A × B) was significant ($p < 0.05$) for the EE of anthocyanins except for del-3-glu. The lineal, quadratic, and cross-product forms of A and B were significant ($p < 0.05$) for the yield (39.6–72.8%). The coefficients of determination (R^2) and R^2-adjusted for the predictive models were higher than 0.9 and 0.8, respectively for each response variable, suggesting that the predictive models seemed to reasonably represent the observed values. The optimal MJ/IN ratio was 1:4.3, the major axial point, at an intermediate inlet air temperature of 162 °C (supplementary material).

In the case of the MJ-ALG system, the anthocyanin EE ranged from 20.4 to 40.3% (del-3-sa-5-gu), 24.3–40.5% (del-3,5-diglu), 22.8–45.7% (cy-3-sa-5-glu + cy-3,5-diglu), 27.9–46.1% (del-3-sa), 25.9–42.4% (del-3-glu), and 25.6–47.3% (cy-3-glu). The yield was not significant ($p < 0.05$) and therefore it was not considered as response variable. Similar to the MJ-IN system, the lineal form of A was significant ($p < 0.05$) for the EE of all of the anthocyanins. The quadratic form (B^2) was only significant ($p < 0.05$) for del-3-sa. The cross-product form (A × B) was significant ($p < 0.05$) for del-3-glu. The coefficients of determination (R^2) and R^2-adjusted for the predictive models were higher than 0.9 and 0.8, respectively for each response variable, suggesting that the predictive models seemed to reasonably represent the observed values. For MJ-ALG (supplementary material), the optimal MJ/ALG ratio was 1:2.2, the major axial point, at an intermediate inlet air temperature of 167 °C. Therefore, the type of EA influences anthocyanin EE because at the same inlet air temperature, IN and ALG showed differences in EE.

3.3. Characterization of MJ microparticles obtained under optimal conditions

Table 1 shows the optimal conditions of the encapsulation of MJ. Although similar optimal inlet air temperatures were obtained using IN (162 °C) and ALG (167 °C) as EA, both MJ-EA systems had different optimum parameters, showing the effect of the nature of biopolymers. Inulin was used at the highest water solubility (10 g/100 g) whereas ALG was used at the highest content that achieved a feed solution with a viscosity that allows the feeding to spray-dyer (3 g/100 g). This resulted in different amounts of EA when IN or ALG was used. In this context, there are few studies using ALG by spray-drying because of high viscosity of ALG in aqueous solution makes it hard to handle (Gharsallaoui, Roudaut, Chanbin, Voilley, & Saurel, 2007).

The anthocyanin profiles by HPLC for MJ microparticles (Fig. 1B and C) were similar to MJ (Fig. 1A), where 3,5-*O*-diglycosylated anthocyanins (72.1%) also predominated over 3-*O*-glycosylated anthocyanins (27.9%). The TA content (Table 1) in both MJ microparticles (2.8 mg/g for MJ-IN and 5.9 mg/g for MJ-ALG) were greater than previous reports for microparticles from other raw materials, which ranged from 0.1 mg cy-3-glu/g for encapsulated Andes berry (Villacrez, Carriazo, & Osorio, 2014) to 0.6 mg cy-3-glu/g for bayberry (Fang &

Table 1
Optimal conditions, physical and chemical characteristics of MJ microparticles obtained under optimal conditions.

System	MJ-IN	MJ-ALG
Inlet air temperature (°C)	162	167
Outlet air temperature (°C)	93	95
MJ/EA ratio	1:4.3	1:2.2
EA (g/100 g)	10	3
Soluble solids (°Brix at 20 °C)	10.8 ± 0.2	4.0 ± 0.1
Total anthocyanins (mg cy-3-glu/g)	2.8 ± 0.1 a	5.9 ± 0.1 b
Encapsulation efficiency (%)	74.4 ± 2.2 b	45.4 ± 3.9 a
Anthocyanin recovery (%)	90.0 ± 2.2 a	100.0 ± 1.6 b
Moisture content (%)	3.5 ± 0.4 a	6.8 ± 0.2 b
Water activity	0.26 ± 0.01 a	0.35 ± 0.02 b
Hygroscopicity (g/100 g)	32.5 ± 4.2 a	91.5 ± 3.2 b
Yield (%)	73.3 ± 2.2 b	47.6 ± 1.0 a

Mean values (n = 3) and standard deviation that are followed by different letters in the same row indicates significant differences ($p \leq 0.05$).

