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Co-encapsulation of bioactive compounds from blackberry juice and probiotic bacteria in biopolymeric matrices

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ABSTRACT

Blackberry juice (BJ) and *Lactobacillus acidophilus* (LA) were co-encapsulated by spray drying in three single materials: gum Arabic (GA), maltodextrin (MD) and whey protein concentrate (WPC), as well as in the 50:50 blends of these materials were also considered. After the spray drying, total phenolic compounds (TPC) and total monomeric anthocyanin content (TMAC) was higher when GA-MD blend was used as an encapsulating agent with 98.4 ± 1.0% and 99.0 ± 1.0%, respectively. FTIR analysis indicated that the BJ affected the structure of the wall material, mainly when WPC was incorporated. The viability of LA was significantly higher when WPC was used (93.3 ± 0.9%) in comparison with a single material or a blend of materials. After storage of microcapsules, the best blend of biopolymers to protect TPC and TMAC was GA-MD with values of 67.9 ± 0.6% and 81.8 ± 0.8% respectively, while WPC presented the highest protection in LA cell survival (81.2 ± 0.7%). The degradation dynamics of compounds in microcapsules was described by first-order kinetics, with degradation rates ranging between 0.06 and 0.30 week^{-1} for bioactives and probiotic bacteria. The overall results showed GA-MD can be considered the most viable formulation to protect bioactives and probiotic bacteria.

1. Introduction

The recent decade has witnessed a huge increase in consumer demand for food products with functional properties. In addition to being a source of essential nutrients and energy, food products are nowadays fortified with additives to improve human health. In particular, antioxidants such as polyphenols and anthocyanins are of particular interest given their presumed effects in the prevention and treatment of some diseases, such as congestive heart failures, inflammation of internal organs, and cancer (Ivanovic et al., 2014). In this regard, natural sources have been considered as suitable and sustainable means for obtaining bioactive compounds with reduced risks and secondary effects. Fruits, tubers and legumes are increasingly studied for this purpose, with promising results and extensive applications (Dimitrios, 2006).

The health benefits of probiotics, which are live microorganisms contained in the gastrointestinal tract, include the control of intestinal infection, serum cholesterol levels, improvements of lactose utilization, and anticarcinogenic activity with proven health benefits, including improvement of the immune system, reduction of gastrointestinal pain, and treatment of infectious bacteria (Iannitti & Palmieri, 2010; Tiihonen, Ouwehand, & Rautonen, 2010). Probiotics can be incorporated in food products for supplementing the activity of natural biotic population in the gastrointestinal tract. Nowadays, probiotics are incorporated in commercial food products, including yogurt and juices (do Espírito Santo, Perego, Converti, & Oliveira, 2011). However, the composition and functionality properties of the food products can be affected by degradation effects (Burgain, Gaiani, Linder, & Scher, 2011). On the other hand, in some instance the ingestion of food products containing probiotic bacteria is not efficient without the adequate protection against adverse conditions (e.g., gastric pH).

Many probiotic bacteria are unable to survive under adverse temperature and acidity environments. For instance, bifidobacteria tend to be less resistant for pH values below < 5.0–5.5. For this reason,

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probiotic bacteria are commonly stored under mild acidic conditions (pH 4.5–5.0) to guarantee their viability for relatively long periods (Damaskos & Kolios, 2008). Microencapsulation is a systematic approach to protect probiotic bacteria against adverse environments. In fact, the microencapsulation process is a useful technology for obtaining powders, reducing the water content of liquid food matrices, and preventing undesirable degradation phenomena. In particular, the spray-drying process has been used for decades for encapsulation of food ingredients such as flavors, lipids, and carotenoids (Anekella & Orsat, 2013; Gaonkar, Vasisht, Khare, & Sobel, 2014; Kingwatee et al., 2015). The spray drying process involves rapid evaporation of solvent (most often water) and quasi-instantaneously entrapment of the interest compound (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). These features make the spray drying process quite attractive for a diversity of applications, from pharmaceutics to entrapment of flavors.

Microencapsulation using a single wall material (gum Arabic, maltodextrin and proteins) has proven to provide protection of probiotic bacteria against adverse conditions. However, the combination of material with different properties confers improved protection properties and acceptable release characteristic under common storage and gastric conditions (Krasaekoopt & Watcharapoka, 2014). For instance, alginate beads coated with chitosan provided enhance protection of *L. casei* Shirota for long storage periods (Chen et al., 2007). The blend of different biopolymeric matrices provides better mechanical and morphological (e.g., porosity) properties, which in principle can be tuned up for producing food products with tailored properties.

The aim of this work was to characterize functional properties of spray-drying microencapsulated blackberry juice containing probiotic bacteria using biopolymer blends. The physicochemical properties of the natural blackberry juice were characterized, and the effects of encapsulating materials on bioactives and the survival of probiotic bacteria in the juice powder, and their stability during storage were studied.

2. Materials and methods

2.1. Materials

The blackberry fruits (*Rubus fruticosus* L.) were obtained in a local supermarket (Walmart S.A. de C.V., Toluca, Mexico). *Lactobacillus acidophilus* LA-5 strain DSM13241 (LA) was provided by Chr. Hansen (Hørsholm, Denmark). The reagents used to determine the total phenolic compounds (TPC) and total monomeric anthocyanin content (TMAC) were purchased from Sigma-Aldrich Corp. (St. Louis, Mo, USA). Biopolymeric materials used for microencapsulation were gum Arabic (GA) food grade E 414 (Distribuidora Química LEFE S.A de C.V., Mexico City, Mexico), maltodextrin DE20 (MD) (CPI Ingredientes S.A de C.V, Toluca, Mexico) and whey protein concentrate (WPC) 7000 (Hilmar Ingredients, Hilmar Cheese Company, Inc., USA).

