Contents lists available at ScienceDirect

Heliyon

Research article

Microencapsulates by spray of *Lacticaseibacillus rhamnosus* GG from fermented whole or skimmed cow's milk added with Mexican honeysuckle (*Justicia spicigera*) extract using mesquite gum as carrier agent

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A B S T R A C T

This work aimed to evaluate the effect of the addition 5°Bx Mexican honeysuckle (*Justicia spicigera*) extract (JSE) in spray dried encapsulates of whole and skimmed unfermented and fermented cow's milk with *Lacticaseibacillus rhamnosus* (LR). All samples were spray dried at 160 °C. Samples were analyzed in physical properties (moisture content, water activity (a_w), L^*, a^*, b^*, Hue, Chroma color parameters, particle size), LR content, and bioactive compounds (total anthocyanins (TA), total phenolic content (TPC), and antioxidant capacity (AC) using the DPPH assay). Results showed that the load of LR was in the range 6.79–7.44 Log_{10} (CFU/mL) cycles after fermentation, lower values were obtained when JSE was added before fermentation. In addition, LR remains after drying in fermented samples but decrease about 1 Log_{10} (CFU/mL) cycle. LR was 4.46 Log_{10} (CFU/mL) in the fermented skimmed milk-*J. spicigera* extract powder. All powders had a_w and moisture content below 0.295 and 6.51%, respectively. Color of powders depended on the moment of addition of JSE and fermentation. Powders from fermented milk were pale brownish/orangey/red (Hue = 44.91–59.7) and unfermented and *J. spicigera* extract-maltodextrin solution (12% w/v) powders were purple (Hue = 314.52–326.68). Higher particle sizes (52.3–104.7 μm) were obtained with whole milk fermented and unfermented powders. On the contrary, skimmed milk and JSE without milk protein had values in the range 15.56–44.0 μm. TPC in powders were higher (16.96–33.81 mg GAE/g powder db) compared with TA (0.27–0.64 mg Peonidin-3,5-diglucoside/g powder db). TPC increased with fermentation and remain after spray drying. The AC and TPCs were highly correlated and had antioxidant capacity of 10.18 mg TE/g powder db. The principal component analysis showed that the type of milk and fermentation separate the powders in four groups, depending on their physical and antioxidant properties. Encapsulated pigments could be used in formulations in the food industry to increase bioactive compounds and pigments in foods.

1. Introduction

Color is one of the most important sensory aspects in foods; this can influence the consumers decision and preference for their consumption. During processing for food preservation, natural pigments are easily destroyed; consequently, the color could be altered (Coultate and Blackburn, 2018), faded or totally lost. Therefore, the development of natural or artificial colorants becomes relevant in the food industry. Different pigments from important natural sources have been used for foods since they stand out for their functional properties (Lima et al., 2019; Sarı et al., 2019). Among the most natural pigments used are anthocyanins. They are responsible for the color of distinct parts of

plants (housed in the vacuoles) such as flowers, leaves, stems and seeds (Horbowicz et al., 2008; Kallam et al., 2017). Anthocyanins are soluble in polar solvents; moreover, thanks to the double bonds in their multiple aromatic rings, they may exhibit antioxidant, anticancer, antimicrobial properties, among others (Giuliani et al., 2016; Sarı et al., 2019; Zhang et al., 2019).