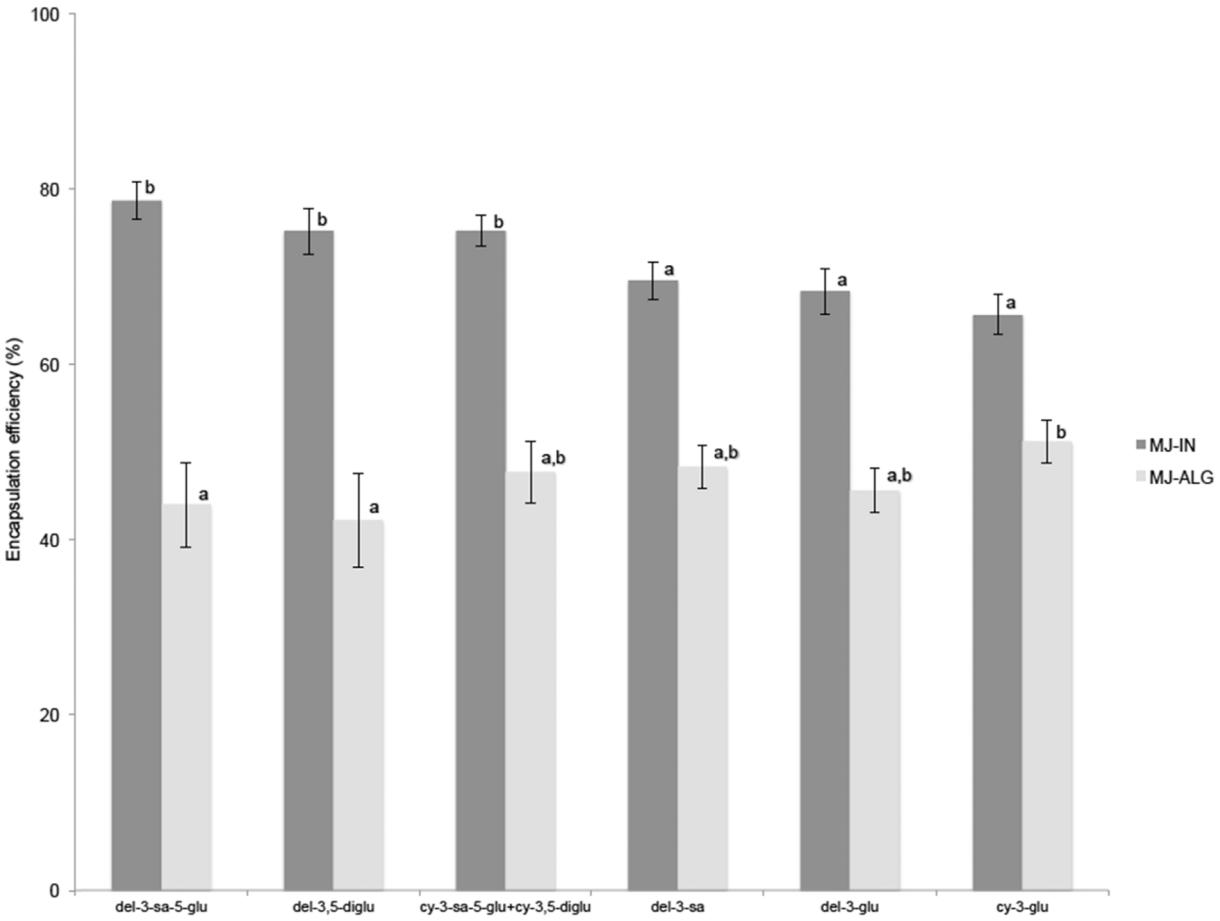

Fig. 2. Encapsulation efficiency (EE) of individual anthocyanins in MJ microparticles obtained under optimal conditions.