2.2. Fruit samples

Blackberry fruits were sanitized by dipping them in water containing a silver colloidal solution (0.35 g/100 g water) for 10 min and then fruits were washed with water after immersion in the silver colloidal solution. Next, the fruits were stored in a freezer at −3.0 °C until their physicochemical analysis.

2.3. Extraction of blackberry juice

The blackberry fruits were thawed in a water bath at 25 °C and homogenized using an Oster blender model 2607 (Jarden Corporation, New York, USA) until the juice and solids were obtained by separation. The mixture was filtered with a Tyler mesh at < 70 μm. The supernatant was centrifuged using an Eppendorf centrifuge model 5430 R (Hamburg, Germany) at 479 × *g* for 15 min.

2.4. Physicochemical analyses

2.4.1. pH, tritable acidity and Brix value

To determine tritable acidity, 20 g of sanitized blackberries were crushed and homogenized. The solution was diluted to 54 mL with distilled water and maintained at 70 °C for 30 min. The mixture was filtered and washed with distilled hot water. The filtered fraction was taken in a volumetric flask (50 mL). 25 mL of the sample was taken and the pH was adjusted to 8.3 with a NaOH solution (0.1 equiv/L). The NaOH volume added was recorded and the titratable acid was calculated using the following equation:

$$\text{Acidity} = [(N \times V_1 \times 100)/m] \times F \quad (1)$$

where N is the normality of NaOH solution, V_1 is the added volume of NaOH (mL), m is the mass of the blackberry juice sample (g) and F is the factor of the predominant acid inside the blackberries (6.7×10^{-3} g of malic acid). The Brix value was determined using an Atago refractometer model RX-500 (Atago Co. Ltd., Tokyo, Japan).

2.4.2. Total phenolic compounds (TPC)

The concentration of phenolic compounds in blackberry juice was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965). The reaction mixture was prepared by mixing 10 μL centrifuged blackberry juice using 1500 μL of a 10% Folin-Ciocalteu reagent. After 5 min, 1500 μL 10% $NaHCO_3$ were added. The reaction was carried out for 90 min. Blank also was prepared, containing 1500 μL of 10% Folin-Ciocalteu reagent and 1500 μL 10% $NaHCO_3$. The absorbance was measured at 750 nm. The same procedure was repeated for the standard solution of gallic acid (GA), and the calibration line was conducted. The phenolic content in the blackberry juice was expressed in terms of the gallic acid equivalent (mg of GA/mL of blackberry juice).

2.4.3. Total monomeric anthocyanin content

The total monomeric anthocyanin content (TMAC) was estimated using the pH differential method (de Souza et al., 2014). An aliquot (10 μL) of centrifuged blackberry juice was diluted with 920 μL pH = 1.0 (0.025 mol/L KCl) and pH = 4.5 (0.4 mol/L CH_3CO_2Na) buffers to achieve the same dilution. The absorbance was measured at 510 nm and 700 nm in both pH = 1.0 and pH = 4.5 buffers and calculated as:

$$A = (A_{510} - A_{700})_{pH=1.0} - (A_{510} - A_{700})_{pH=4.5} \quad (2)$$

where A_{510} is the absorbance at 510 nm, and A_{700} is the absorbance at 700 nm. Then, the total monomeric anthocyanin content (expressed in terms of cyanidin-3-glucoside) was calculated using the following formula:

$$TMAC = (A \times MW \times DF \times Ve \times 1000)/(\varepsilon \times M) \quad (3)$$

where A is the absorbance determined by using Eq. (2), MW is the molecular weight of cyanidin-3-glucoside (449 g/mol), DF is the dilution factor, Ve is the extract volume, ε is the molar extinction coefficient of cyanidin-3-glucoside (29600), and M is the mass of the blackberries extracted. The results were expressed as mg cyanidin-3-glucoside equiv/g of blackberries.

2.5. Probiotic strain and growth conditions

A sample of dry probiotic cells was rehydrated in 200 mL of Man Rogosa and Sharpe (MRS) broth and incubated anaerobically at 37 °C for 24 h (Kingwatee et al., 2015) in order to obtain the final concentration of cell cultures at least 1×10^{10} CFU/mL. Then, the culture was centrifuged using an Eppendorf centrifuge model 5430 R (Hamburg, Germany) at 1465 × *g* for 30 min. The precipitate resulting from the centrifugation was removed and the cells in the supernatant were washed with 0.9% sterile saline water and completed to 100 mL volume.

2.6. Zeta potential

The ζ-potential measurements of the mixtures used for feeding the spray dryer were determined using a Zetasizer Nano ZSP (Malvern Instruments Ltd., Malvern, Worcestershire, UK). 1 mL of each sample was put into a disposable folded capillary cell for analysis. The equipment software converted the electrophoretic mobility measurements into ζ-potential values using the Smoluchowsky mathematical model.

2.7. Spray drying

A mini spray dryer model B-290 (Büchi Labortechnik AG, Flawil, Switzerland) was equipped with a fluid atomizer (inside diameter of 5 mm) operated in current mode. The drying conditions for the experiment were adjusted as follows: 130 °C ± 5 °C hot-air inlet temperature and 60 ± 5 °C hot-air outlet temperature, and 40–50 mBar atomizing pressure, pumped to 15% and aspirator 100%. These conditions were selected on the basis of previous reports (Lapsiri, Bhandari, & Wanchaitanawong, 2012; Rodea-González et al., 2012) and tests for obtaining stable (e.g., reduced material aggregation in the nozzle) equipment operation. Pasteurized blackberry juice (70 °C in a warm water bath for 15 min) was blended with each biopolymer or a 50:50 blend of them (GA, MD, WPC, GA-MD, GA-WPC, MD-WPC) until reaching 20% of the total solid content. Each mixture consisted of 100 mL pasteurized blackberry juice, 100 mL of biopolymer dissolved in water, and 20 mL lactic bacteria in an isotonic solution. The pH of the feed mixture was about 3.5, determined mainly by the fraction of blackberry juice (~45% of the total drying medium). For stability tests under storage conditions, the water activity of all samples was adjusted to 0.3 ± 0.02, and the samples were stored in a chamber with relative humidity (about 25%) control.