Various natural sources (parts of plants) have been used for the extraction, encapsulation and addition of anthocyanins for coloring foods (Jiménez-González and Guerrero-Beltrán, 2021). However, the pigments from *Justicia spicigera* (a shrub) have been studied by few researchers. The *J. spicigera* plant is native to Mexico and has been used for dyeing fabrics (Baqueiro-Peña and Guerrero-Beltrán, 2017;

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Chan-Bacab et al., 2015), make paintings, codices, murals (Arberet et al., 2021; Casanova-González et al., 2012), painting crafts (Baqueiro-Peña and Guerrero-Beltrán, 2017), and coloring *tortillas* (traditional Mexican corn flat bread) (Alvarez-Poblano et al., 2020). In addition, it has been used in traditional medicine as an anxiolytic (García-Ríos et al., 2019), anti-inflammatory (Real-Sandoval et al., 2020), analgesic (Zapata-Morales et al., 2016), antipyretic (Baqueiro-Peña and Guerrero-Beltrán, 2014), among others. Its efficacy as an antimicrobial against pathogenic bacteria and fungi (Bernardo-Mazariegos et al., 2019; Jacobo-Salcedo et al., 2011) and against cancer cells (Jacobo-Salcedo et al., 2011) has also been reported.

On the other hand, one way to fortify foods is by adding prebiotic microorganisms. Most of these probiotics belong to the group of lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium*, they are characterized by promoting health benefits when consumed and reach the intestines (Varzakas et al., 2018). One way to transport these probiotics to the intestine is by adding them to foods; however, it is necessary that they be viable during processing, storage and consumption (Shori, 2015; Syngai et al., 2016; Zhang et al., 2017). Therefore, encapsulation can help to protect probiotic microorganisms from being incorporated during processing.

During the fermentation some secondary metabolites could be generated, such as some phenolic compounds or organic acids (Filannino et al., 2018). These compounds could work as co-pigments, and forming a non-covalent complex (anthocyanin-secondary metabolite) (Sun et al., 2022). As a result, co-pigmentation prevent the degradation of anthocyanin by blocking water nucleophilic attack or the formation of chalcone structures (Sun et al., 2022). Last prevent or minimize color changes due to chemical reactions (Fan et al., 2008).

Therefore, encapsulation can help to protect probiotic microorganisms before being incorporated to the food or during processing. Encapsulation has been used to protect a large variety of compounds, including anthocyanins (Jiménez-González and Guerrero-Beltrán, 2021). This technique adds a thin layer that cover the active ingredient, extending its shelf life, facilitate the incorporation to the food, and may the reduce cost of storage. The encapsulation of anthocyanins-rich-probiotic bacteria systems has been reported only by few authors. Bolea et al. (2021) encapsulated anthocyanins from black rice (*Oryza sativa* L.) mixed with *Lactobacillus paracasei*. They observed that the black rice extract could be mixed with lactic acid bacteria. Anekella and Orsat (2013) performed the encapsulation of raspberry juice with *L.* rhamnosus. However, in the two studies, researchers did not perform the growth and development of probiotics in anthocyanin extract before encapsulation. Which is why, the development of encapsulates of probiotic-pigments mixtures could be an attractive way to deliver bioactive compounds into the gastrointestinal tract.

The objective of this work was to obtain encapsulates of fermented and unfermented whole and skimmed milk containing *Lactobacillus rhamnosus* GG and pigments from *Justicia spicigera* by spray drying.

2. Materials and methods

2.1. Materials

Leaves of *Justicia spicigera* were purchased at a local market in the city of Puebla, Mexico. Ultra-pasteurized commercial cow's milk (30 g of protein/L) with 30 or 5 g of fat/L (Santa Clara, The Coca-Cola Company, Mexico) was used for the fermentation and encapsulation of the pigments. Maltodextrin was purchased from Ingredion S.A. de C.V. (Mexico). Mesquite (*Prosopis* spp.) gum, harvested from mesquites of the northern Mexico, was purified according to the procedure reported by Beristain et al. (2002).

2.2. Justicia spicigera concentrated extracts

J. spicigera leaves were dried for 48 h at room temperature (25 ± 2 °C) and then ground. 0.3 g of powder was mixed with 25 mL of 50% (v/v)

ethanol. The extraction of pigments was conducted for 2 h in the dark with agitation according to the Baqueiro-Peña and Guerrero-Beltrán (2017) methodology. The powder-ethanol mixture was centrifuged at 4, 500 rpm for 10 min (4 °C) for removing solids. The centrifuged extract was concentrated in a Büchi rotary evaporator (Büchi, Switzerland) at 45 °C and 54 cmHg until obtaining a concentrate of about 5% total soluble solids. The final concentrate was adjusted at 5°Bx and stored at 4 °C for later use.