Bhandari, 2011). The highest TA content in ALG microparticles can be expected because the amount of EA used was less than half than what was used with IN.

The EE represents the anthocyanin-polymer interaction. In this study, the EE of TA was higher with IN than ALG, which could be attributed to anthocyanin-polymer interaction that may occur by electrostatic interactions or hydrogen bonding, and/or the EA content that affect the formation rate of a crust. The EE of individual anthocyanins ranged from 65.6 to 78.6% in the MJ-IN system (Fig. 2) where the EE of 3,5-*O*-diglycosylated anthocyanins were significantly higher than the EE of 3-*O*-glycosylated anthocyanins. These differences among anthocyanins can be attributed to the high number of hydroxyl groups of 3,5-*O*-diglycosylated anthocyanins which help form intermolecular hydrogen bonds with IN. Thus, the del-3-sa-5-glu has the highest number of hydroxyl groups (OH 14) and the highest EE (78.6%), whereas the cy-3-glu has the lowest number of hydroxyl groups (OH 8) and the corresponding lowest EE (65.6%). Contrary to these results, in the MJ-ALG system, the number of hydroxyl groups showed no effect on the EE of anthocyanins. The EE of del-3-sa-5-glu (44.0%) was significantly lower than cy-3-glu (51.2%). In this context, Sun-Waterhouse, Wadhwa, and Waterhouse (2013) indicate that the type of polyphenol, especially its structural features, influences the EE when the same EA is compared. However, the magnitude of the EE depends on the biopolymer used as EA.

Anthocyanin recovery demonstrates the effect of the spray-drying process on anthocyanin content. These results were high for both MJ-IN (90.0%) and MJ-ALG (100.0%) at similar temperatures (162 and 167 °C, respectively) (Table 1), showing the effect of the short exposure time to high temperature and/or rapid crust formation (Gharsallaoui et al., 2007). Our results are in agreement with Fang and Bhandari (2011) and Silva, Stringheta, Teófilo, and de Oliveira (2013). who reported similar anthocyanin recovery in the encapsulation of bayberry and jaboticaba (94 and 80%) at similar inlet air temperatures (150 and 160 °C, respectively).

Regarding the physical characteristics of microparticles (Table 1), those prepared with IN had a lower moisture content than those prepared with ALG because the higher the solid content in the feed solution, the lower the moisture content in microparticles. Nevertheless, these results were in the range acceptable for food powders (3–10%). In line with the moisture content, the a_w of microparticles with IN was lower than with ALG, but both a_w values were below the maximum (0.60) accepted for avoiding microbial food spoilage (Villacrez et al., 2014). In agreement with our results, Sun-Waterhouse et al. (2013) described lower a_w values with IN (0.25 and 0.24) than with ALG (0.32) for quercetin and vanillin microparticles. Hygroscopicity results indicated that the microparticles with ALG had significantly greater hygroscopicity than the microparticles with IN. These results were in agreement with Ferrari, Pimentel, Germer, and de Aguirre (2012) who suggest that hygroscopicity of the microparticles decreases when the concentration of EA in the feed solution is increased.

For the MJ-ALG system, the high viscosity observed in the feed solution may explain the lowest yield. According to Cai and Corke (2000), feed solutions with high viscosity can cause more solids to stick to the wall of the dryer chamber, resulting in less powder at the end of the process.

