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The encapsulation of purple cactus pear (*Opuntia ficus-indica*) pulp by using polysaccharide-proteins as encapsulating agents

Paz Robert [a, *], Victoria Torres [a], Paula García [a], Cristina Vergara [a], Carmen Sáenz [b]

[a] Depto. Ciencia de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, Santiago, Chile
[b] Depto. Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile

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ABSTRACT

Cactus pear (*Opuntia ficus-indica*) pulp (CP) was encapsulated with a soybean protein isolate (SPI) and an SPI blend with maltodextrin (MD) or inulin (I). A 2^2 statistical factorial design for each system (CP-SPI, CP-(SPI + MD) and CP-(SPI + I)) was used. The independent variables were the CP/encapsulating agent ratio (1:1–5:1) and inlet air temperature (100–140 °C), and the dependent variables were the polyphenol, betacyanin and betaxanthin encapsulation efficiencies.

The CP total polyphenol, betacyanin and betaxanthin contents were 73.2 ± 1.0 mg gallic acid equivalent/100 g, 22.4 ± 0.31 mg/100 g and 7.6 ± 0.12 mg/100 g, respectively.

A 5:1 ratio of CP/encapsulating agent at 100 °C and 140 °C inlet air temperatures were the optimal conditions for the CP-SPI and CP-(SPI + MD) systems, respectively; for the CP-(SPI + I) system, the ratio and the inlet optimal air temperature were 4:1 and 105 °C, respectively.

The stability of the powders obtained under optimal conditions for each system was studied at 60 °C in the dark. Increased polyphenols and decreased betalains were observed in all systems during storage, and the yellow pigments (betaxanthin) were more stable than the red pigments (betacyanin).

1. Introduction

The red and purple cactus pear (*Opuntia* spp.) is one of the few sources of betalains in nature and is therefore an attractive alternative for replacing synthetic red additives (Castellar, Obon, & Fernández-López, 2006; Díaz, Santos, Kerstupp, Villagómez, & Scheinvar, 2006; Tesoriere, Fazzari, Allega & Livrea, 2005). Cactus pears could have a double application, both as an option for obtaining natural colouring and for providing health benefits from its antioxidant function (Azeredo, 2009; Stintzing & Carle, 2004; Tesoriere et al., 2005).

Commercial betalains are extracted from beetroot (*Beta vulgaris*) and used as a natural colourant in the food industry; they are approved for use in the United States (Title 1 of the Code of Federal Regulations, 21 CFR 73, 40) and the European Union (E-162) (Serries & Biliaderis, 2001; Moreno, García-Viguera, Gil, & Gil-Izquierdo, 2008). Cactus fruit extracts have a fresh odour and flavour and are free of nitrates, and these features are advantages with respect to red beet extracts, making them suitable as potential food additives (Azeredo, Santos, Souza, Mendes, & Andrade, 2007). *Opuntia ficus-indica* pulp has exhibited high levels of betalains (40 mg/100 g) similar to some commercial red beetroot (40–60 mg/100 g fresh fruit) (Castellar, Obon, Alacid, & Fernández-López, 2003). Betanin and indicaxanthin are the main colourant components of *O. ficus-indica*, and isobetanin has been detected at a low level (Sáenz, Tapia, Chávez, & Robert, 2009).

The consumption of cactus pear fruit was shown to affect the body's redox balance in a positive manner and to decrease oxidative damage in lipids. In addition, the intake of red beetroot juice has delayed LDL oxidation modification (Sembries, Dongowski, Mehrländer, Will, & Dietrich, 2006; Tesoriere, Butera, Pintaudi, Allegra, & Livrea, 2004). Thus, betalains have been associated with protection against oxidative stress-related disorders (Tesoriere et al., 2004) because they are cationised compounds that can increase the membrane's affinity to them (Kanner, Harel, & Granit, 2001).

The literature includes few scientific studies on the presence of phenols and other antioxidant compounds in cactus pear fruits. A high concentration of total polyphenols has been reported for a purple cultivar of *O. ficus-indica* (660–900 mg/L) in comparison with other coloured varieties (Sáenz et al., 2009; Stintzing et al., 2005). Flavonoids, primarily flavonol glycosides such as

* Corresponding author. Tel.: +56 2 9781666; fax: +56 2 2227900.
E-mail address: proberts@uchile.cl (P. Robert).

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isorhamnetin-3-rutinoside, rutin, kaempferol-3-rutinoside and quercetin, have been reported in a blend of yellow and red cultivars (Galati et al., 2003; Kuti, 2004; Stintzing, Schieber, & Carle, 2001; Yeddes, Chérif, Guyot, Sotin, & Ayadi, 2013).

Polyphenol intake is widely recognised for its positive health effects, and it has been inversely correlated with the incidence of several chronic diseases related to oxidative stress such as cancer and cardiovascular disease (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005; Mertens-Talcott, Zadezensky, De Castro, Derendorf, & Butterweck, 2006).

