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RESEARCH ARTICLE

Spray drying microencapsulation of purple cactus pear (*Opuntia ficus indica*) peel extract and storage stability of bioactive compounds

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ABSTRACT

Purple cactus pear (*Opuntia ficus indica*) is a fruit found in Mexico that is mainly consumed fresh. The fruit has a peel, which is a non-usable by-product that can represent up to 52% of the fruit´s total weight. This peel is rich in phenolic compounds (PC) and betalain pigments (betacyanins (BC) and betaxanthins (BX)), with important antioxidant capacity (AC), making this waste product an interesting source to obtain extracts rich in bioactive compounds that can be utilized by food industry. Since extracts are liable to degradation, they require protection through techniques such as spray drying microencapsulation. Therefore, this study evaluated the retention of bioactive compounds during spray drying microencapsulation of purple cactus pear peel extract using 10, 15, and 20%$_w$ (weight percentage) of maltodextrin (MDX) and Gum Arabic (GA) solutions as encapsulating agents, under different drying conditions. Storage stability during 90 days was also studied for powders obtained at the best drying conditions with both encapsulating agents. The best drying conditions were 170-80 °C (inlet-outlet temperature), in which retention efficiencies for MDX were: 95.5 % (PC), 100.5% (BC), 103.5% (BX) using 20%$_w$ MDX, and 117.9% (AC) using 15%$_w$ MDX; for GA retentions were 92.4% (PC) and 107% (AC) with 20%$_w$ GA and 103.4% (BC) and 93.4% (BX) with 10%$_w$ GA. Under storage for 90 days at 22-25 °C, 10%$_w$ of encapsulating agent protected microcapsules in presence or absence of light, having the advantage of containing higher concentration of bioactive compounds per gram of dry solid.

Keywords: Spray drying; Microencapsulation; Phenolic compound; Betalain; Purple cactus pear peel

INTRODUCTION

Cactus pear fruit, a member of the Cactaceae family, is found in Mexico and also grows in many countries around the world. The fruit has a wide spectrum of colors such as orange, red and purple (Barba *et al.*, 2017), the latter being the most attractive due to its color (Saénz and Sepúlveda, 2001). This fruit has gained considerable attention due to the bioactive compounds with antioxidant capacity (AC), which is mainly attributed to the presence of bioactive substances such as phenolic compounds (PC) and betalains. Betalains are water-soluble pigments that can be divided in red-colored betacyanins (BC), and yellow-colored betaxanthins (BX) (Pinedo-Espinoza *et al.,* 2017; Aparicio-Fernández *et al.*, 2017; Rodriguez-Amaya, 2018). Antioxidant capacity of PC is important because it protects human health against degenerative diseases (de Wit *et al.*, 2019). In the case of betalains, this capacity relates to its potential to reduce or prevent problematic oxidative stress in the human body (Carillo-López and Yahia, 2017).

Most of the research done on this fruit is centered on its pulp but PC and betalains of cactus pears are also richly distributed in its peel (Namir *et al.,* 2016; Chougui *et al.,* 2015). The peel is a non-usable by-product that can represent up to 52% of the fruit´s total weight (Mahmoud *et al.,* 2018), making it a good source of bioactive compounds that can be extracted for different purposes by the food industry.

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Extracts of bioactive compounds are liable of degradation by factors such as humidity, light, ambient oxygen, and heat; therefore they require protection that can be given by microencapsulation. There are several techniques that have been used to microencapsulate bioactive compounds such as spray drying, freeze drying, coacervation, liposomes, polymeric micelles, and molecular inclusion, among others. Each of these techniques presents their own advantages and disadvantages; therefore, it is important to use them according to the food ingredient that will be submitted to the microencapsulation process (Gómez *et al.,* 2018). Briefly, during microencapsulation, a wall of certain material (encapsulating agent) protects bioactive compounds (core) from environmental factors (Ballesteros *et al.,* 2017).

Among all the microencapsulation techniques, spray drying has become a successful industrial drying process that has evolved as the main technique used for the microencapsulation of food ingredients due to its low processing cost, rapid water evaporation, and easy scaling-up (Gómez *et al.,* 2018). Spray drying is based on the conversion of a fluid feed to a powder, by the continuous atomization of the feed into a hot dry medium (Wilkowska *et al.,* 2016). The quality of the powdered microcapsules can be affected by the encapsulating agent and by the spray drying operating conditions, such as inlet and outlet temperatures (Kha *et al.,* 2014).

Selecting the appropriate encapsulating agent is very important since it must protect the core from deterioration, allow controlled release of the bioactive compounds, and have thermal properties compatible with the intended product (Gómez *et al.,* 2018). Some of the ideal properties for an encapsulating agent used in spray drying microencapsulation include: having low viscosity, low hygroscopicity (to avoid agglomeration and easier manipulation), high solubility, a film-forming ability, provide high protection, and have favorable economic costs (Franco *et al.,* 2017). There are many encapsulating agents used in food industry. Maltodextrins (MDX) are one of the most utilized agents because they have high water solubility, low viscosity at high solid concentrations, and low cost (Šturm *et al.,* 2019; Cian *et al.,* 2018). Gum Arabic (GA) is another type of encapsulating agent also used because it has a good emulsifying capacity and presents low viscosity in aqueous solutions (Šturm *et al.,* 2019).

Due to the functional properties that PC and betalains have, and since they are highly susceptible to degradation, it is interesting to study the microencapsulation of these compounds from new, low-cost sources, such as purple cactus pear peel, so that they can be utilized by the food industry.

The aim of the present study was to evaluate the effect of microencapsulation by spray drying on PC, BC, BX and AC from purple cactus pear (*Opuntia ficus indica*) peel extract, using different drying temperatures and concentrations of MDX. A comparative study was also made using GA as encapsulating agent using the best drying conditions found for MDX.