Scanning electron microscopic photographs showed that the MJ microparticles with either IN (Fig. 3A) or ALG (Fig. 3B) had spherical morphology and particles with indented surfaces, obviating their agglomerating tendency. The MJ microparticles with IN appear with a smooth surface whereas MJ microparticles with ALG appear with a

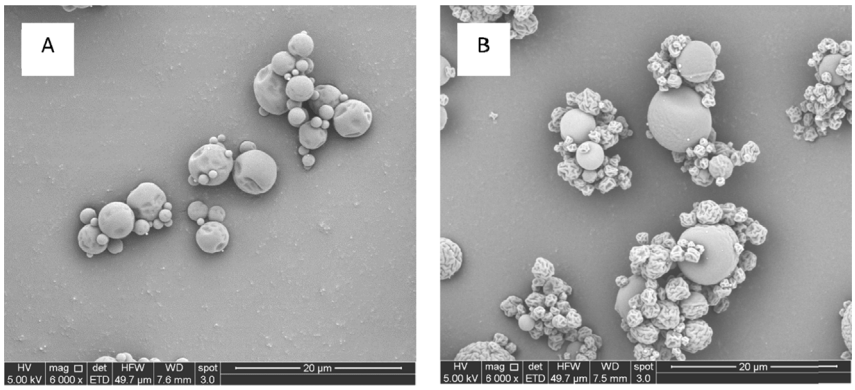

Fig. 3. Scanning electron microscopic (SEM) photographs for MJ microparticles with IN (A) and ALG (B) under optimal conditions.

rough surface. These observations are in agreement with Robert et al. (2010) and Sun-Waterhouse et al. (2013) who reported that the appearance of polyphenol microparticles as seen in SEM microphotography depends on the type of EA, independent of the encapsulated polyphenols.

Fig. 4 shows the evolution of individual anthocyanins retention vs. time (days) for MJ microparticles during storage at 60 °C. The del-3-sa-5-glu showed the highest retention (75.3% and 95.0% for MJ-IN and MJ-ALG, respectively), whereas cy-3-glu the lowest retention (55.4% and 53.7% for MJ-IN and MJ-ALG, respectively). These results are in agreement with Brauch et al. (2015) who indicated that maqui anthocyanins (non-encapsulated) with two (3,5-*O*- or 3,7-*O*-) diglycosylated hydroxyl groups had half-life values 2.4 times higher than those with one (3-*O*-) glycosylated hydroxyl group at 25 °C.

The degradation of individual anthocyanins followed pseudo first-order kinetics. The correlation coefficient was used as parameter to determine the reaction order. In this study the r^2 values were higher than 0.95, indicating a good data fit to the first-order kinetic model in agreement with previous reports (Brauch et al., 2015; Harbourne et al., 2008; Rhim, 2002; Robert et al., 2010). The degradation rate constants of each individual anthocyanins (k_{obs}) were only calculated for 3-*O*-glycosylated anthocyanins because their retention value was less than 50%. The half-life values of del-3-sa, del-3-glu and cy-3-glu were 198 ± 10, 173 ± 8, and 150 ± 9 days, respectively for MJ-IN microparticles and 198 ± 8, 182 ± 9, and 135 ± 8 days, respectively for MJ-ALG. This result showed that the half-life values were independent of the EA nature. However, the structural features of anthocyanins influenced their degradation, and the use of either IN or ALG favors the production of stable MJ microparticles with half-life values over 135 days at 60 °C. Several factors such as pH, temperature, light, oxygen, ascorbic acid, metal ions, sugars and enzymes affect the stability of anthocyanins during processing and storage (Rhim, 2002). In this sense, the first step in the degradation of anthocyanins by heat is the hydrolysis of the glycosidic bond (Rhim, 2002). This can explain the differences between 3-*O*-glycosylated and 3,5-*O*-diglycosylated anthocyanins stability. Moreover, along with degradation, losses of monomeric anthocyanins in processing blueberries into various forms may be by the polymerization reactions of anthocyanins with other phenolic compounds (Brownmiller, Howard, & Prior, 2008). Thus, further analysis are necessary to elucidate the possible condensation reactions of maqui anthocyanins with other polyphenols during thermal treatment that may explain more complex reactions involved in the degradation of maqui anthocyanins as suggested by Reed, Krueger, and Vestling (2005).