Stability is an important parameter to consider when using these pigments as antioxidants and colourants in foods. However, the stability of betalains is affected by pH, water activity, exposure to light, oxygen, metals, antioxidants, temperature and enzymatic activity, and the temperature is the most decisive factor for betalain degradation (Azeredo, 2009; Castellar et al., 2003). Hence, betalain stabilisation could be improved by using microencapsulation technologies, such as spray-drying.

Betalain encapsulation has been primarily undertaken with red beetroot by spray-drying (Azeredo et al., 2007; Pitalua, Jimenez, Veron-Carter, & Beristain, 2010; Ravichandran et al., 2012) and by freeze-drying (Serries & Biliaderis, 2001). However, little research has been reported on the encapsulation of betalains by spray-drying when using purple cactus pear as the betalain source (Sáenz et al., 2009; Vergara, Saavedra, Sáenz, García, & Robert, 2014).

An encapsulation of betalains from purple cactus pear polysaccharides employed maltodextrin and inulin (Sáenz et al., 2009) and Capsul (Vergara et al., 2014) as encapsulating agents. Soybean protein isolate (SPI) and an SPI blend with polysaccharides (maltodextrin (MD) and inulin (I)) were evaluated in this study. SPI is one of the most popular plant protein sources used in food formulation. The globulins glycinin (11S) and β-conglycinin (7S) are the major components of soybean isolates. These two globulins have different structures and functional properties (Petruccelli & Añón, 1996). Soybean protein isolate has been used as an encapsulating agent with its binding and emulsifier properties in orange oil microparticles, and it exhibits higher oil retention than whey protein isolate and arabic gum (Kim & Morr, 1996). Inulin is a fructooligosaccharide (FOS), and it is composed of fructose units with β(2−1) links. It is only hydrolysed in small amounts in the stomach and is fermented by the microflora of the large intestine, creating prebiotic effects (Stevens, Meriggi, & Booten, 2001). In addition, there is one study in which inulin was used as an encapsulating agent for purple cactus pear fruit (Sáenz et al., 2009). Maltodextrin (MD) is obtained by the acid hydrolysis of different starch sources (corn, potato or other), and it is the most common biopolymer to be used as an encapsulating agent by spray-drying because MD has high solubility in water (Gibbs, Kermasha, Alli, & Mulligan, 1999).

In summary, the aim of this work was to evaluate how cactus pear microparticles that are obtained by spray-drying can be influenced by soybean protein isolate and its blending with maltodextrin or inulin, on betalain and polyphenol encapsulation efficiency, and on stability during storage at 60 °C.

2. Materials and methods

2.1. Materials

Cactus pear fruits (*O. ficus-indica*) were obtained from a plantation located in the Antumapu Experimental Station, University of Chile, Santiago, Chile. Encapsulating agents (EA): Soybean protein isolate (SPI) (Prinal, Santiago, Chile); maltodextrin (MD) (Globe®, 10 DE) (Inducorn, Santiago, Chile) and inulin (I) HP (DP > 23) (Raftilina) (Alfa-Chilena, Santiago, Chile).

2.2. Pulp preparation

The fruits (13.75 kg) were manually peeled after being washed and pulped in a screw press (Alexanderwerk, AG, Remscheid, Germany) with a 2 mm screen, yielding 11 kg of pulp. The cactus pear pulp (CP) was packed in polypropylene bags and frozen at −20 °C.

2.3. Cactus pear pulp analysis

The moisture contents (AOAC method 925.40), soluble solids (AOAC method 970.59), pH (AOAC method 981.12), and acidity (AOAC method 935.57) were determined according to AOAC methods (1996). The total sugars were determined by using the Antrona method (Osborne & Voogt, 1986) in a UNICAM UV3 UV/Vis spectrometer (Rochester, U.S.A.).

The polyphenol contents were determined according to the Folin-Ciocalteau method (Singleton & Rossi, 1965), and the results were expressed in gallic acid equivalents according to a calibration curve (133.8−428.0 μg/mL; $r^2 = 0.9901$).

The betalain analyses were performed spectrophotometrically according to the methods of Stintzing et al. (2005). Colour parameters (L^*, a^*, b^*) were determined with MINOLTA CR-200b equipment (Osaka, Japan). The hue angle ($h^0 = \tan^{-1}(b^*/a^*)$) and the chroma (C^*) value were calculated according to McGuire (1992).

2.4. Preparing the microparticles

Microparticles with SPI, SPI + MD (1:1) or SPI + I (1:1) were prepared in 100 g solutions as follows: CP (12 g) was mixed with SPI (2.4−12 g) or SPI + MD (1:1) (1.2−6 g SPI + 1.2−6 g MD) and water (85.6−76 g) with constant stirring. For SPI + I (1:1), I (1.2−6 g) was hydrated for 12 h, heated at 60−70 °C (5 min), and then cooled to 40 °C prior to the addition of SPI (1.2−6 g) and CP (12 g b.h). Each preparation was homogenised with an Ultraturrax IKA T50 at 1000 g for 5 min. The resulting solutions were fed into a mini spray-dryer B191 (Buchi, Flawil, Switzerland). The spray-dryer was operated at inlet air temperatures ranging from 100 to 140 ± 5 °C. The air flow, rate of feeding, and atomisation pressure were 600 L/h, 3 mL/min and 0.14 MPa, respectively, for all encapsulation systems. The resulting powders were protected from light exposure and stored at −20 °C for subsequent analysis.