MATERIALS AND METHODS

Materials and reagents

Purple cactus pears were obtained from a local market (Central de Abastos, Ciudad de México, MX). Fruits had no physical external damage and were washed with water, removing thorns with a soft brush. Fruits were packed in sealed polyethylene bags and frozen at -20 °C until use. The encapsulating agents were MDX DE10 (Amidex-10, Estado de México, MX) and GA (Reasol, Ciudad de México, MX). All other reagents were analytical grade.

Preparation of extracts

Cactus pears were thawed at room temperature and peels were manually separated from the pulp. Peels were dried in a conventional oven at 60 °C for 24 h and then pulverized with a food processor. Bioactive compounds were extracted by mixing 1 g of the peel powder with 10 g of 50%$_w$ (weight percentage) aqueous ethanol in sealed amber glass bottles. This mixture was then kept at 40 °C in a conventional oven for 2 h, hand-shaking every 15 min. After this period, the extract was filtered with Whatman filter paper No. 1 (UK) to separate the solid residue. The solid residue was re-extracted under the same conditions, filtered and finally both extracts were mixed together. The extract was concentrated in a rotary evaporator (Buchi R, Flawil, CH) to a concentration of 2 mg of gallic acid equivalents (GAE) per mL. This concentration was set as an indicator because it was similar to the quantity of PC found in pomegranate juice (2.13 ± 0.01 mg EAG/mL), which is considered an excellent source of PC (Robert *et al.,* 2010).

Microencapsulation by spray drying

Microencapsulation experiments using MDX as encapsulating agent were performed with a 3x2x3 factorial design, with replica in all points. The independent variables considered were inlet drying temperature (160, 170, and 180 °C), outlet drying temperature (70 and 80 °C), and encapsulating agent concentration in the solution to be dried (10, 15, and 20%$_w$). Response variables were: content and retention efficiency of PC, BC, BX, and AC. From the results obtained with MDX, specific inlet-outlet temperatures (160-80, 170-70 and 170-80 °C) were used to compare with GA as encapsulating agent. Retention efficiency was obtained by determining the PC, BC, BX or

the AC content in solutions before and after spray drying, expressing the result in percentage.

The preparation of the solutions to be spray dried (SSD) was the same for both encapsulating agents. First, the encapsulating agent solution (EAS) was prepared at 30%$_w$ and left to stand overnight to eliminate air bubbles. Then, in order to obtain 10, 15 and 20%$_w$ of encapsulating agent in SSD, the quantity of EAS added to the concentrated extract (CE) (2 mg GAE/mL extract) was calculated with an overall material balance and a solids balance, considering negligible the solids content in the CE, obtaining Equation 1.

$$EAS = \frac{CE\left(\%S_{SSD}\right)}{\left(\%S_{EAS} - \%S_{SSD}\right)} \quad (1)$$

Where, *EAS*: mass of encapsulating agent solution (30%$_w$), *CE*: mass of concentrated extract (2 mg GAE/mL extract), $\%S_{SSD}$: solids percentage in SSD (10, 15 or 20%$_w$ of encapsulating agent), and $\%S_{EAS}$: solids percentage in EAS (30%$_w$).

Once the EAS was added to the CE, the SSD obtained were mixed with a magnetic stirrer at 25 °C and dried with a semipilot scale Mobile Minor spray-dryer (Niro Atomizer, Copenhagen, DK). Powders were stored in sealed amber glass bottles and kept at -20 °C until analysis.

Preparation of powder samples for analyses

For all analyses, the microcapsule powders were reconstituted in water to the same solids content present in SSD (concentrated extract + encapsulating agent) prior to the spray drying process, considering the moisture content in the powders.

Phenolic compounds content determination

Phenolic compounds content was determined according to Singleton and Rossi (1965). For the analysis, an aliquot of 200 µL of each reconstituted powder was placed in a separate test tube, adding 7.8 mL of distilled water and 500 µL of Folin-Ciocalteu reagent; after 8 min, 1.5 mL of sodium carbonate (20%$_w$) was added and left to stand for 60 min in the dark. Absorbance was measured at 750 nm in a spectrophotometer (Jenway 6320D, UK). Results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g$_{dw}$). All analyses were carried out in triplicate.

Betalain determination

Betalain content was quantified by determining the absorbance at 535 (betacyanins) and 480 nm (betaxanthins), according to Castellanos and Yahia (2008). Equation 2 was used to determine the results:

$$B\left(\frac{mg}{g}\right) = \left(\frac{\left(A \times DF \times M \times V\right)}{\left(\mu \times W \times L\right)}\right) \times 100 \quad (2)$$

Where B: betacyanin or betaxanthin, A: absorbance at 535 (betacyanin) or at 480 nm (betaxanthin); DF: dilution factor; M: molecular weight (Betanin: 550 g/mol or Indicaxanthin: 308 g/mol); V: extract volume; ε: molar extinction coefficient (Betanin: 60,000 L/mol.cm or Indicaxanthin: 48,000 L/mol.cm); W: sample quantity (g) and L: cuvette length (1 cm). Results were expressed, for BC as mg of betanin equivalents (mg BE/g$_{dw}$), for BX as mg of indicaxanthin equivalents (mg IE/g$_{dw}$), and for total betalains as mg of total betalains per gram of dry weight (mg TB/g$_{dw}$).

Antioxidant capacity determination

AC was determined using the ABTS (2, 2´-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical (Rufino *et al*., 2007). Trolox (6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard. The radical ABTS was generated combining 7 mM stock solution of ABTS and 140 mM of potassium persulfate and keeping it in the dark for 16 h, at room temperature. Then the radical was diluted with ethanol in order to obtain an absorbance of 0.7 (±0.05) at 734 nm. Aliquots of 30 µL of each sample were mixed with 3 mL ABTS solution; absorbance was monitored after 6 min. For the solutions before spray drying and reconstituted powders, a previous precipitation of the encapsulating agent was made with ethanol because it is insoluble in this solvent. Then the solution was filtered through a glass microfiber filter paper 0.6 µm pore size (ADVANTEC, JP). Aliquots of the filtrate were taken for analysis.