2.3. Preparation of inoculum

Lacticaseibacillus rhamnosus GG (ATCC 53103) was acquired from the Food Microbiology Laboratory Collection at the Universidad de las Americas Puebla (UDLAP) (Cholula, Puebla, Mexico). *L. rhamnosus* was previously cultured in 100 mL of sterilized de Man, Rogosa and Sharpe (MRS) broth (MERCK, Germany) until reaching the growing early stationary phase; therefore, it was mixed with glycerol in a 1:1 (v/v) ratio. The mixture was placed in vials of 1 mL and stored at -20 °C until use. Before using the inoculum for fermentation, a vial of inoculum of *L. rhamnosus* was thawed and added to 50 mL of whole milk and incubated at 37 °C for 3 days (inoculum for fermentation). The microbial counts of lactic acid bacteria in MRS agar were performed to determine the number of colonies in the inoculum: 7.26 ± 0.20 log cycles (CFU/mL).

2.4. Treatments

The following treatments were performed: 1) fermented whole milk-*J. spicigera* extract (FWJ), 2) fermented skimmed milk-*J. spicigera* extract (FSJ), 3) fermented whole milk with subsequent addition of *J. spicigera* extract (FWSAJ), 4) fermented skimmed milk with subsequent addition of *J. spicigera* extract (FSSAJ), 5) unfermented whole milk-*J. spicigera* extract (WJ), 6) unfermented skimmed milk-*J. spicigera* extract (SJ), and 7) *J. spicigera* extract-maltodextrin solution (12% w/v) (JM). Each treatment was prepared in a 1:3 v/v ratio (*J. spicigera* extract: fermented or unfermented milk or maltodextrin solution). 3% (w/v) of mesquite gum was added to all treatments as carrier agent.

2.5. Fermentation

Fermentation was conducted in four treatments: FWJ, FSJ, FWSAJ, FSSAJ. The fermentation process was conducted adding the inoculum in a 1:10 (v/v) ratio. The inoculated milk, with or without *J. spicigera* extract, was placed in sterile bottles and fermented for 3 days at 37 °C with an agitation of 80 rpm using a shaking bath. The fermentation was conducted in the dark to avoid the degradation of the pigments that were added to the milk before fermentation.

2.6. Spray drying

Spray drying of 200 mL of each treatment was conducted in a Mini spray-dryer B-290 (Büchi, Switzerland) at 160 ± 3 and 93 ± 3 °C of inlet and outlet temperatures, respectively. Drying was conducted at 3.5 m/s (100%) and 1.05 bar at a feed flow of 3.21 ± 0.42 mL/min using a 0.7 mm spray nozzle.

2.7. Characterization of encapsulates

2.7.1. Moisture content and water activity (a_w)

Moisture content was determined by drying 2 g of powder at 75 ± 2 °C at vacuum conditions, until reaching constant weight, for avoiding burning of the sample, avoid the damage of chemicals such as antioxidants, pigments among other compounds and more important, avoid to "kill" or inhibit the bacteria. Water activity (a_w) was determined using a hygrometer (AQUALAB 4TE, Pullman, WA, USA).