2.5. Statistical design

The experiments were performed in a 2^2 factorial experimental design with 10 experiments for each encapsulating agent system (CP-SPI, CP-(SPI + MD) and CP-(SPI + I)). The independent variables were the cactus pear pulp/encapsulating agent ratio (1:1−5:1) and inlet air temperature (100−140 °C). The dependent variables were the polyphenol, betacyanin and betaxanthin encapsulation efficiency (EE). A response surface methodology was applied to optimise the EE.

2.6. Microparticle powder analysis

2.6.1. Total polyphenol, betacyanin and betaxanthin determination

The total polyphenols: microparticles (200 mg) were dispersed in 1 mL of acetonitrile and 1 mL of methanol:acetic acid:water (50:8:42 v/v/v). This dispersion was stirred with a vortex (1 min), ultrasonicated twice for 20 min each, centrifuged at 112,000 g for 5 min, and was then filtered (0.22 μm Millipore filter). The polyphenol content was quantified by Folin-Ciocalteau method (Singleton & Rossi, 1965).

The total betacyanins and betaxanthins: microparticles (200 mg) were dispersed in 1 mL of methanol:acetic acid:water

(50:8:42 v/v/v), stirred with a vortex (1 min), ultrasonicated twice for 20 min each, and then centrifuged at 112.000 g for 5 min and filtered (0.22 μm Millipore filter). The betalain (betacyanin and betaxanthin) contents were spectrophotometrically quantified by the Stintzing et al. (2005) method.

2.6.2. Surface polyphenol, betacyanin and betaxanthin determination

Surface polyphenols: microparticles (200 mg) were treated with 2 mL of ethanol:methanol (1:1). The dispersion was stirred in a vortex at room temperature for 1 min and then filtered (0.22 μm Millipore filter). The polyphenol content was quantified by Folin-Ciocalteau (Singleton & Rossi, 1965) method.

Surface betacyanin and betaxanthin: microparticles (100 mg) were treated with 10 mL of ethanol:methanol (1:1), stirred on a vortex mixer for 1 min and centrifuged at 112,000 g for 5 min. The betalain (betacyanin and betaxanthin) content was spectrophotometrically quantified by using the Stintzing et al. (2005) method.

The encapsulation efficiency (EE) for polyphenols or betalains (betacyanins or betaxanthins) was calculated according to the following equation:

$$EE(\%) = \frac{\text{experimental polyphenols or betalains} - \text{surface polyphenols or betalains}}{\text{experimental total polyphenols or betalains}} \times 100$$

2.7. An analysis of microparticle powder obtained under optimal conditions

A moisture content determination was performed according to AOAC method 925.40 (1996), and the total and surface polyphenols, betacyanins and betaxanthins, and colour parameters were determined as described above.

2.7.1. Scanning electron microscopy (SEM)

The outer structures of the microparticles obtained under optimal conditions were studied by SEM. The samples were coated with gold/palladium by a Varian Vacuum Evaporator PS 10E and analysed with a JEOL JSM-25SII (Jeol, Tokyo, Japan) scanning electron microscope operated at 30 KV. The images were obtained with a Mamiya Roll Film Holder camera (Model 2) coupled to the microscope by using Kodak 120 T-Max ISO 100 film.

2.8. Accelerated storage stability test

Microparticles obtained under optimal conditions (CP-SPI, CP-(SPI + I) and CP-(SPI + MD)) and unencapsulated pulp (CP) were stored at 60 °C in a forced-air oven (Memmert, model BE 500, Schwabach, Germany) at a controlled temperature in the absence of light for 56 days. Samples of 0.2 g for each powder were transferred to 450 × 250 mm clear glass vials. To determine the polyphenols and betalains (betacyanin and betaxanthin), the vials were removed every 7 days until the study was completed.

2.9. Statistical analysis

A linear regression (95 % confidence limit) was used to determine the reaction order and the degradation rate constants. A one-way analysis of variance was performed to determine the significant differences between the parameters. Statistical analyses were performed with Statgraphics software version 7.0 (Manugistics Inc., Statistical Graphics Corporation, 1993, Rockville, MA).

3. Results and discussion

3.1. Cactus pear pulp characterisation

The CP total soluble solids (15.33 ± 0.6 g/100 g), total sugars (16.85 ± 0.2 g/100 g), pH (5.69 ± 0.03), acidity (0.21 ± 0.002 g citric acid/100 g) and moisture contents (85.3 ± 0.03 g/100 g) were consistent with previously reported data (Castellar et al., 2003; Morales, Sáenz, & Robert, 2008; Sáenz & Sepúlveda, 2001; Piga, 2004; Stintzing et al., 2005).