The inhibition percentage (%) was determined with Equation 3:

$$\% = \left(\frac{Control\,A - Sample\,A}{Control\,A}\right) \times 100 \quad (3)$$

Results were expressed as Trolox equivalents µmol per gram dry weight (TE µmol/g$_{dw}$).

Moisture content and total solids

Moisture content was determined using a thermobalance (OHAUS MB200, USA), placing 0.5 g of the sample at 105 °C until there was no change in weight greater than 0.01 g in 90 s (Cárdenas-Bailón *et al*., 2015). Duplicated analysis was made. Total solids were determined by difference.

Particle size distribution and morphology

Particle size distribution was determined in a laser diffraction particle size analyzer (Malvern IM 026, 2006 series, Malvern, UK). Samples were dispersed in hexane under constant stirring.

For particle morphology, microcapsules were put in double-faced adhesive tape stubs and coated with gold and observed in a scanning electron microscope (SEM), operating at 15 kV using a 1000, 1500, 2000 y 3000x magnification (Jeol, JSM-5800LV, Peabody, USA).

Storage stability test

Microcapsules obtained under the best drying conditions for each encapsulating agent (10, 15, and 20%$_w$) were stored at room temperature (22-25 °C). Samples of 1.5 g were placed in sealed amber glass bottles. Samples with 10%$_w$ of encapsulating agent in the solution to be dried were also placed in clear glass bottles to determine whether light had an effect in PC, BC, BX and AC degradation. This was carried out in order to determine if the lowest concentration of encapsulating agent studied was enough protection for bioactive compounds from light. Analyses were made every 15 days and results were expressed as percentages of preserved bioactive compounds (PC, BC, BX and AC%).

Statistical analysis

Results were expressed as the average ± standard deviation of all determinations. Analysis of variance (ANOVA) was performed with Minitab statistical software, version 17. Mean comparisons were performed using Tukey´s test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Bioactive compounds and antioxidant capacity in purple cactus pear peel

The extraction procedure used in this work extracted 95% of the PC present in the purple cactus pear peel. Bioactive compounds and antioxidant capacity in purple cactus pear peel are shown in Table 1. PC content in the purple cactus pear peel was 16.7 ± 0.108 mg GAE/g_{dw}; total betalain content was 0.428 mg TB ±0.002/g_{dw}, 55% belonging to BC and 45% to BX. These values are comparable to the ones reported for red skinned cactus pear (Fernández *et al*., 2010), Spanish purple cactus pear (Gandía *et al*., 2010), red cactus pear peel (Li *et al*., 2015) and amaranth (Aparicio-Fernández *et al*., 2017). The AC of the extract was 192.69 ± 4.35 TE µmol/g_{dw}, which lies between values reported for tropical fruits considered to have high antioxidant activity, as acai and jaboticaba (Rufino *et al*., 2010). With these results it is clear that purple cactus pear peel is a by-product rich in bioactive compounds that can be exploited for use in the food industry.

Table 1: Bioactive compounds and antioxidant capacity in purple cactus pear peel

Component	Result
Phenolic compounds (mg GAE/g_{dw})	16.7±0.108
Total betalain content (mg TB/g_{dw})	0.428±0.002
Antioxidant capacity (µmol/g_{dw})	192.69±4.35

The reported values are the mean±standard deviation, n=3

MICROENCAPSULATION BY SPRAY DRYING WITH MDX

Phenolic compounds in microcapsules using MDX as encapsulating agent

Retention efficiency of PC (PC%) was significantly higher with 20%$_w$ MDX compared to 10 and 15%$_w$ MDX (Fig. 1A) which are in agreement with other studies that indicate the important effect of encapsulating agent´s concentration in %PC (Saénz *et al*., 2009; Robert *et al*., 2010). Statistical analysis indicated that inlet and outlet temperature´s interaction affected PC% (Fig. 2A), obtaining the highest retentions with 160-80 and 170-70 °C. An interaction between inlet temperature and MDX was also present (Fig. 2B), obtaining higher retentions with 160 °C-20%$_w$ MDX, followed by 170 °C-20%$_w$ MDX.

Regarding PC content, drying conditions had no effect, on the other hand, PC content decreased as encapsulating agent concentration increased ($P<0.05$), due to dilution effect (Fig 1B). So even though PC% is higher when using 20%$_w$ MDX, PC content in such samples is approximately three times smaller than the content obtained in samples with 10%$_w$ MDX. Bioactive compound content in powders that have good retention efficiencies is very important when determining the best conditions for the encapsulation.

Regarding the values of PC% over 100% in microcapsules with 20%$_w$ MDX, these high values may be due to the hydrolysis of polyphenol conjugates during the drying process (Turkmen *et al*., 2005), as it has been previously reported during the encapsulation of PC from cactus pear pulp extract with MDX (112%) by Saénz *et al*. (2009).

Betalains (BC and BX) in microcapsules using MDX as encapsulating agent

BC content and retention efficiency were only significantly affected by MDX concentration ($P<0.05$) (Fig. 1 C-D and Fig. 2 D-E). The highest retention (101.32%) was obtained with 20%$_w$ MDX; on the other hand, the highest content (0.0873 mg BE/g_{dw}) was obtained with 10%$_w$ MDX (Fig. 1 C-D). Regarding BC%, other works also reported that there was no significant effect of drying temperatures (Saénz *et al*., 2009; Azeredo *et al*., 2007).

BX results were very similar to BC, with significant effect only from MDX concentration ($P<0.05$) obtaining the highest BX% but lowest concentration with 20%$_w$ MDX (Fig. 1E-F and Fig. 2F-G).