2.7.2. Color

The $L*$, $a*$, and $b*$ color parameters were measured using a Konica Minolta (Osaka, Japan) portable colorimeter in the transmittance mode to calculate Hue (Eq. 1) and chroma (Eq. 2) values.

$$Hue = \tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{1}$$

$$Croma = (a^{*2} + b^{*2})^{1/2} \tag{2}$$

2.7.3. Particle size

The particle size distribution was determined using a laser diffraction particle size analyzer (Bluewave, Microtrac, USA). This is based on the intensity of light scattered when a laser beam passes through the sample. The measurement was made on approximately one gram of dry sample. The d_{10}, d_{50} and d_{90} values were determined automatically by the program of the equipment; these values were used for calculating size distribution (Eq. 3). The De Brouckere mean diameter (D[4, 3]) (the mean of a particle size distribution) and the Sauter Mean Diameter (D[3, 2] (an average of particle size) were calculated by the software of the equipment.

$$Span = \frac{d90 - d10}{d50} \tag{3}$$

2.8. Lacticaseibacillus rhamnosus counts

The contents of *L. rhamnosus* were determined by plate count using MRS agar (MERCK, Germany). The plates were kept in anaerobic jars (AnaeroJar, Oxoid, Thermo Scientific) for 48 h at 37 °C.

2.9. Bioactive compounds

For the determination of bioactive compounds, 100 mg of each powder was dissolved in 10 mL of distilled water, kept under agitation using a vortex until complete homogeneity, and centrifuged at 5,000 rpm for 5 min at 4 °C.

2.9.1. Total phenolic compounds

The amount of total phenolic compounds was determined using the method of Singleton and Rossi (1965) and Singleton et al. (1999) with some modifications. 200 µL of sample were mixed with 1000 µL of Folin-Ciocalteu reagent (0.4 M aqueous solution) and allow to stand for 3 min. Then 1000 µL of Na_2CO_3 solution (5% w/v) was added and kept in the dark for 30 min. The absorbance of the samples was read at 765 nm using a Shimadzu UV-1900i spectrophotometer (Tokyo, Japan). To calculate the concentration, a standard curve ($R^2 = 0.987$) was made with different concentrations of gallic acid (0–50 ppm). The content of total phenolic compounds was calculated as mg gallic acid equivalents (GAE) per gram of sample (mg GAE/g) in dry basis (db) and was calculated using Eq. (4).

$$TPC\left(\frac{mg\ GAE}{g}\right) = \left(\frac{Abs - b}{m}\right) \times DF \tag{4}$$

where Abs is the absorbance of the sample, b the intercept (0.0064), m the slope (0.0008 1/ppm), and DF is the dilution factor.

2.9.2. Total monomeric anthocyanins

The content of total monomeric anthocyanins was assessed using the differential pH spectrophotometric method (Lee et al., 2005). Briefly, one mL of sample was mixed with 4 mL of two different buffers separately (0.025M potassium chloride, pH 1.0, and 0.4M acetate buffer, pH 4.5). Mixtures were allowed to stand for 15 min in the dark at room temperature. The absorbance of each sample was then read at the different pHs

at 520 and 700 nm using a Shimadzu UV-1900i spectrophotometer (Tokyo, Japan). The absorbances were calculated using Eq. (5).

$$Abbs = (Abs_{520} - Abs_{700})_{pH\ 1.0} - (Abs_{520} - Abs_{700})_{pH\ 4.5} \tag{5}$$

The total monomeric anthocyanin content was calculated as the equivalents of peonidin-3,5-diglucoside (Pnd-3,5-diglu) per gram of sample (mg Pnd-3,5-diglu/g) in dry basis (db) using Eq. (6).

$$AT\left(\frac{mg\ Pnd - 3,5 - diglu}{g}\right) = \frac{Abs \times MW \times DF \times V \times 1000}{\varepsilon \times l} \tag{6}$$

where Abs is the difference of absorbances, MW the molecular weight of Pnd-3,5-diglu (625.4 g/mol), DF is the dilution factor, V is the volume of the sample, 1000 is a conversion factor to mg, ε the molar absorptivity coefficient (36.654 L/cm mol) and l the pathway of light in the cell.