3.4. Stability under in vitro digestion conditions

BA was calculated in order to quantify the inhibition of the degradation of anthocyanins. The BA of TA in MJ microparticles (43.0% for MJ-IN and 44.1% for MJ-ALG systems) was significantly higher than in MJ (31.1%). Moreover, significant differences between both MJ-IN and MJ-ALG microparticles were not found. The expected low BA of TA in the MJ can be attributed to the high degradation of anthocyanins under GI conditions. It is well known that anthocyanins are unstable at high pH, and the shift from the acidic pH (pH 2) of the stomach to the almost neutral pH in the duodenum (pH 6) may be responsible for their specific hydrolysis and/or degradation (Lila et al., 2012; Flores, Singh, Kerr, Pegg, & Kong, 2014; Mosele, Macià, Romero, & Motilva, 2016; Lucas-González et al., 2016). Nevertheless, when anthocyanins are encapsulated, they are more protected in the GI tract (Oidtmann et al., 2012) as was also shown in this study. Thus, our results suggest that the higher BA of MJ microparticles is due to lower exposure times of anthocyanins to gut conditions because of the protective effects of IN or ALG.

In concordance with the results of TA, BA of individual anthocyanins from MJ microparticles (Table 2) was significantly higher than MJ (without microparticles), del-3-sa-5-glu: 35.0%, del-3,5-diglu: 36.4%, cy-3-sa-5-glu + cy-3,5 diglu: 48.9%, del-3-sa: 21.9%, del-3-glu: 20.7%, cy-3-glu: 24.0%). Moreover, there were significant differences in the BA among anthocyanins, where cy-3-sa-5-glu + cy-3,5-diglu had the highest BA (83.5% for MJ-IN and 74.0% for MJ-ALG) and del-3-glu had the lowest BA (19.6% for MJ-IN and 21.2% for MJ-ALG), demonstrating differences in their stability after their release from the microparticles. In agreement with our results, Lila et al. (2012) suggest that the highest BA of cy-3-sa-5-glu plus cy-3,5-diglu is attributed to the combination of two cyanidins with two linked sugars, generating a more stable anthocyanin structure. Furthermore, our results showed that the BA of both MJ microparticles and MJ were higher than that described by Lila et al. (2012) for a semi-purified maqui extract (4%). These differences may be explained by the additional protector effect that MJ, as a food matrix, gives to anthocyanins. McDougall, Fyffe, Dobson, and Stewart (2005) suggested that polyphenols generate linkages with the food matrix during digestion that may protect more labile anthocyanins against degradation.

In conclusion, the encapsulation technology using either IN or ALG can be used as a protection strategy for MJ anthocyanins by producing stable anthocyanin-rich microparticles. Moreover, both EA improves the BA by 10% comparing to MJ. The use of MJ as a raw material offers an additional protection effect for anthocyanins while following a more sustainable process. These results contribute to the development of new maqui fruit-based products as health ingredients in powder formulations.