With these results, it can be seen that even though betalains are heat sensitive compounds, spray drying is a technique that offers protection, since the evaporation process is

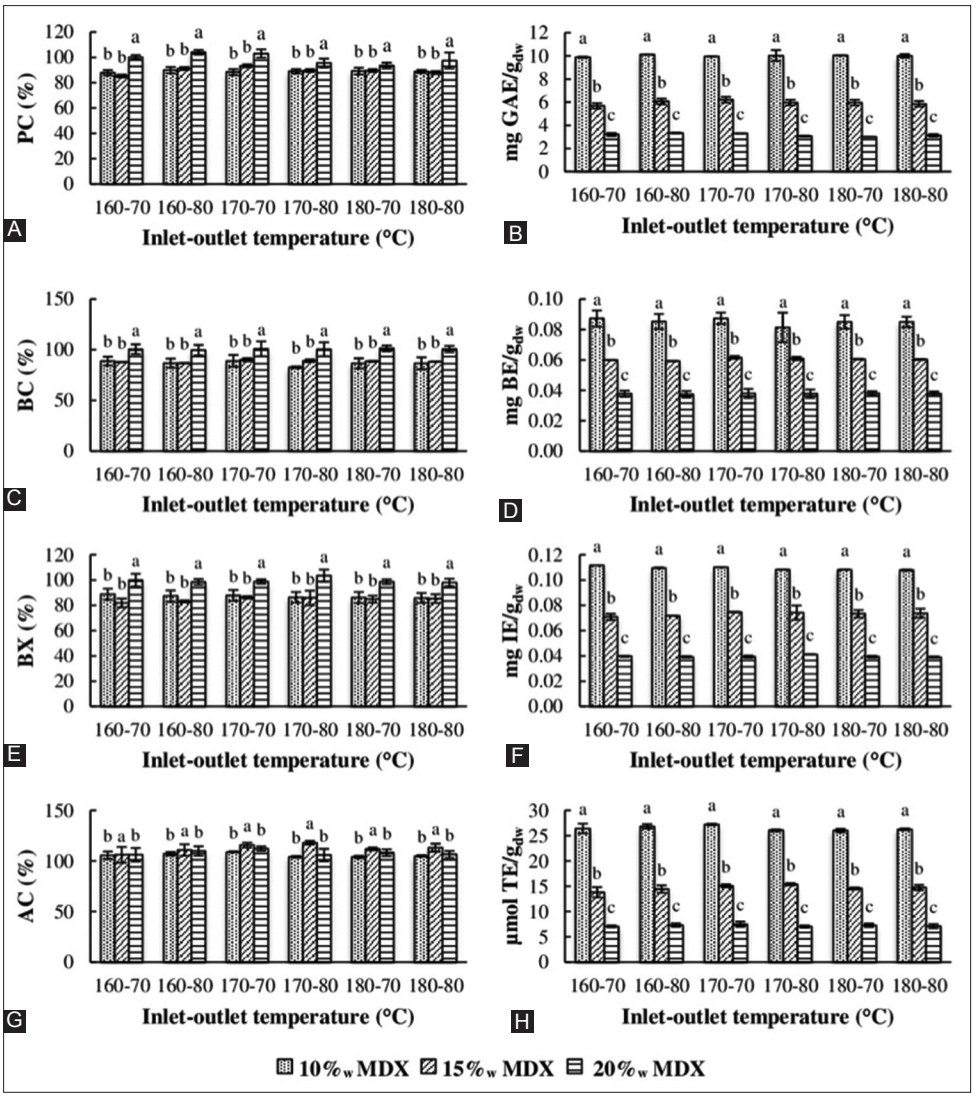

Fig 1. Retention efficiency (%) and content of: PC (A-B), BC (C-D), BX (E-F) and AC (G-H), respectively, in microcapsules obtained with MDX. Values are mean ± standard deviation. Mean (n = 2) values with different letters represent significant differences (P<0.05).

carried out so fast, droplets remain cool until they are completely dry (Gharsallaoui *et al.,* 2007).

Antioxidant capacity of microcapsules using MDX as encapsulating agent

AC value decreased as encapsulating agent concentration increased (Fig. 1H and Fig. 2I) due to effect of dilution previously mentioned. Statistical analysis indicated that 15%$_w$ MDX microcapsules were significantly different from the other samples (P<0.05) on AC% (Fig. 2H), nevertheless; overall AC% exceeded 100% for all conditions (Fig. 1G). AC% exceeding 100% can be explained by the formation of new compounds with greater AC, during the drying process, due to possible hydrolysis of complex structures (Robert *et al*., 2010).

Based on the results obtained in the microencapsulation of purple cactus pear peel extract with MDX as encapsulating agent, the drying conditions of 160-80 and 170-70 °C (inlet-outlet temperature) were selected as the best drying conditions since they provided the highest PC%, as shown in Fig. 2A. The combination 170-80 °C was also included because 170°C is part of the interaction inlet temperature-20%$_w$ MDX, which presented high PC% (Fig.2B). These drying temperatures were used with 10, 15, and 20%$_w$ GA in the solution to be dried, for comparison.

MICROENCAPSULATION BY SPRAY DRYING WITH GA

Phenolic compounds in microcapsules with GA as encapsulating agent

GA concentration had a significant effect on %PC and content (P<0.05), obtaining higher PC% when using 20 and 15%$_w$ GA but a lower content, compared with 10%$_w$ (Fig. 3A-B). Comparing with MDX, PC% was in general lower when using GA, being 10% lower when comparing