2.8. Particle size

The particle size of microcapsules was measured using a laser diffraction instrument (Mastersizer 3000, Malvern Instruments Ltd., Malvern, Worcestershire, UK) equipped with a Malvern Aero S unit for analysis of dry powders. Air pressure, feed and obscuration were 1×10^5 Pa, 30%, 0.5–5.0%, respectively. The particle size measurements are reported as volume-weighted mean diameter ($d_{4,3}$).

2.9. Scanning electron microscopy (SEM)

The morphology of the microcapsules was analyzed by means of a scanning electron microscope (JEOL JMS 7600F, Akishima, Japan) with the BEC mode at 15 kV accelerating voltage at room temperature under 22 Pa. The samples were mounted on circular pans with double-sided adhesive carbon tape and sputtered with 20 nm of gold using a Denton Vacuum DESK IV device. Micrographs at 3000× magnification were presented.

2.10. FTIR

The FTIR spectrum of the dried samples was obtained using a Perkin Elmer spectrometer (Spectrum 100, Perkin Elmer, Waltham, MA, USA) equipped with a crystal diamond universal ATR element and a Spectrum 10 Software (Perkin Elmer). First, a spectrum of the empty cell was measured in order to correct the background effect. Three replicates were measured for each film sample (at room temperature), with each spectrum averaged over three scans collected in the range from 400 to 4000 cm^{-1}, at 1 cm^{-1} resolution.

2.11. Glass transition temperature (Tg)

The glass transition temperature of the microcapsules was determined using a Mettler-Toledo model 822E differential scanning calorimetry (DSC) (Mettler-Toledo GmbH, Greifensee, Switzerland) previously calibrated with indium. Five milligrams of microcapsules were weighed in aluminum hermetic pans. An empty aluminum hermetic pan was used as a reference. Samples were heated from 25 °C to 120 °C at a heating rate of 5.0 °C/min. The instrument was purged with nitrogen at a flow rate of 100 mL/min. The data was analyzed using the equipment software. The glass transition temperature (Tg) was taken as the midpoint of the baseline shift obtained in DSC. All measurements were made by triplicate.

2.12. Encapsulation efficiency

The estimation of the encapsulation efficiency was made by means of the procedure described by Maciel, Chaves, Grosso, and Gigante (2014). 1 g of microcapsules was dispersed in 99 mL of peptone solution (10 g/100 mL). The powder and solution mixture were homogenized. The quantification of the viable bacteria was performed by the traditional plate counting method by means of dilutions. To this end, 1 mL of each dilution was put in a Petri dish containing Man Rogosa and Sharpe (MRS) broth. Samples were incubated at 37 °C for 48 h. A correction of the dilutions was made after each counting, and units forming colonies (CFU)/g of powder were reported. The estimation of total concentrations was corrected for the moisture content (3.0%) in each sample. The percentage (%) of efficiency was determined according the following equation:

$$EE(\%) = (N/N_0) \times 100 \quad (4)$$

Here, N is the number of viable cells (CFU/g) in the powder, and N_0 is the number of viable cells in the solution before the spray drying process.

2.13. Kinetics parameters

2 g of microcapsules having water activity of 0.30 ± 0.02 were stored in dark and hermetic bags at 20 °C (30 small bags) for 10 weeks. Each week, a bag was withdrawn to determine the total phenolic compounds, the total monomeric anthocyanin content, and the survival of probiotic bacteria. Variations in the concentration of encapsulated bioactives and cell survival probiotic bacteria for all samples over time were fitted to a first-order kinetic model. The kinetics rate constant of each sample by effect of storage conditions was calculated by means of the following expression:

$$\ln C_t = \ln C_0 - kt \quad (5)$$

where t is the storage time (weeks), k is the first-order rate constant ($week^{-1}$), C_0 is the initial concentration of bioactives in the microcapsules, and C_t is the concentration of bioactives as a function of time. In the case of probiotic bacteria, C_t and C_0 are the CFU/g of microcapsules at the initial time of the study and in function of the storage time, respectively.

2.14. Statistical analyses

All experiments were performed for three samples, and values were expressed as mean values ± SD. Data were analyzed using the one-way analysis of variance (ANOVA) and a Tukey's test for a statistical significance of $p \leq 0.05$, using the SPSS Statistics 19.0.

3. Results and discussion

3.1. Characterization of the blackberry juice

Table 1 presents the results of physical and chemical analyses of the blackberry juice. The Brix value was slightly lower (9.23 ± 0.21) than the value reported by de Souza et al. (2014). On the other hand, the presence of organic (e.g., malic, tartaric and citric) acids contributes to

Table 1
Chemical composition of blackberry juice extracted from fresh fruits purchased from a local market. The extracted juice was homogenized and filtered to obtain particles of size < 70 μm.

Parameter	Values
pH	3.08 ± 0.05
Total soluble solids (°Brix)	9.23 ± 0.21
Titratable acidity (g malic acid/100 g fresh juice)	429 ± 7
Total phenolic compounds (mg GA/100 g fresh juice)	344 ± 62
Total monomeric anthocyanins (mg cyanidin 3-glucoside/100 g fresh juice)	206 ± 7

Values are means ± standard error, of three replicates.

the stickiness of the powder (Bhandari, Datta, & Howes, 1997), an effect that potentially hampers the operation of the spray drying process. The titratable acidity was 429 ± 7 mg malic acid/100 g of fresh juice. The values reported for the total phenolic compounds and total monomeric anthocyanins were in the range of the values reported by Acosta-Montoya et al. (2010) and Wu, Frei, Kennedy, and Zhao (2010). Most of the anthocyanins corresponded to cyanidin-3-glucoside. In general, the results reported in Table 1 are in line with the values previously reported (Wang & Xu, 2007).