The CP betacyanin (22.4 ± 0.3 mg betanin equivalent/100 g) and betaxanthin (7.6 ± 0.1 mg indicaxanthin equivalent/100 g) contents were lower than Stintzing et al. (2005) reported 41.05 mg/100 g and 18.65 mg/100 g, respectively, and Sáenz et al. (2009) 28.09 mg/100 g and 9.96 mg/100 g, respectively, but greater than those reported by Morales et al. (2008) at 11.10 mg/100 g and 2.93 mg/100 g, respectively. These differences in betalain contents could be attributed to factors such as the cultivar or variety, stage of maturity, and climate or geographic site of production (Stintzing & Carle, 2004). The CP total polyphenol contents (73.2 ± 1.0 mg gallic acid equivalent/100 g) were higher (62.4 mg/100 g), similar (73.5 mg/100 g) and lower (85.9 mg/100 g) than those reported by Stintzing et al. (2005), Morales et al. (2008) and Sáenz et al. (2009), respectively. Similar CP total polyphenol contents were found in grapes (50–490 mg/100 g fresh matter), but they were lower than in blackcurrants (140–1200 mg/100 g fresh matter) or blueberries (135–280 mg/100 g fresh matter) (Bravo, 1998).

The CP colour parameters L^* (21.3 ± 0.2), a^*(2.4 ± 0.3), b^*(1.2 ± 0.1), h° (25.8 ± 3.4) and C^* (2.7 ± 0.3) were in accordance with the red-purple colour of the *Opuntia* fruit pulp, which was associated with the betacyanin content (Felker et al., 2008).

3.2. The encapsulation of polyphenols, betacyanin and betaxanthin from cactus pear pulp

A 2^2 factorial experimental design for each system (CP-SPI, CP-(SPI + MD) and CP-(SPI + I)) was applied to evaluate the effect of the inlet air temperature and CP/encapsulating agent ratio on the betacyanin, betaxanthin and polyphenol encapsulation efficiency. The betacyanin and betaxanthin encapsulation efficiencies both ranged from 98 to 100 % for all studied systems. A similar behaviour was previously reported for the encapsulation of cactus pear pulp with MD and I (Sáenz et al., 2009) and Capsul (Vergara et al., 2014). The polyphenol encapsulation efficiency ranged from 68 to 80 %, 76–85 % and 78–86 % for CP-SPI, CP-(SPI + MD) and CP-(SPI + I), respectively. Zhang, Mou, and Du (2007) reported that the encapsulation of procyanidins from grape seeds with arabic gum-maltodextrin reached encapsulation efficiency values of up to 88.8%, and Kosaraju, D'ath, and Lawrence (2006) reported 27 % polyphenols in olive leaf extract with chitosan, both by spray drying. Moreover, other techniques for polyphenol encapsulation have been reported, such as quercetin nanoprecipitation (Wu et al.,