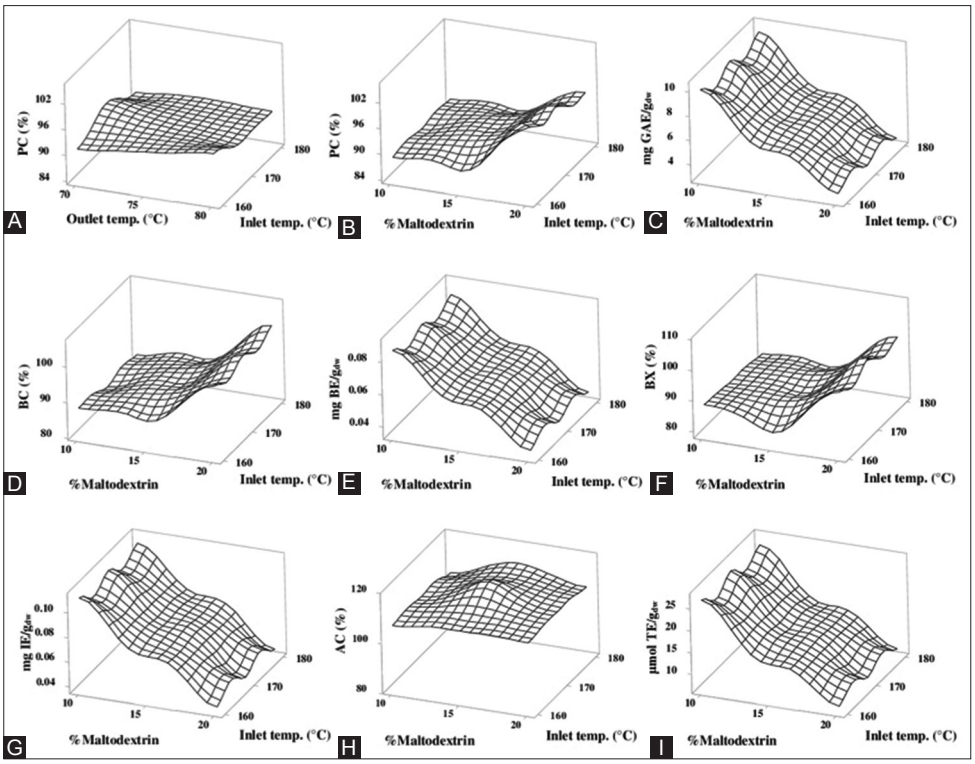

Fig 2. Surface plots for PC (A, B, and C), BC (D and E), BX (F and G) and AC (H and I) retention and content of microcapsules obtained with MDX as encapsulating agent.

20%$_w$ MDX. This indicates that MDX has a better protection against oxidation during the drying process. These results are in agreement with those reported by Tonon *et al.* (2010), which showed that MDX presented better protection for PC during the drying process.

Betalains in microcapsules with GA as encapsulating agent

Statistical analysis indicated that the effect of 10%$_w$ GA was significant ($P<0.05$) for BC content and retention (Fig. 3C-D). However, regarding BC%, with all GA concentrations, retention was above 92%. BC content was slightly higher (up to 0.036 mg BE/g_{dw}) when using GA (Fig. 3D), as compared with MDX (Fig. 1D). BC% was also higher for GA as compared to MDX (16.9, 8.6, and 3.6% higher for 10, 15, and 20%$_w$ GA, respectively). This may occur because of the good filmmaking property of GA, which provides a greater stability for betacyanins in microcapsules (Gharsallaoui *et al.*, 2007) and because GA is more hydrophilic than MDX (Janiszewska, 2014). Janiszewska (2014) also reported that microcapsules with GA showed higher BC% than MDX.

BX content and retention were significantly affected by GA concentration ($P<0.05$) (Fig. 3E-F). The highest BX% was obtained with 10 and 20%$_w$ GA, being significantly different than 15% GA ($P<0.05$). Similar to BC results, BX content presented up to 0.016 mg IE/g_{dw} more when using GA than the microcapsules obtained with MDX but BX% with GA was similar to MDX, resulting only 7.5% higher when using 20%$_w$ MDX.

Antioxidant capacity in microcapsules with GA as encapsulating agent

AC% was significantly higher when using 20%$_w$ GA but due to the dilution effect; AC value was the lowest ($P<0.05$) (Fig. 3G-H). %AC is consistent with PC% (higher retention when increasing encapsulating agent concentration), indicating PC play an important role in AC of purple cactus pear peel extract. Comparing with results obtained for 10 and 15%$_w$ MDX (Fig. 1G), less AC% was obtained when using GA, in average 35.3 and 26.4% less, respectively. These results may be due to the higher retention efficiency of PC presented in MDX microcapsules, as shown previously. These results are in agreement with the encapsulation of açai extract with GA and MDX, reported by Tonon *et al.* (2010) where a higher AC% was obtained when using the latter, indicating that MDX gives a good protection against oxidation during the drying process.

Based on the comparative study on drying conditions using MDX and GA, the combination 170-80 °C (inlet-outlet temperature) at 10, 15 and 20%$_w$ of encapsulating agent was selected to determine size distribution and morphology of the particles and to study the storage stability because