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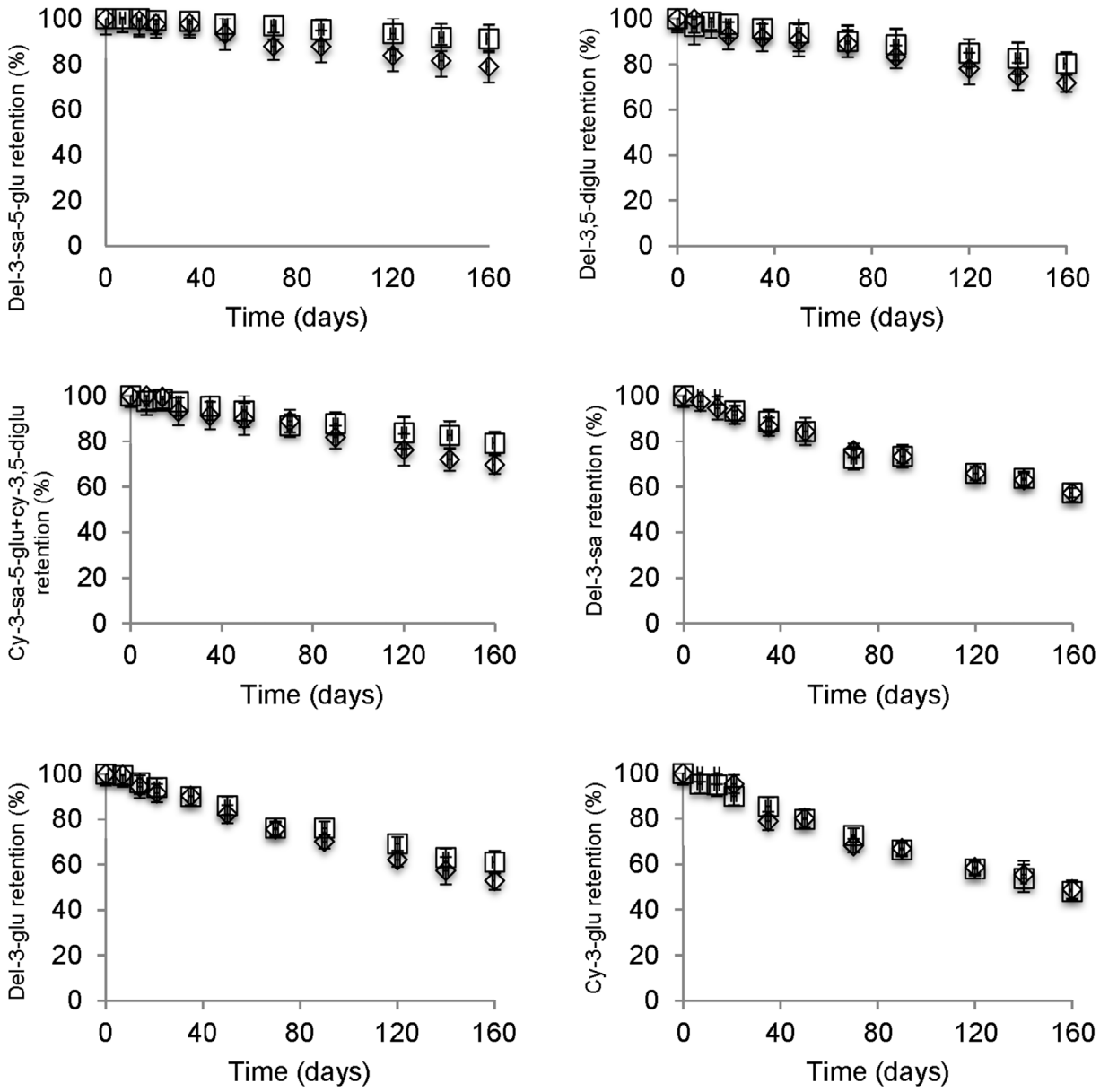

Fig. 4. Evolution of individual anthocyanin retention of MJ microcapsules obtained under optimal conditions during storage at 60 °C. MJ-IN (◇) and MJ-ALG (□).

Table 2
Bioaccessibility of MJ anthocyanins using *in vitro* digestion model.

	MJ-IN		MJ-ALG	
	mg/g in microparticle	Bioccesible %	mg/g in microparticle	Bioccesible %
Del-3-sa-5-glu	1.1 ± 0.03	43.7 ± 3.8cd	2.1 ± 0.06	57.2 ± 2.5c
Del-3,5-diglu	0.5 ± 0.01	35.7 ± 4.4bc	1.0 ± 0.02	52.9 ± 5.6c
Cy-3-sa-5-glu + cy-3,5-diglu	0.6 ± 0.02	83.5 ± 6.6e	1.1 ± 0.01	74.0 ± 7.2d
Del-3-sa	0.4 ± 0.01	25.7 ± 2.3 ab	0.8 ± 0.01	24.8 ± 1.0 ab
Del-3-glu	0.3 ± 0.01	19.6 ± 2.1a	0.5 ± 0.01	21.2 ± 1.6a
Cy-3-glu	0.2 ± 0.01	49.6 ± 2.7d	0.4 ± 0.01	34.4 ± 2.1b

Mean values (n = 3) and standard deviation that are followed by different letters in the same column indicates significant differences ($p \leq 0.05$).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2018.01.090.