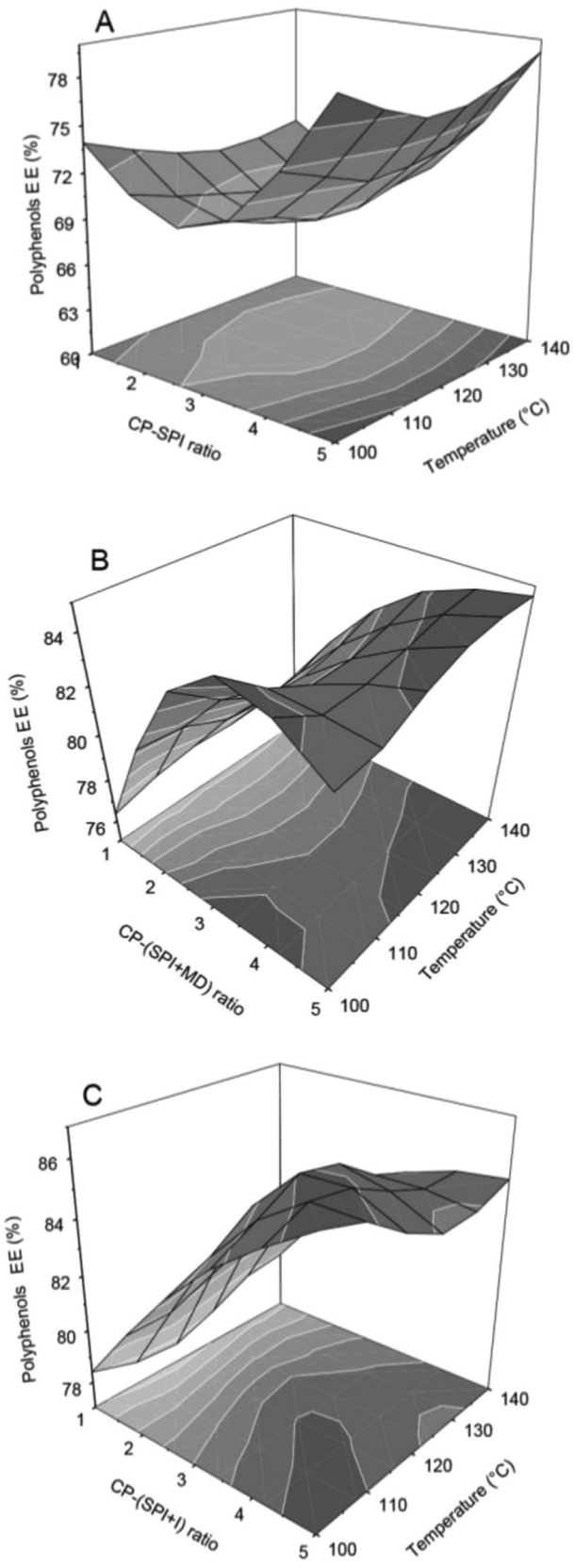

Fig. 1. Graphs obtained by response surface methodology for cactus pear pulp (CP) microparticles with soybean protein isolate (SPI) and its blend with maltodextrin (MD) or inulin (I), under optimal conditions: CP-SPI (A), CP-(SPI + MD) (B), and (CP-(SPI + I) (C).

2008) and tea catechins by ionic gelation (Hu et al., 2008) with encapsulation efficiency values of 99 and 24–53 %, respectively.

An analysis by response surface methodology (RSM) for the betacyanin and betaxanthin encapsulation efficiency showed that the inlet air temperature and CP/EA ratio had no significant effect ($p < 0.05$) for all the systems under study. For the polyphenol encapsulation efficiency, the CP/EA ratio showed a significant effect on the three systems studied, but the inlet air temperature was significant ($p < 0.05$) only for CP-SPI. According to these results, the optimisation for the three systems under study was performed by only considering the polyphenols. Fig. 1 shows the graphs obtained by RSM for the three systems.

3.3. A characterisation of the microparticles obtained under optimal conditions

Table 1 shows the optimal conditions and betacyanin, betaxanthin and polyphenol encapsulation efficiency in cactus pear pulp microparticles. The optimal CP/EA ratio was 5:1 for CP-SPI and CP-(SPI + MD) and 4:1 for CP-(SPI + I), showing that the optimal CP/EA ratio was obtained at high CP values within the range studied. The systems containing SPI had better binding properties (higher pulp incorporation) than the systems reported by Sáenz et al. (2009) where polysaccharides were used as an encapsulating agent (CP-MD and CP-I). However, the optimal temperatures were dependent on the encapsulating agent; 100 °C for CP-SPI, 105 °C for CP-(SPI + I) and 140 °C for CP-(SPI + MD). Therefore, the microparticle systems had different optimum parameters in spray-drying, primarily because of the nature of the polymer.

High betacyanin and betaxanthin encapsulating efficiencies were found in this study for CP-SPI, CP-(SPI + MD) and CP-(SPI + I). These results are consistent with studies in which polymers with different natures have been used (maltodextrin, inulin, pullulan, Capsul, corn starch and gum arabic) (Azeredo et al., 2007; Gandía-Herrero, Jiménez-Atienzar, Cabanes, García-Carmona, & Escribano, 2010; Pitalua et al., 2010; Sáenz et al., 2009). This behaviour could be related to the cationic features of betalains (betacyanin and betaxanthin) (Moreno et al., 2008), allowing for high betalain-polymer interactions because of electrostatic interactions or hydrogen bonding. However, the SPI-polysaccharide (MD or I) blends improved the polyphenol encapsulation efficiency to a significant extent, and they were said to form a new system with different properties with respect to each single polymer as has also been reported for hydrophobic molecules (Benichou, Aserin, & Garti, 2002; Young, Sarda, & Rosenberg, 1993).

Table 2 shows the physical and chemical characteristics of CP-SPI, CP-(SPI + MD) and CP-(SPI + I) microparticles. The betalain recovery was significantly higher in the CP-SPI (lower inlet air temperature system) than in the CP-(SPI + MD) and CP-(SPI + I). Moreover, the betaxanthin recovery was slightly greater than the betacyanin recovery, which was in line with the better temperature stability of betaxanthin (Gandía-Herrero et al., 2010; Azeredo et al., 2007). These results may be explained by the encapsulating agent properties (viscosity, solubility) and/or the inlet air temperature that affect the formation rate of a crust on the particle surface and active recovery (Gharsallaoui, Roudat, Chambin, Voilley & Saurel, 2007). However, the temperature is the most important factor in betalain (betacyanins and betaxanthins) degradation (Herbach, Stintzing & Carle, 2006) and therefore in the recovery of betalains.