2.9.3. Antioxidant capacity

The antioxidant capacity (AC) of all powders was determined by the inhibition of the DPPH$^\bullet$ radical (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, MO, USA) method. 100 µL of each solution of powder was mixed with 2000 µL of DPPH ethanolic (0.04 M) solution. The DDPH-solution mixtures were kept in the dark for 30 min at room temperature. Subsequently, the absorbance at 517 nm was read in a Shimadzu UV-1900i spectrophotometer (Tokyo, Japan). The absorbance values were used to calculate the percentage of inhibition (I%) with Eq. (7).

$$I\% = \left(\frac{Abs_{DPPH} - Abs_{sample}}{Abs_{DPPH}}\right) \times 100 \tag{7}$$

where Abs_{DPPH} is the absorbance value of 2,000 µL of DPPH with 100 µL of water, Abs_{Sample} is the absorbance of the DPPH-sample mixture after 30 min. The results of the antioxidant capacity were calculated as mg of Trolox equivalents (TE) per gram of sample (mg TE/g) in dry basis (db) and were calculated with Eq. (8).

$$AC\left(\frac{mgTE}{g}\right) = \left(\frac{I\% - b}{m}\right) \times DF \tag{8}$$

where b is the intercept (1.107), m the slope (0.780 1/ppm) of a standard curve ($R^2 = 0.990$) with different concentrations of Trolox (0–100 ppm), and DF is the sample dilution factor.

2.10. Statistical analysis

All results were expressed as the mean ± standard deviation. Analysis of variance (ANOVA) was used to establish significant differences (p < 0.05) within means of samples. The differences within the means of the samples were establish with the Tukey test. All statistical tests were performed using Minitab 18 software (Minitab LLC, USA). Principal component analysis (PCA) was carried out to observe the relationship between microbiological, physical (moisture content, a_w, $L*$, $a*$, $b*$, Hue, Chroma and particle size) and antioxidant (total monomeric anthocyanins, total phenolic compounds and antioxidant capacity) characteristics. The PCA analysis was based on the Pearson's correlation and was performed in R version 4.1.1 and the programming environment Rstudio (version 1.4.1717, USA) using normalized data with the z-score normalization method (value = (value - average)/standard deviation). All graphs were made with KyPlot 6.0 software (Kyens Lab Inc, Japan) except the Biplot of PCA.

3. Results and discussion

3.1. Lacticaseibacillus rhamnosus retention

Table 1 shows the counts of *Lacticaseibacillus rhamnosus* before and after encapsulation. It can be seen that *L. rhamnosus* carried fermentation

Table 1. Content of *Lacticaseibacillus rhamnosus* in solutions (before drying) and microencapsulated (powders) from fermented and unfermented milk added with *J. spicigera* extracts.

Sample	Before drying	After drying
	(Liquid mixture)	(Powder)
	Log_{10} (CFU/mL)	Log_{10} (CFU/g)
FWJ[a]	6.81 ± 0.11bA	6.07 ± 0.35aB
FSJ[b]	6.79 ± 0.2bA	4.46 ± 0.12bB
FWSAJ[c]	7.28 ± 0.05aA	6.31 ± 0.29aB
FSSAJ[d]	7.44 ± 0.12aA	6.38 ± 0.35aB
WJ[e]	<1.0	<1.0
SJ[f]	<1.0	<1.0
JM[g]	<1.0	<1.0

[a] FWJ: fermented whole milk-*J. spicigera* extract.

[b] FSJ: fermented skimmed milk-*J. spicigera* extract.

[c] FWSAJ: fermented whole milk with subsequent addition of *J. spicigera* extract.

[d] FSSAJ: fermented skimmed milk with subsequent addition of *J. spicigera* extract.

[e] WJ: unfermented whole milk-*J. spicigera* extract.

[f] SJ: unfermented skimmed milk-*J. spicigera* extract.