3.2. ζ-potential

The ζ-potential of the feeding mixtures provides some insights on the electrostatic environment surrounding the biopolymer molecules used for microencapsulation. The first column of Table 2 shows the values of the ζ-potential for individual biopolymers. GA and MD presented negative values (−2.54 and 3.34 mV, respectively), reflecting the anionic nature of these biopolymers. On the other hand, the WPC presented positive charge (3.51 mV), which is indicating the well-known fact that proteins exhibit cationic behavior. The blending of the two anionic materials GA and MD discards their chemical interactions, such that the resulting microcapsules resulted from the physical aggregation of these materials (Fig. 1d and 1 e). In contrast, the incorporation of WPC led to the formation of smaller and disaggregated microcapsules (Fig. 1f), an effect that can be attributed to the formation complex coacervates between polysaccharides (either GA or MD) and proteins (Espinosa-Andrews, Sandoval-Castilla, Vázquez-Torres, Vernon-Carter, & Lobato-Calleros, 2010). Here, electrostatic forces promote the chemical interaction between the two electrostatically antagonist blending materials, resulting in microcapsules with improved cohesiveness properties. Interesting, cationic potentials dominate the electrostatic characteristic of the microcapsules GA-WPC and MD-WPC.

3.3. Glass transition temperature of microcapsules

The glass transition temperature of microcapsules is presented in Table 2. During storage and in the processing, juice powder becomes agglomerated. Caking can occur as consequence of the plasticizing effects of water on the particle surface. The mechanisms involved in caking can be characterized in terms of the glass transition effects (Chuy & Labuza, 1994). Microcapsules made with WPC presented the lowest glass transition (46.12 °C), suggesting that these microcapsules are susceptible of caking at relatively low temperature values. The combination of WPC with other biopolymers increased the glass transition, with 51.66 °C for GA-WPC and 42.32 °C for MD-WPC. In turn, the increased glass transition temperature should improve the stability of protein-based microcapsules.

Table 2
ζ-potential of the different mixtures used for feeding the spray dryer. The particle size and glass transition temperature (Tg) of microcapsules are also presented.

Sample	ζ-potential (mV)	$d_{4,3}$ (μm)	Tg (°C)
GA	-2.54 ± 0.07^{b}	11.15 ± 0.25^{e}	56.10 ± 0.11^{e}
MD	-3.34 ± 0.06^{a}	9.94 ± 0.21^{d}	54.87 ± 0.09^{d}
WPC	3.51 ± 0.05^{f}	5.05 ± 0.10^{a}	46.12 ± 0.07^{a}
GA-MD	-2.01 ± 0.03^{c}	8.97 ± 0.17^{c}	52.98 ± 0.17^{c}
GA-WPC	3.07 ± 0.04^{e}	8.71 ± 0.15^{c}	51.66 ± 0.15^{c}
MD-WPC	1.04 ± 0.02^{d}	5.65 ± 0.12^{b}	49.32 ± 0.05^{b}

Values are means ± standard error, of three replicates. Superscripts with different letters in same column indicate significant differences ($p \leq 0.05$).

3.4. Particle size and morphology of microcapsules

The mean particle size of microcapsules is presented in Table 2. GA led to large microcapsules (11.15 μm), an affect that could be induced by the plasticizing effect of juice sugars in maltodextrin molecules. The smallest size was obtained for WPC (5.05 μm) and MD-WPC (5.65 μm). In general, the combination of different materials led to a reduction of the particle size, an effect that is likely linked to electrostatic forces of antagonist materials. It has been reported that the size (e.g., mean diameter) of particles obtained with spray drying methods is affected by the properties of the material, the concentration and viscosity of the encapsulated material, as well as on the drying conditions (Jafari, Assadpoor, He, & Bhandari, 2008). The issue takes importance as the encapsulation efficiency depends on the particle size (de Barros Fernandes, Borges, & Botrel, 2014). The results in Table 2 showed that the mean particle size was significantly affected ($p \leq 0.05$) by the type of wall material, with the largest size using only GA and MD. Particles with high mean size can be explained from the high viscosity of the dispersions of these materials (Jafari et al., 2008). In contrast, MD dispersions exhibit lower viscosity values. The combination of high and low viscosity materials led to particles with size between the values of individual materials.

The morphology of microcapsules was evaluated by means of SEM images (Fig. 1). Gum Arabic (GA) and maltodextrin (MD) produced dried powders with a compact microstructure, large cavities and a coarse porous network (Fig. 1a and 1.b). In general, particles exhibited an oval, irregular like shape. In contrast, whey protein concentrate (WPC) led to more granular powder particles, with spherical shape (Fig. 1c). The combination of these materials modified the morphology of the dried encapsulation systems. The GA-MD blend still produced coarse grained material (Fig. 1d), although with reduced pore size. The incorporation of WPC (Fig. 1e and 1.f) modulated the microstructure by inducing fine grained morphologies linked possibly to the interaction of proteins and polysaccharides. In particular, particles obtained with blends of materials showed no evidence of cracking, an issue that is important to guarantee low gas permeability and protection of encapsulated material. The formation of voids and cracks are commonly related to the expansion of the particles due to thermal effects, and effect depending on the drying rate and the viscosity of the matrix material (Teixeira, Andrade, Farina, & Rocha-Leão, 2004). The blend of materials with different thermal and viscosity properties enables the modulation of the particle size, as shown by results in Table 1.