The polyphenol recovery was high for all the systems at over 98 %, showing that the differences in the optimal inlet air temperatures are not associated with polyphenol stability. The polyphenol recovery values were approximately 100 % for all the systems. The same results in CP-MD and CP-I have been previously reported (Sáenz et al., 2009).

Table 1
Optimal conditions and betacyanin, betaxanthin and polyphenol encapsulation efficiency in cactus pear pulp microparticles.

System	Inlet air temperature (°C)	EA (g/100 g)	CP/EA	Betacyanins encapsulated (% EE)	Betaxanthins encapsulated (% EE)	Polyphenols encapsulated (% EE)
CP-SPI	100	2.4	(5:1)	99.6 ± 0.02b	98.1 ± 0.07b	79.7 ± 0.15c
CP-(SPI + MD)	140	2.4	(5:1)	99.9 ± 0.03a	99.5 ± 0.09a	84.7 ± 0.05b
CP-(SPI + I)	105	3.0	(4:1)	99.9 ± 0.02a	99.3 ± 0.08a	86.5 ± 0.14a
CP-MD*	140	10	(3:1)	99.3 ± 0.02c	96.5 ± 0.08c	72.8 ± 0.11e
CP-I*	120	10	(3:1)	99.4 ± 0.02c	97.7 ± 0.08c	74.6 ± 0.11d

CP: cactus pear pulp; SPI: soybean protein isolated; MD: maltodextrin; I: inulin; EA: encapsulating agent; EE: encapsulation efficiency; $n = 3$; different letters show significant differences between systems ($p < 0.05$); *From reference: Sáenz et al. (2009).

Table 2
Physical and chemical characteristics of cactus pear pulp microparticles with soybean protein isolate and its blend with maltodextrin or inulin, obtained under optimal conditions.

	CP-SPI	CP-(SPI + I)	CP-(SPI + MD)
Moisture content (g/100 g)	7.7 ± 0.2a	7.0 ± 0.1b	5.6 ± 0.2c
Colour parameters			
L^* (lightness-darkness)	69.9 ± 0.5c	71.4 ± 0.5b	73.6 ± 0.5a
a^* (redness-greeness)	29.0 ± 0.2a	27.3 ± 0.3b	26.7 ± 0.4c
b^* (blueness-yelowness)	−7.9 ± 0.2b	−9.0 ± 0.1c	−6.6 ± 0.1a
h° (hue)	344.8 ± 0.2b	341.7 ± 0.3c	346.0 ± 0.1a
C^* (chroma)	30.1 ± 0.2a	28.7 ± 0.3b	27.5 ± 0.4c
Betacyanins (BE mg/g)	0.45 ± 0.004a	0.33 ± 0.001b	0.34 ± 0.002b
Betacyanin recovery (%)	70 ± 1.0a	60 ± 1.6b	54 ± 1.4c
Betaxanthins (IE mg/g)	0.18 ± 0.001a	0.13 ± 0.0b	0.14 ± 0.01b
Betaxanthin recovery (%)	86 ± 1.2a	68 ± 0.6b	67 ± 1.3b
Total polyphenols (GAE mg/g)	2.31 ± 0.15a	2.19 ± 0.14b	2.23 ± 0.05b
Poyphenols recovery (%)	100 ± 0.8a	100 ± 0.1a	98 ± 0.2b

CP: cactus pear pulp; SPI: soybean protein isolate; I: inulin; MD: maltodextrin; BE: betanin equivalent; IE: indicaxanthin equivalent; GAE: gallic acid equivalent; $n = 3$.

The moisture of the cactus pear pulp microparticles that were obtained under optimal conditions was within the range described for microparticles obtained by spray-drying (Gharsallaoui et al., 2007). For the CP microparticles, the L^* and a^* values increased and the b^* values decreased with respect to CP, which could be explained by the influence of the encapsulating agent on the powder colour.

Fig. 2 shows the external structure of microparticles that were obtained under optimal conditions for the three systems in the study. SEM micrographs showed microparticles that were irregular in shape and particles with indented surfaces, obviating their agglomerating tendency. Cai and Corke (2000) studied *Amaranthus* microparticles with maltodextrins of different dextrose equivalents (10 DE, 20–23 DE and 28–31 DE), and they reported that when higher DE maltodextrin was used, less surface indentation and cracks in the wall system were observed. The formation of indented surfaces in the spray-dried particles was attributed to particle shrinkage during the drying process, which can occur at low or high inlet temperatures. There is less water diffusion at low inlet temperatures, and the particles have more time to shrink. At high inlet temperatures, the rapid evaporation and high pressure inside the