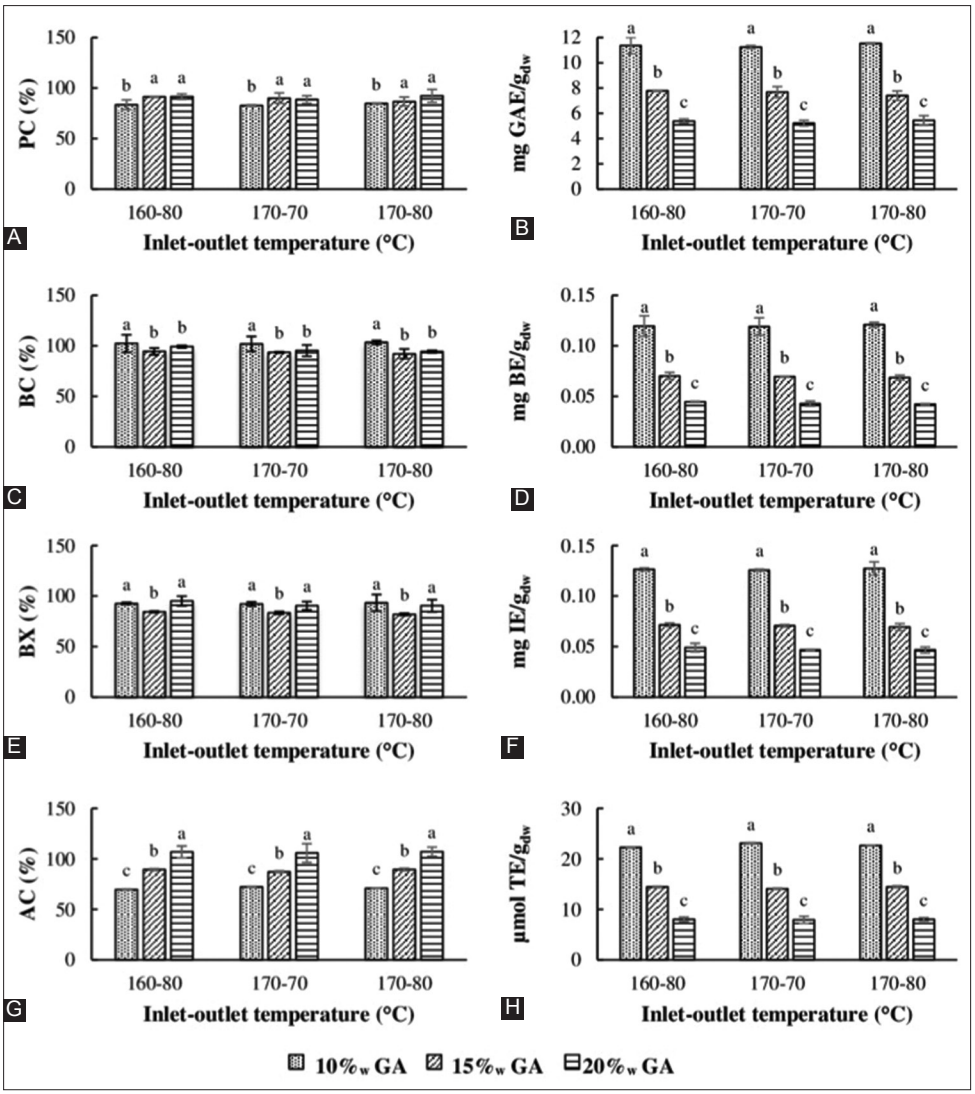

Fig 3. Retention efficiency (%) and content of: PC (A-B), BC (C-D), BX (E-F) and AC (G-H), respectively, in microcapsules obtained with GA. Values are mean ± standard deviation. Mean (n = 2) values with different lower case letters represent significant differences (P<0.05).

it was one of the conditions in which the drying process was carried out faster.

Particle size distribution and morphology of microcapsules

Particle size distribution was dependent on the MDX concentrations employed (Fig. 4A). A bimodal distribution can be observed and particle size spans from 5-40 µm for 10%$_w$ MDX and 8-60 µm for 15 and 20%$_w$ MDX. For 10 and 15%$_w$ GA (Fig. 4B), particle size distribution presented a slightly wider dispersion (8 to around 75 µm) than those obtained with MDX, probably due to the higher viscosity of GA solutions. Particle size distribution was also bimodal with GA. Bimodal distribution was also reported by Tonon *et al.* (2010) for microcapsules of açai juice with MDX and GA.

Particle morphology images by SEM (Fig. 5A-F) show that the majority of the microcapsules, regardless concentration and type of encapsulating agent, present a shrinked sphere form with a dented surface. This is attributed to the permeability of the microcapsule wall to water vapor during the drying process (Saénz *et al.*, 2009). Small and big particles can be observed, which corresponds to the bimodal distribution previously shown.

STORAGE STABILITY OF MICROCAPSULES

Storage stability test of phenolic compounds

PC% in microcapsules (Fig. 6A) remained constant for 10 and 15%$_w$ MDX until the 60th day, where a slight decrease was observed, reaching a maximum of 7.1% total loss for 15%$_w$ MDX during the 90-day storage study. When using 20%$_w$ MDX, losses were registered from the 15th day, remaining constant until the end of the study, losing only 2.5% of the original value. Tonon *et al.* (2010) worked

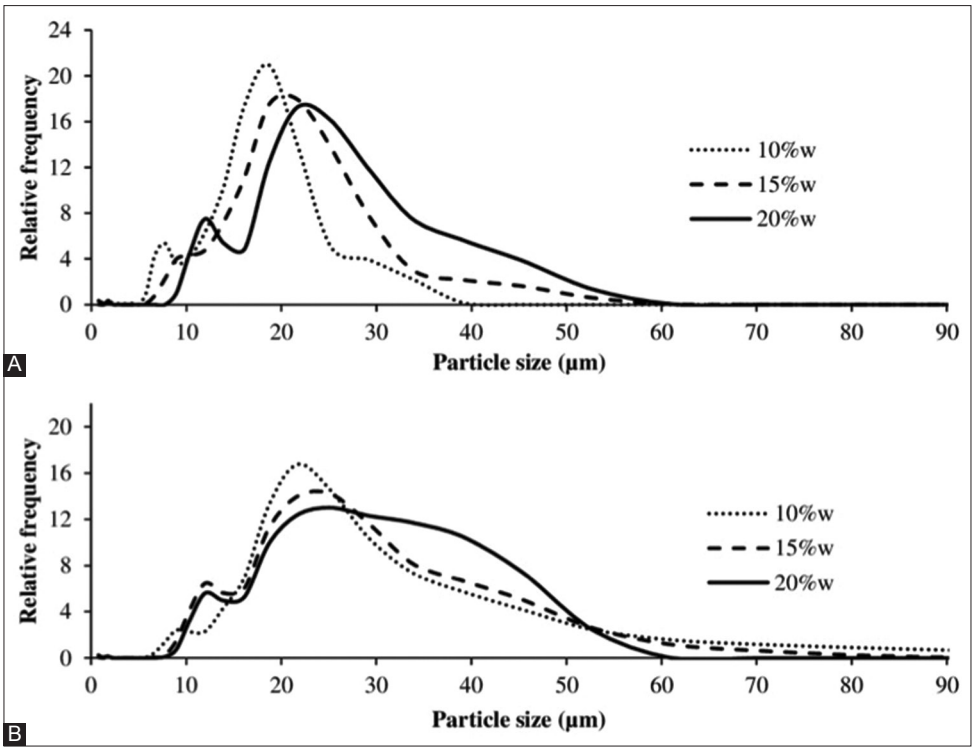

Fig 4. Particle size distribution of microcapsules obtained at 170-80 °C using MDX (A) and GA (B) as encapsulating agents.