3.5. FTIR

FTIR analysis was used for exploring possible interactions between the encapsulation material and compounds from the blackberry juice. Fig. 2 presents the FTIR spectra for microcapsules with (dashed line) and without (solid line) blackberry juice. For wavenumbers higher than 1800 cm^{-1}, the spectrum containing blackberry juice was similar to the spectrum of raw microcapsules. However, some subtle differences were detected for the wavenumber range from 1800 to 900 cm^{-1}. For AG and MD (Fig. 2a and 2.b), the spectrum showed a shift of the intensity peak at about 1150 cm^{-1}. In fact, the incorporation of blackberry juice smoothed the intensity of the peak, suggesting that the blackberry juice

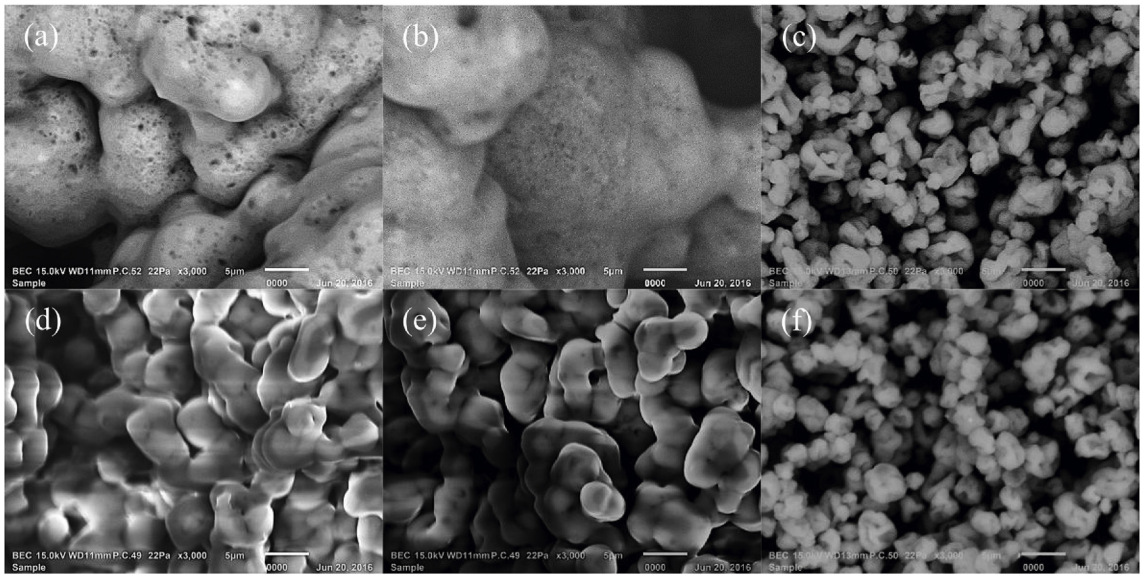

Fig. 1. SEM images of spray-dried encapsulation material containing blackberry juice and *Lactobacillus acidophilus* LA-5 strain DSM13241 (LA). (a) Gum Arabic (GA), (b) maltodextrin (MD), (c) whey protein concentrate (WPC), (d) GA-MD, (e) GA-WPC and (f) MD-WPC.

induced slight modifications of the encapsulation material. The peak at about 1150 cm^{-1} can be ascribed to C-O stretching of the polysaccharide molecules. Its shift and smoothing could be caused by the acidity of the blackberry juice, which induced weak modifications to the polysaccharide structure. However, the interaction between wall material and encapsulated compounds cannot be discarded (Janjarasskul & Krochta, 2010). Similar results were obtained for the GA-MD blend (Fig. 2d) where the modification of the intensity peak at about 1150 cm^{-1} was also observed. More pronounced modifications by blackberry juice were observed for microcapsules containing WPC.