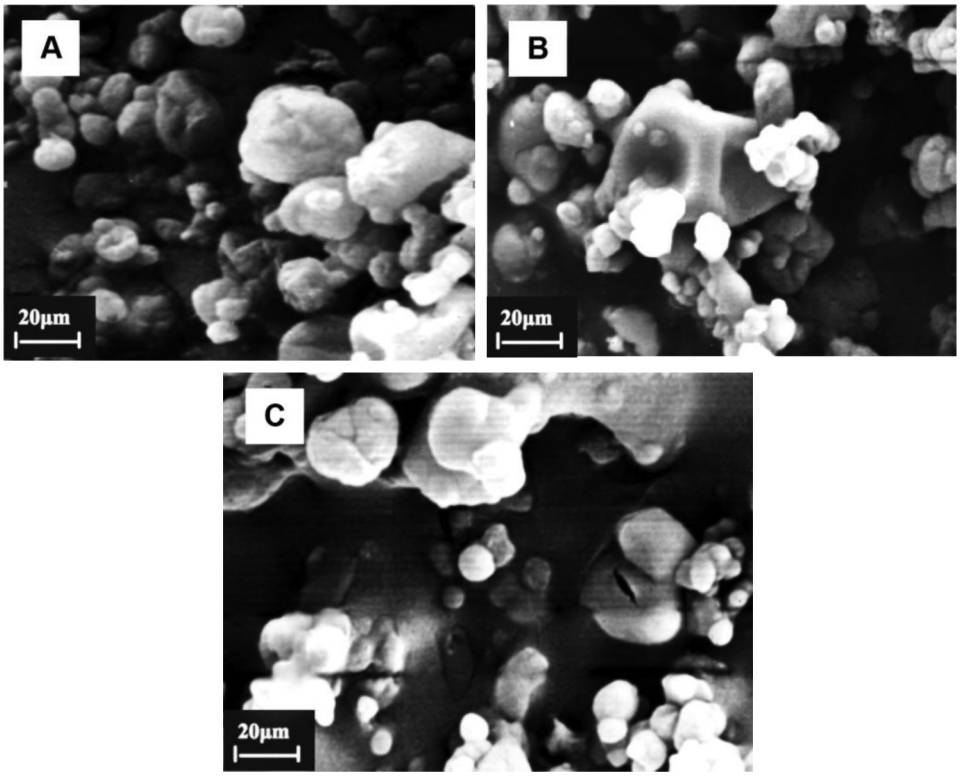

Fig. 2. Scanning electron microscopic photographs for cactus pear pulp (CP) microparticles with soybean protein isolate (SPI) and its blend with maltodextrin (MD) or inulin (I), under optimal conditions: CP-SPI (A), CP-(SPI + MD) (B), and (CP-(SPI + I) (C).

Table 3
Evaluation of betacyanin, betaxanthin and polyphenol contents from cactus pear pulp microparticles with soybean protein isolate and its blend with maltodextrin or inulin, obtained under optimal conditions and stored at 60 °C.

System	Time (days)	0	7	14	21	28	35	42	49	56
		$X \pm 10^2$ SD (mg/g)								
CP-SPI	Betacyanin*	0.45 ± 0.3	0.25 ± 1.2	0.23 ± 0.4	0.21 ± 2.1	0.14 ± 1.8	0.17 ± 1.1	0.16 ± 3.3	0.15 ± 0.8	0.14 ± 0.1
CP-(SPI + MD)		0.34 ± 0.3	0.30 ± 0.8	0.30 ± 0.2	0.28 ± 1.1	0.18 ± 0.4	0.20 ± 0.1	0.22 ± 0.4	0.17 ± 0.2	0.18 ± 0.3
CP-(SPI + I)		0.33 ± 0.1	0.26 ± 1.6	0.22 ± 1.6	0.12 ± 1.3	0.17 ± 2.3	0.18 ± 7.2	0.16 ± 1.3	0.11 ± 1.5	0.15 ± 0.0
CP-SPI	Betaxanthin**	0.18 ± 0.1	0.13 ± 0.8	0.13 ± 0.1	0.13 ± 0.7	0.10 ± 1.3	0.12 ± 0.9	0.12 ± 2.2	0.15 ± 1.0	0.12 ± 0.8
CP-(SPI + MD)		0.14 ± 0.1	0.14 ± 0.5	0.15 ± 0.1	0.15 ± 0.2	0.13 ± 0.1	0.14 ± 0.1	0.14 ± 0.6	0.14 ± 0.1	0.13 ± 0.0
CP-(SPI + I)		0.13 ± 0.0	0.13 ± 0.7	0.12 ± 0.6	0.07 ± 1.1	0.12 ± 0.7	0.11 ± 3.6	0.11 ± 0.5	0.11 ± 1.6	0.11 ± 0.0
CP-SPI	Polyphenol***	2.31 ± 0.15	3.15 ± 0.91	3.71 ± 0.12	3.70 ± 0.23	3.85 ± 0.24	8.40 ± 0.35	9.08 ± 0.08	10.01 ± 0.40	9.20 ± 0.34
CP-(SPI + MD)		2.23 ± 0.05	2.88 ± 0.21	3.22 ± 0.46	3.30 ± 0.46	3.47 ± 0.11	7.35 ± 0.36	8.62 ± 1.01	8.18 ± 0.20	8.07 ± 0.38
CP-(SPI + I)		2.19 ± 0.14	2.96 ± 0.44	3.20 ± 0.58	3.06 ± 0.14	3.34 ± 0.11	5.55 ± 0.73	8.11 ± 1.46	10.02 ± 0.05	8.67 ± 0.00