with açai extract spray dried with MDX and GA stored at 25 °C during 120 days; these authors explained that when powders present degradation on the first days of storage, it is because some of the bioactive compounds of these powders remain on the surface of the microcapsules, which have contact with oxygen, and therefore they are degraded faster. This could be a possible explanation as to why PC in powders obtained with 20%$_w$ MDX started to degrade before PC of powders obtained with 10 and 15%$_w$ MDX. Statistical analysis showed no significant difference among the samples ($P>0.05$), regardless the concentration of encapsulating agent and the presence or absence of light. These results indicate that even though after the drying process, retention efficiencies can be slightly lower when using 10%$_w$ MDX compared to 20%$_w$ MDX, it is sufficient to protect PC and that a higher amount of encapsulating agent, in this case is unnecessary. Similar results to MDX were obtained when using GA (Fig. 6B). There was no significant difference among GA concentration, and the major loss was for microcapsules with 10%$_w$ GA in presence of light (4.2%). MDX and GA results showed no significant difference between them with the exception of 20%$_w$ GA, for which there was no loss. These results were similar to a 12-month storage stability study at 25 °C of black currant PC encapsulated with MDX reporting up to 11% of loss, indicating that encapsulated PC have a high degree of stability (Bakowska and Kolodziejczyk, 2011).

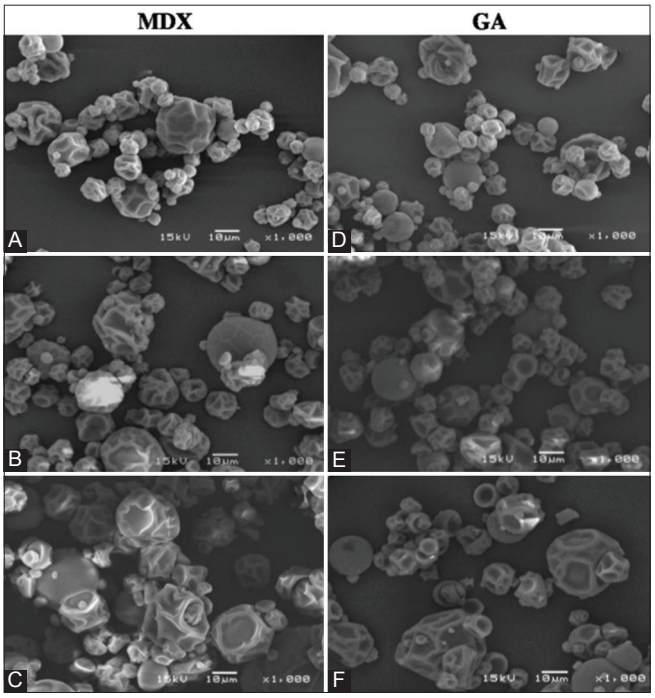

Fig 5. Micrographs of microcapsules obtained with 10, 15 and 20%$_w$ MDX (A, B, and C, respectively) and 10, 15 and 20%$_w$ GA (D, E, and F, respectively).

Storage stability test of betalains

BC% (Fig. 6C) was stable during the 90-day storage with 10%$_w$ MDX, in absence or presence of light, the latter losing just 2.8%, indicating that light does not affect the stability