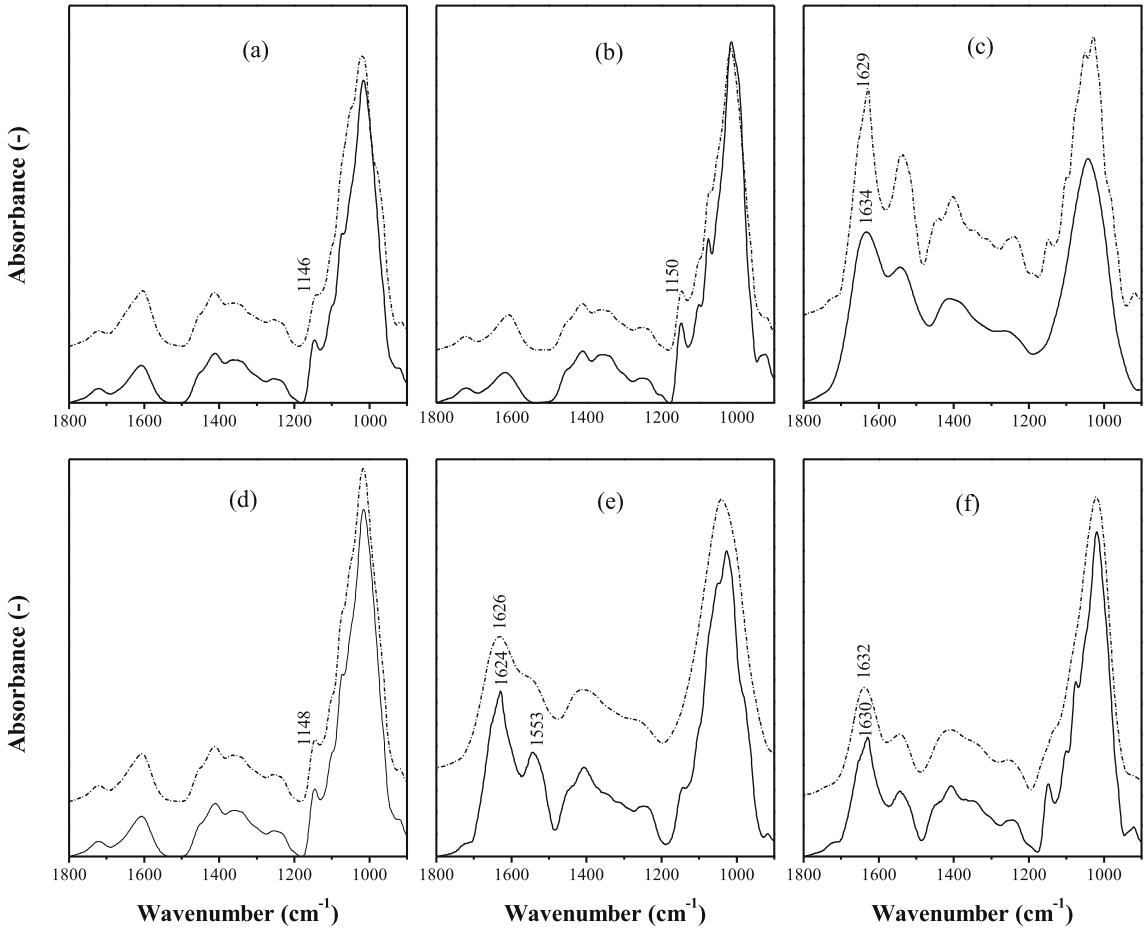

Fig. 2. FTIR spectrum of encapsulation material with (dashed line) and without (solid line) blackberry juice. (a) GA, (b) MD, (c) WPC, (d) GA-MD, (e) GA-WPC, and (f) MD-WPC.

Fig. 2.c presents the FTIR spectra for microcapsules containing only WPC. For instance, the peak located at about 1634 cm^{-1} is linked to Amide I, a distinctive band of proteins (Byler & Susi, 1986). The incorporation of blackberry juice modified the shape of this band, shifting the peak to smaller wavenumber values (about 1134 cm^{-1}). The effect was also observed for the region at about 1400 cm^{-1}, which is linked to the Amide III group of proteins. The variations of the FTIR spectrum in the amide groups reflect that the blackberry juice incorporation induced important modifications in the structure of microcapsules. In turn, these modifications could be linked to changes of the secondary structure of proteins, which is quite sensible the pH of the surrounding environment (Chen et al., 2019). Similar results were obtained for blends containing WPC and polysaccharides (Fig. 2e and 2.f), although the changes after blackberry juice incorporation were smaller. Overall, the FTIR results showed that the incorporation of blackberry juice induced only slight changes in microcapsules made only with polysaccharides. However, more pronounced changes were observed for blends containing WPC, and the effects can be attributed to variations of the protein secondary structure by the juice acidity.

3.6. Total phenolic compounds

Fig. 3 shows the degradation kinetics from total phenolic content (TPC). Microcapsules made with GA-MD showed the highest values ($p \leq 0.05$) of phenolic compounds, in comparison with microcapsules made with the GA-WPC blend, MD-WPC or unblended polymers (GA, MD or WPC). In fact, about 35% of the initial content of AG (70 mg AG/g of microcapsules) was retained after ten weeks of storage (Table 3). This indicates that the blend of biopolymeric materials used to encapsulate polyphenols was the better formulation for protection of phenolic compounds. As shown by Table 4, the highest TPC after the storage period was obtained from microcapsules made with GA-MD, since the kinetics constant of degradation exhibited the lowest value (0.06 $week^{-1}$). The chemical composition of the bioactive compounds (i.e., negative charge for polyphenols) and polyelectrolyte structure (type and density charge) are likely to be involved in bioactive-polymer interactions (Robert et al., 2010). It has been reported that polysaccharides such as GA contain several hydroxyl groups (OH-) in their molecular structure, improving the hydrophilic nature of the molecule. In fact, the presence of hydroxyl groups makes possible the formation of hydrogen bonds with water from blackberry juice. In this way, hydroxyl groups promote the interaction between wall material and encapsulated

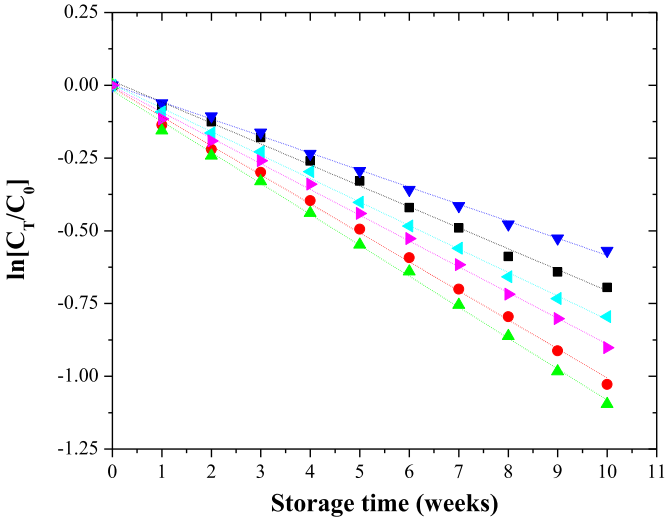

Fig. 3. Total phenolic compounds content (TPC) degradation kinetics of spray-dried encapsulation material containing blackberry juice and *Lactobacillus acidophilus* LA-5 strain DSM13241 (LA). ■ GA, ● MD, ▲ WPC, ▼ GA-MD, ◀ GA-WPC and ▶MD-WPC.

Table 3
Encapsulation efficiency of total phenolic compounds, anthocyanins and probiotic bacteria after the spray drying process and during storage of microcapsules.