References

AOAC - Association of Official Analytical Chemists (1996). Fruits & fruit products. In P. Cunniff (Ed.). *Official methods of analysis*(16th ed.). Gaithersburg: AOAC International.

Aravena, G., García, O., Muñoz, O., Pérez-Correa, J. R., & Parada, J. (2016). The impact of cooking and delivery modes of thymol and carvacrol on retention and bioaccessibility in starchy foods. *Food Chemistry, 196*, 848–852.

Brauch, J. E., Kroner, M., Schweiggert, R. M., & Carle, R. (2015). Studies into the stability of 3-O-glycosylated and 3,5-O-diglycosylated anthocyanins in differently purified liquid and dried maqui (*Aristotelia chilensis* (Mol.) Stuntz) preparations during storage and thermal treatment. *Journal of Agricultural and Food Chemistry, 63*, 8705–8714.

Brownmiller, C., Howard, L. R., & Prior, R. L. (2008). Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blueberry products. *Journal of Food Science, 5*, 72–79.

Cai, Y. Z., & Corke, H. (2000). Production and properties of spray-dried Amaranthus betacyanin pigments. *Journal of Food Science, 65*, 1248–1252.

Céspedes, C. L., Valdez-Morales, M., Avila, J. G., El-Hafidi, M., Alarcón, J., & Paredes-