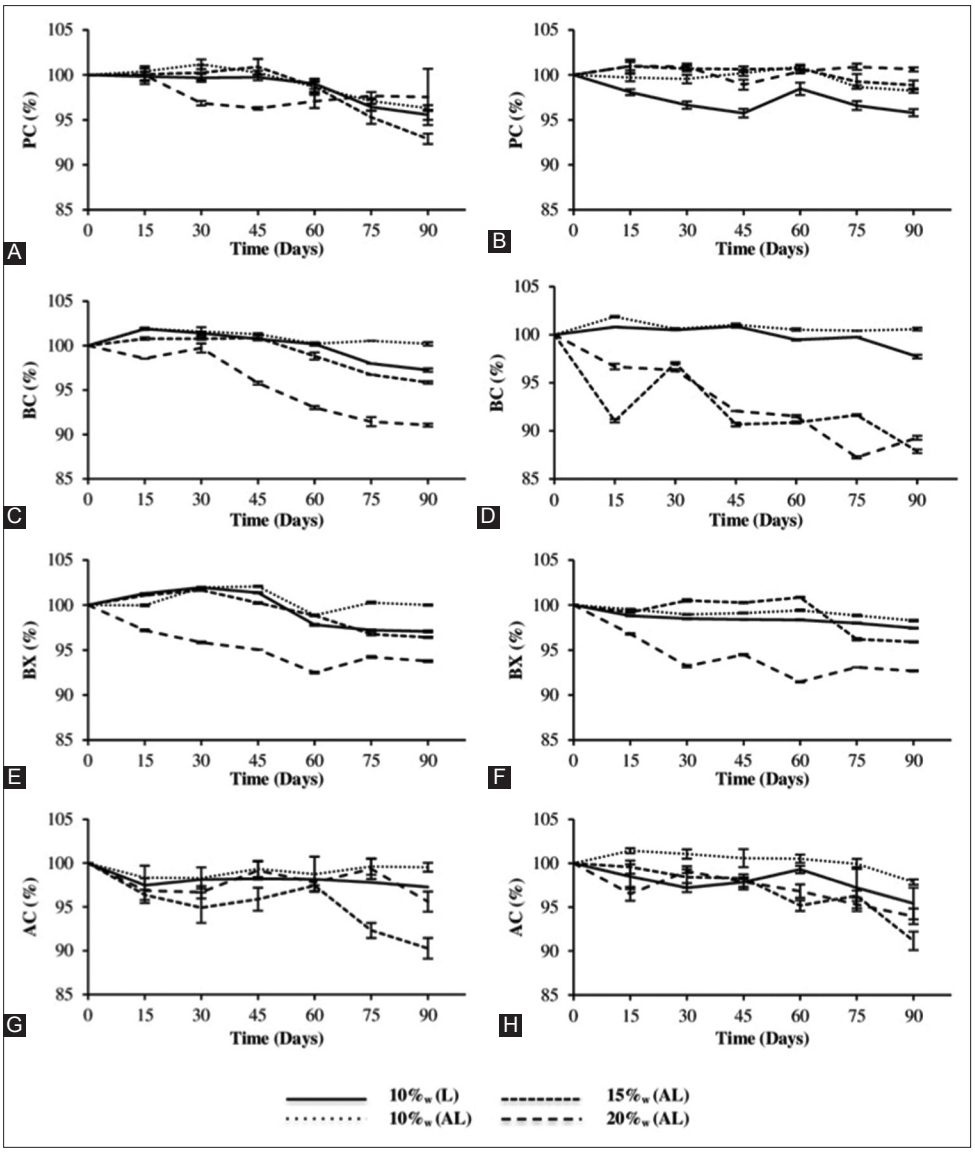

Fig 6. Preserved bioactive compounds during storage stability test. MDX: A, C, E, and G, and GA: B, D, F, and H. (L: presence of light, AL: absence of light).

of microencapsulated BC with MDX. 15 and 20%$_w$ MDX presented degradation from the 45th and 30th days, respectively, but only losing 4.14 and 8.95%, respectively. Contrary to what it was expected, at higher encapsulating agent concentration, less protection was provided against oxidation. A possible explanation may be that at higher solid concentration, certain amount of pigments stays on the surface of the particles and eventually degrades. BC encapsulated with 10%$_w$ GA had minimal loss either in presence or absence of light and similar to MDX microcapsules, higher loss was observed (12.12 and 10.71%, respectively) with 15 and 20%$_w$ GA. Statistical analysis indicated 10%$_w$ GA was different than 15 and 20%$_w$ GA, providing a higher protection for BC. In general, both 10%$_w$ MDX and GA protected very well BC during the 90-day storage, having the advantage of presenting a higher BC content.

Regarding BX%, (Fig. 6E), the major loss (6.23%) was observed again for 20%$_w$ MDX microcapsules, which were statistically different ($P<0.05$) from 10 and 15%$_w$ MDX. Clearly, 10%$_w$ MDX protects BX, even in presence of light. Similar behavior was observed for BX encapsulated with GA (Fig. 6F), being the microcapsules obtained with 20%$_w$ GA the ones with major loss (6.38%) and significantly different ($P<0.05$) from the rest of the samples. Results for BC and BX are comparable to the ones reported by Janiszewska (2014) for a 6-month storage of microcapsules of beet juice obtained with MDX and GA. Through the results previously mentioned, it can be observed that both encapsulating agents may help the pigments stability throughout storage. It is also important to mention that *Opuntia ficus indica* fruits contain natural gummy substances, which may act as intrinsic agents for stability of betalains

(Khan, 2016), which may be a reason why such a good stability was observed for the microencapsulated pigments in the present work. Evidently there is still further research needed regarding betalains stability, in order to take major advantage in the use of these pigments in the food industry.

Storage stability test of antioxidant capacity

Figure 6G shows that when 10%$_w$ MDX was used, AC maintained stable in presence and absence of light, being the total loss of only 2.73% and 0.46%, respectively. For 15 and 20%$_w$ MDX, there was a total loss of 9.73 and 4.41%, respectively, indicating that in general, AC had a good stability throughout the test. This can be related to the satisfactory results obtained for bioactive compounds, therefore not affecting AC. Statistical analysis showed that there was no significant differences between 10 and 20%$_w$ MDX whereas 15%$_w$ MDX was significantly different ($P<0.05$), since it had the major losses when comparing to the other samples.

For samples encapsulated with 10%$_w$ GA (Fig. 6H), total loss was 2.14 and 4.59% in absence and presence of light, respectively, without significant difference between them ($P>0.05$). When using 15 and 20%$_w$ GA, total loss increased to 8.84% and 6.05%, respectively, being only significantly different from the sample in absence of light with 10%$_w$ GA. Comparing MDX and GA (Fig. 6), results are very similar, and statistical analysis indicates no significant difference between them. It is interesting to observe that for both encapsulating agents, for some samples, like 15%$_w$ MDX and 20%$_w$ MDX and GA, degradation started on the 15th day. Çam *et al.* (2014) discussed this phenomenon for spray dried extracts (without adding encapsulating agent) stored a 4 °C and explained that bioactive compounds on the surface of the powders degrade much faster due to a major exposure to oxygen. This can be applied to microcapsules of bioactive compounds obtained with encapsulating agents, since some of the bioactive compounds that remain on the surface could be degrading rapidly, and therefore, early degradation is observed during storage. Since antioxidant capacity depends on bioactive compounds stability, it can also present early degradation, as in the present work. Despite early degradation, antioxidant capacity remained stable during overall storage for both encapsulating agents so it can be asserted that the use of any of these encapsulating agents is effective for the preservation of AC during a 90-day storage at 22-25 °C, either in presence or absence of light.

Results show that 10%$_w$ of encapsulating agent is sufficient for such protection, recalling that with such concentration of encapsulating agent, a good retention efficiency is obtained with the advantage of a higher content of bioactive compounds. On a practical basis, the use of MDX is recommended as an encapsulating agent because it is easier to prepare in solution, has low viscosity and it is less expensive than GA.

CONCLUSION

As a conclusion, purple cactus pear peel is a rich by-product source of PC and betalains with important AC that can be exploited by food industry. Microencapsulation by spray drying with 10%$_w$ MDX or GA as encapsulating agents can be used to obtain a high concentration of bioactive compounds giving good protection during drying and storage processes, extending shelf life of bioactive compounds with a simple but effective process technology.

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Author's contributions

G. I. Osorio-Revilla, K. I. Toledo-Madrid and T. G. Gallardo-Velázquez: Design of the work. K. I. Toledo-Madrid: Data collection. K. I. Toledo-Madrid, F. Terrazas-Valencia and G. I. Osorio-Revilla: Data analysis and interpretation. K. I. Toledo-Madrid: Drafting the article. K. I. Toledo-Madrid and G. I. Osorio-Revilla: Critical revision of the article. All authors approved the final version to be published.

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