Sample	Time (week)	Phenolic compounds (%)	Anthocyanins (%)	Probiotic bacteria (%)
GA	0	95.1 ± 0.9^{e}	$88.4 \pm 0.9^{c, d}$	89.4 ± 0.8^{d}
	10	62.9 ± 0.6^{E}	35.8 ± 0.4^{A}	35.3 ± 0.3^{B}
MD	0	81.1 ± 0.8^{b}	90.4 ± 1.0^{d}	79.7 ± 0.8^{b}
	10	36.9 ± 0.1^{B}	40.4 ± 0.7^{B}	30.2 ± 0.2^{A}
WPC	0	75.7 ± 0.8^{a}	80.2 ± 0.8^{a}	93.3 ± 0.9^{e}
	10	31.1 ± 0.2^{A}	55.4 ± 0.4^{C}	81.2 ± 0.7^{F}
GA-MD	0	98.4 ± 1.0^{f}	99.0 ± 1.0^{e}	85.9 ± 0.7^{c}
	10	67.9 ± 0.6^{F}	81.8 ± 0.8^{F}	71.0 ± 0.7^{E}
GA-WPC	0	90.1 ± 0.8^{d}	85.9 ± 1.0^{b}	73.3 ± 0.8^{a}
	10	53.9 ± 0.3^{D}	62.1 ± 0.6^{D}	55.6 ± 0.6^{D}
MD-WPC	0	87.1 ± 0.7^{c}	$87.3 \pm 0.9^{b, c}$	71.9 ± 0.7^{a}
	10	48.0 ± 0.2^{C}	70.6 ± 0.5^{E}	50.3 ± 0.5^{C}

Values are means ± standard error, of three replicates. Superscripts with different lower-case letters in same column at 0 weeks indicate significant differences ($p \leq 0.05$). Superscripts with different capital letters in same column at 10 weeks indicate significant differences ($p \leq 0.05$).

Table 4
Kinetic constants (Eq. (5)) of total phenolic compounds, anthocyanins and probiotic bacteria during storage of microcapsules.

Sample	Kinetic constant ($week^{-1}$)	Phenolic compounds	Anthocyanins	Probiotic bacteria
GA	k_1	$0.07 \pm 0.01^{b, c}$	$0.10 \pm 0.01^{c, d}$	$0.14 \pm 0.01^{b, c}$
	k_2		0.26 ± 0.02^{g}	0.33 ± 0.02^{g}
MD	k_1	$0.10 \pm 0.01^{e, f}$	$0.08 \pm 0.01^{b, c}$	$0.13 \pm 0.01^{a, b}$
	k_2		$0.30 \pm 0.02^{g, h}$	$0.36 \pm 0.02^{g, h}$
WPC	k_1	$0.11 \pm 0.01^{f, g}$	0.17 ± 0.01^{f}	0.11 ± 0.01^{a}
	k_2			
GA-MD	k_1	$0.06 \pm 0.01^{a, b}$	0.05 ± 0.01^{a}	$0.15 \pm 0.01^{c, d}$
	k_2			
GA-WPC	k_1	$0.08 \pm 0.01^{c, d}$	0.14 ± 0.01^{e}	$0.17 \pm 0.01^{d, e}$
	k_2			
MD-WPC	k_1	$0.09 \pm 0.01^{d, e}$	0.11 ± 0.01^{d}	$0.19 \pm 0.01^{e, f}$
	k_2			

Values are means ± standard error, of three replicates. Superscripts with different letters in same column indicate significant differences ($p \leq 0.05$).

compounds to form the coating matrix, and at the same time to protect the encapsulated material (Janjarasskul & Krochta, 2010).

3.7. Total monomeric anthocyanin content (TMAC)

After the encapsulation process, the percentage of retention of anthocyanins was in the range from 80.2% to 99.0% for fresh microcapsules (Table 3). However, the values decreased after 10 weeks of storage time where the retention of anthocyanins decreased to levels of about 35% for, e.g., AG. Fig. 4 shows the degradation kinetics of total monomeric anthocyanin content as a function of time in the storage microcapsules. The blend GA-MD was the system that presented the best protection of TMCA as resulting from synergic effects of the encapsulation materials. Table 4 presents the degradation rates with the GA-MD blend exhibiting significant differences ($p \leq 0.05$) with respect to the other encapsulation matrices. The degradation kinetics of anthocyanins for single GA and MD materials presented two stages, each one with a first-order kinetics pattern (Tao et al., 2017). The first stage started right after the encapsulation process and presented higher stability of anthocyanins until the fourth week of storage. The formation of complex GA-anthocyanin was studied by Guan and Zhong (2015), who found a strengthened binding between these the gum and the anthocyanin fraction. In the case of MD, the TMAC could be explained by the

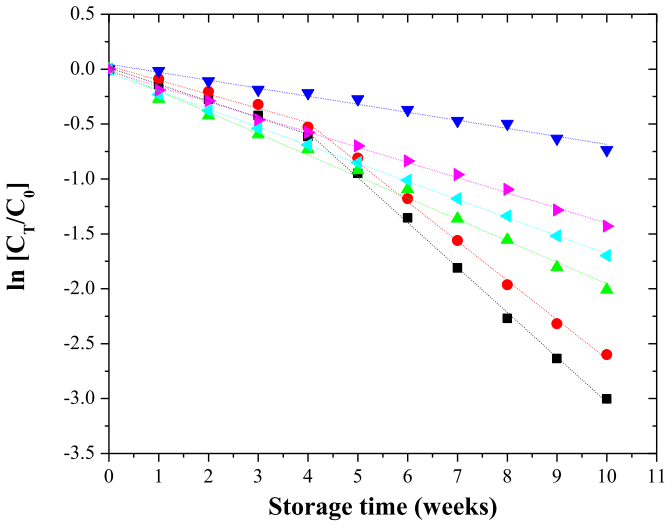

Fig. 4. Total monomeric anthocyanins content (TMAC) degradation kinetics of spray-dried encapsulation material containing blackberry juice and *Lactobacillus acidophilus* LA-5 strain DSM13241 (LA). ■ GA, ● MD, ▲ WPC, ▼ GA-MD, ◀ GA-WPC and ▶MD-WPC.