CP: cactus pear pulp; SPI: soybean protein isolate; I: inulin; MD: maltodextrin; X: mean; SD: standard deviation, * expressed as betanin equivalent; ** expressed as indicaxanthin equivalent; *** expressed as gallic acid equivalent; $n = 3$.

particles also produce shrinkage (Alamilla-Beltrán, Chanona-Perez, Jimenez-Aparicio & Gutierrez-Lopez, 2005; Gandía-Herrero et al., 2010).

3.4. Storage stability evaluation

The evaluation of betacyanin, betaxanthin and polyphenol contents from CP-SPI, CP-(SPI + MD) and CP-(SPI + I) microparticles that were obtained under optimal conditions and stored at 60 °C is shown in Table 3. Polyphenol retention remained constant until the 28th day and then increased in the three systems, without significant effects from the encapsulating agent. Isorhamnetin derivatives have been identified as the dominant flavonol glycoside in cactus pear pulp (isorhamnetin-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, and isorhamnetin diglycoside) (Yeddes et al., 2013; Galati et al., 2001). Thus, the increased total polyphenol contents during storage (Table 3) could be attributed to the hydrolysis of polyphenol glycosides and/or condensed into aglycones, leaving a higher number of free hydroxyl groups (Sáenz et al., 2009; Turkmen, Sari, & Velioglu, 2005).

The betacyanin and betaxanthin retention ((betacyanin or betaxanthin content at day 56/betacyanin or betaxanthin content at day zero) × 100) (Table 3) were significantly higher in CP-(SPI + MD) (53 and 93 %, respectively) than in CP-(SPI + I) (45 % and 85 %, respectively) and CP-SPI (31 % and 67 %, respectively), showing that incorporated polysaccharides most likely increased the betacyanin and betaxanthin stability because of their higher film-forming properties (Desai & Park, 2005). By contrast, the betacyanins exhibited higher degradation than the betaxanthins. The yellow pigments were more resistant to temperature treatment than the red ones. The same behaviour was observed when maltodextrin or inulin was used as an encapsulating agent (Sáenz et al., 2009).

Table 4
Betacyanin degradation rate constants from cactus pear pulp microparticles with soybean isolate protein and its blend with maltodextrin or inulin, obtained under optimal conditions and stored at 60 °C.

Systems	$10^2 k_{(obs)} \pm 10^2$SD (days^{-1})
CP	173.9a
CP-SPI	0.9b
CP-(SPI + MD)	0.5d
CP-(SPI + I)	0.8c
CP-MD*	1.06b
CP-I*	1.07b

CP: cactus pear pulp; SPI: soybean protein isolate; MD: maltodextrin; I: inulin; SD: standard deviation; $n = 3$; *From reference: Sáenz et al. (2009); different letters show significant differences among systems ($p < 0.05$).

The degradation of betacyanins followed pseudo first-order behaviour for CP, CP-SPI, CP-(SPI + MD) and CP-(SPI + I) during storage at 60 °C (Cai & Corke, 2000; Sáenz et al., 2009; Serries & Biliaderis, 2001; Vergara et al., 2014). Betacyanin degradation rate constants were obtained from the slope of a natural log plot from the percentage retention of betacyanins vs. time (days), and the values are shown in Table 4. CP had the highest betacyanin degradation rate constant. As expected, the encapsulation of CP showed a protective function against betacyanin degradation (hydrolysis and/or oxidation) (Gandía-Herrero et al., 2010; Sáenz et al., 2009; Vergara et al., 2014). Furthermore, the betacyanin degradation rate constant was significantly lower when the EA was made of SPI-polysaccharide (MD or I) blends in comparison with SPI, MD and I as shown in Table 4. On the other hand, betacyanin degradation rate constant for CP-(SPI + MD) microparticles was significant lower than CP-(SPI-I), showing the effect of type protein-polysaccharide blends. Maltodextrin and inulin are both polysaccharides, but they have different structural features. Inulin is a fructo-oligosaccharide (FOS) composed of fructose units with β-(1–2) and mainly linear (Stevens et al., 2001), while maltodextrin is a glucopyranose with mainly α-(1–6) and three to seventeen glucose units long. these structural differences may explain the differences on the betacyanin storage stability.

4. Conclusions

The protein and polysaccharide blends (CP-(SPI + MD)) and (CP-(SPI + I)) used as encapsulating agents for cactus pear pulp improved the polyphenol encapsulation and betalain stability at 60 °C as shown by the lower degradation rate constant. The cactus pear microparticles could be used as food ingredients for functional foods because of their antioxidant content and colourant properties.

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