López, O. (2010). Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae). *Food Chemistry, 119*, 886–895.
Escribano-Bailón, M. T., Alcalde-Eon, C., Muñoz, O., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (2006). Anthocyanins in berries of maqui [*Aristotelia chilensis* (Mol.) Stuntz]. *Phytochemical Analysis, 17*, 8–14.
Fang, Z., & Bhandari, B. (2011). Effect of spray drying and storage on the stability of bayberry polyphenols. *Food Chemistry, 129*, 1139–1147.
Ferrari, C. C., Pimentel, S., Germer, M., & de Aguirre, J. M. (2012). Effects of spray-drying conditions on the physicochemical properties of blackberry powder. *Dry Technology, 30*, 154–163.
Flores, F., Singh, R., Kerr, W., Pegg, R., & Kong, F. (2014). Total phenolics content and antioxidant capacities of microencapsulated blueberry anthocyanins during in vitro digestion. *Food Chemistry, 153*, 272–278.
Fredes, C., & Robert, P. (2014). The powerful colour of the maqui (*Aristotelia chilensis* [Mol.] Stuntz) fruit. *Journal of Berry Research, 4*, 175–182.
Fredes, C., Yousef, G. G., Robert, P., Grace, M. H., Lila, M. A., Gómez, M., et al. (2014). Anthocyanin profiling of wild maqui berries (*Aristotelia chilensis* [Mol.] Stuntz) from different geographical regions in Chile. *Journal of the Science of Food and Agriculture, 94*, 2639–2648.
Gharsallaoui, A., Roudaut, G., Chanbin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International, 40*, 1107–1121.
Gironés-Vilaplana, A., Baenas, N., Villaño, D., Speisky, H., García-Viguera, C., & Moreno, D. A. (2014). Evaluation of Latin-American fruits rich in phytochemicals with biological effects. *Journal of Functional Foods, 7*, 599–608.
Harbourne, N., Jacquier, J. C., Morgan, D. J., & Lyng, J. G. (2008). Determination of the degradation kinetics of anthocyanins in a model juice system using isothermal and non-isothermal methods. *Food Chemistry, 111*, 204–208.
Lila, M., Ribnicky, D., Rojo, L., Rojas-Silva, P., Oren, A., Havenaar, R., et al. (2012). Complementary approaches to gauge the bioavailability and distribution of ingested berry polyphenolics. *Journal of Agricultural and Food Chemistry, 60*, 5763–5771.
Lucas-Gonzalez, R., Navarro-Coves, S., Pérez-Álvarez, J. A., Fernández-López, J., Muñoz, L. A., & Viuda-Martos, M. (2016). Assessment of polyphenolic profile stability and changes in the antioxidant potential of maqui berry (*Aristotelia chilensis* (Molina) Stuntz) during *in vitro* gastrointestinal digestion. *Industrial Crops and Products, 94*, 774–782.
Mahdavi, S. A., Jafari, S. M., Ghorbani, M., & Assadpoor, E. (2014). Spray-drying microencapsulation of anthocyanins by natural biopolymers: A review. *Drying Technology, 32*, 509–518.
McDougall, G., Fyffe, S., Dobson, P., & Stewart, D. (2005). Anthocyanins from red wine. Their stability under simulated gastrointestinal digestion. *Phytochemistry, 66*, 2540–2548.
Mosele, J. I., Macià, A., Romero, M. P., & Motilva, M. J. (2016). Stability and metabolism of *Arbutus unedo* bioactive compounds (phenolics and antioxidants) under *in vitro* digestion and colonic fermentation. *Food Chemistry, 201*, 120–130.
ODEPA-Oficina de Estudios y Políticas Agrarias (2017). Boletín de frutas y hortalizas procesadas. Available from http://www.odepa.gob.cl/wp-content/files_mf/1490990342BFrutaprocesada0317.pdf, Accessed date: 9 May 2017.
Oidtmann, J., Schantz, M., Mäder, K., Baum, M., Berg, S., Betz, M., et al. (2012). Preparation and comparative release characteristics of three anthocyanin encapsulation systems. *Journal of Agricultural and Food Chemistry, 60*, 844–851.
Osborne, D. R., & Voogt, P. (1986). *Análisis de los nutrientes de los alimentos.* Zaragoza: Acribia.
Reed, J. D., Krueger, C. G., & Vestling, M. M. (2005). MALDI-TOF mass spectrometry of oligomeric food polyphenols. *Phytochemistry, 66*, 2248–2263.
Rhim, J.-W. (2002). Kinetics of thermal degradation of anthocyanin pigment solutions driven from red flower cabbage. *Food Science and Biotechnology, 11*, 361–364.
Robert, P., García, P., & Fredes, C. (2017). Drying and preservation of polyphenols. In J. Cuevas-Valenzuela, J. R. Vergara Salinas, & J. R. Pérez-Correa (Eds.). *Advances in technologies for producing food-relevant polyphenols* (pp. 281–302). Boca Raton: CRC Press, Taylor and Francis Group.
Robert, P., Gorena, T., Romero, N., Sepulveda, E., Chavez, J., & Saenz, C. (2010). Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. *International Journal of Food Science and Technology, 45*, 1386–1394.
Rojo, L. E., Ribnicky, D., Logendra, S., Poulev, A., Rojas-Silva, P., Kuhn, P., et al. (2012). *In vitro* and *in vivo* anti-diabetic effects of anthocyanins from maqui berry (*Aristotelia chilensis*). *Food Chemistry, 131*, 387–396.
Silva, P. I., Stringheta, P. C., Teófilo, R. F., & de Oliveira, I. R. N. (2013). Parameter optimization for spray-drying microencapsulation of jaboticaba (*Myrciaria. jaboticaba*) peel extracts using simultaneous analysis of responses. *Journal of Food Engineering, 117*, 538–544.
Sun-Waterhouse, D., Wadhwa, S. S., & Waterhouse, G. I. N. (2013). Spray-drying microencapsulation of polyphenol bioactives: A comparative study using different natural fibre polymers as encapsulant. *Food and Bioprocess Technology, 6*, 2376–2388.
Villacrez, J. L., Carriazo, J. G., & Osorio, C. (2014). Microencapsulation of Andes Berry (*Rubus glaucus* Benth.) aqueous extract by spray drying. *Food and Bioprocess Technology, 7*, 1445–1456.