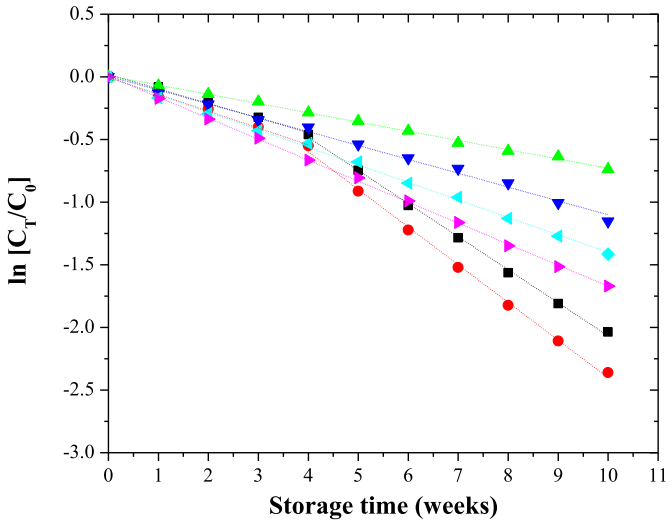

Fig. 5. Probiotic bacteria degradation kinetics of spray-dried encapsulation material containing blackberry juice and *Lactobacillus acidophilus* LA-5 strain DSM13241 (LA). ■ GA, ● MD, ▲ WPC, ▼ GA-MD, ◀ GA-WPC and ▶MD-WPC.

formation of a complex between the flavylium cation form of the anthocyanins and dextrin molecules, which prevented the transformation of anthocyanins to other forms of reduced stability (Duangmal, Saicheua, & Sueeprasan, 2008). It is apparent that the interaction of anthocyanins with dextrins was mediated by a nucleophilic substitution of hydroxyl groups of dextrin in an acid medium. On the other hand, the structure presented by cyanidin-3-glucoside exhibits enhanced stability with respect to the other anthocyanin forms in raw juice, because of the glucose substitution in the C-3 carbon. It has been reported that MD offered better protection results compared with SPI (soy protein isolate) for polyphenols and anthocyanins from pomegranate (Robert et al., 2010).

The second kinetics stage was characterized by a sharp decrease after four weeks of storage in GA and MD matrices, while WPC, MD-WPC and GA-WPC presented a slight but sustained decrease. Differences in bioactive compound retention during storage could be caused by the affinity of biopolymers by moisture content, giving place to the degradation of compounds. It has been reported that GA combined with MD can induce improvements of the anthocyanin stability (Idham, Muhamad, & Sarmidi, 2012). However, the electrostatic interaction between anthocyanin-GA in the formulation of this work can be considered weak compared to interactions anthocyanin-GA-MD. In fact, the GA-MD biopolymeric matrix was the formulation with the highest anthocyanin retention values. The encapsulation materials GA, and MD are more hydrophilic than WPC. WPC is a blend of low hydrophobicity globular proteins (lactalbumin, β-lactoglobulin, immunoglobulin and albumin), with a low tendency to capture moisture, while GA and MD are more hydrophilic molecules. It is apparent that WPC affected the retention of anthocyanins in storage time, exhibiting a constant degradation rate.

3.8. Probiotic bacteria encapsulation

The encapsulation efficiency is described in Table 3, showing a strong dependence on the wall material. WPC and GA exhibited the highest encapsulation efficiencies, with 93.3 ± 0.9% and 89.4 ± 0.8%, respectively. In contrast, the lowest value was exhibited by the MD-WPC blend with 71.9 ± 0.7%. The relatively low value presented by the MD-WPC blend can be explained by the morphology of microcapsules, since SEM images showed the presence of fractures and cavities. The irregular morphology of microcapsules reduced the ability of the wall material to retain the probiotic cells. Overall, the results showed that WPC was the biopolymer that allowed a higher ($p \leq 0.05$) retention of probiotic bacteria after encapsulation and during storage. In general, all encapsulation materials showed only a reduction of 1 log CFU/g of microcapsules immediately after the spray drying process, except the MD-WPC blend, which lost 3 log CFU/g microcapsules. The kinetics of the survival of probiotic bacteria after storage is presented by Fig. 5. Four biopolymer matrices (GA, GA-MD, MD and WPC) were capable to preserve higher than 6 log CFU/g microcapsules after two weeks of storage at 20 °C and less than 6.0% of moisture content, while WPC in combination with GA or MD retained about 6 log CFU/g microcapsules only for one week. It can be concluded that WPC and GA-MD biopolymeric matrices are suitable materials to preserve probiotic bacteria (Table 3). Interactions of WPC with the phospholipid of polar groups of probiotic membrane cells could be involved in the encapsulation system, limiting an excessive loss of water and avoiding the evaporation of water in the spray drying process. These changes of phase could cause damage to membrane probiotic cells and reduce the protection of probiotic bacteria (Santivarangkna, Higl, & Foerst, 2008).

4. Conclusions

The stability of bioactive compounds and probiotic bacteria (*Lactobacillus acidophilus*) protected by biopolymeric wall material was evaluated after the spray-drying encapsulation process and during storage. The main conclusion of the present study was that the type of encapsulation material has a strong effect in stability and encapsulation efficiency. The SEM analysis showed compact microstructure with large cavities and a coarse porous network in GA-MD powders, while a more granular structure was observed for WPC. The GA-MD blend showed the better conditions to protect bioactive compounds (TPC and TMAC). In contrast, WPC offered the better protection conditions for probiotic bacteria. FTIR analysis indicated that the modification of the structure of proteins by the effect of blackberry juice acidity could be responsible of the improved encapsulation properties by WPC blends. However, the issue deserves detailed analysis, which should be reported in a future study. In particular, the modification of the protein secondary structure by the effect of acidity and spray drying conditions should provide valuable insights on the mechanisms involved in the improved encapsulation stability by wall materials containing whey proteins.

Conflicts of interest

The authors declare no conflict of interest.

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