[g] JM: *J. spicigera* extract-maltodextrin solution (12% w/v). Lowercase letters in the same column mean significant differences within samples. Capital letters in the same row mean significant differences before and after drying.

out even with the addition of the concentrated extract of *J. spicigera*. However, a greater amount of *L. rhamnosus* was observed when the fermentation was carried out without the addition of the *J. spicigera* extract and its subsequent incorporation. This could be due to the anthocyanins (Awad et al., 2015) and other phenolic compounds found in the extract of *J. spicigera* (Baqueiro-Peña and Guerrero-Beltrán, 2017) that could inhibit or slow down the growth of *L. rhamnosus*. Similar to that, Sun et al. (2018) observed that the increase in the concentration of anthocyanins extracted from sweet purple potato (*Ipomoea batatas* L.) decreased the growth of lactic acid bacteria such as *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Bifidobacterium infantis* and *Lactobacillus acidophilus*. In addition, it has been observed that *J. spicigera* extracts can inhibit the growth of Gram-negative and Gram-positive pathogenic bacteria and some molds (Jacobo-Salcedo et al., 2011). Also, it was not observed any influence by the type of milk (whole and skimmed) on the growth of *L. rhamnosus*. On the other hand, the milk that was not fermented, but added with *J. spicigera* extract, did not show any growth of bacteria (<10 CFU/mL or g) in the MRS culture media. Microencapsulation might protect cells from external factors and prevents the loss of probiotic cells during intestinal passage due to bile acids (Burgain et al., 2015; Kil et al., 2020). In addition, it has been observed that the use of dairy base protein for the encapsulation of probiotics might have a significant improvement in the survival and tolerance of bacteria to bile acids (Kil et al., 2020). A greater reduction of probiotic bacteria was observed in skimmed milk (FSJ) after spry drying. This may be because of the glass transition temperature, which may change due to the fat content (Hu et al., 2009); therefore, a change in heat capacity (Cp) may occur, requiring greater amount of energy to carry it out the evaporation for obtaining the particles. However, in the four fermented samples (FWJ, FSJ, FWSAJ, FSSAJ) enriched with *J. spicigera* extract, the probiotic bacteria were maintained.

3.2. Characteristics of powders

3.2.1. Moisture content and water activity

Table 2 shows the moisture content and a_w values of the powders. The moisture content and a_w, for fermented and unfermented milk-based powders, were in the ranges 1.39–6.51% and 0.201–0.295, respectively. Slight variations (p < 0.05) in the two characteristics were observed between powders of fermented and unfermented milk. In powders of unfermented milk, the moisture content was lower compared to that of powders of fermented milk. This coincides with that reported in previous works, where the moisture value of powdered milk is around 1.5%; however, the moisture content may varies depending on the drying temperature or the concentration of solids in the milk (Langrish et al., 2006; Shrestha et al., 2008). On the other hand, yogurt in powder contains 3.5–5.0% moisture content, depending on the solids content (Sunitha et al., 2016), or 3.98–7.17%, depending on the drying conditions (Koc et al., 2010). In this study, a a_w value of 0.468 ± 0.034 in the powder JM was observed, this value of a_w may be due to the high content of solids in the feed mixture before spray drying. Similar behavior has been observed by da Costa et al. (2015), they studied the effect of the concentration of the encapsulating agent in Swiss cheese bioaroma and observed that the a_w increased and the humidity decreased. Low a_w values indicate that products may be more stable to oxidation (Khwanpruk et al., 2018) or any other physical or microbiological change. Also, low a_w values can increase the shelf life of probiotics (Muhammad et al., 2021).

Table 2. Physical properties of microencapsulates of *Lacticaseibacillus rhamnosus* from fermented or unfermented milks added with *J. spicigera* extract.

Sample	Moisture content (%)	a_w	L*	a*	b*	Hue	Chroma
FWJ[a]	3.55 ± 0.73bc	0.272 ± 0.001b	68.40 ± 0.64a	9.36 ± 0.19b	13.66 ± 0.23b	55.6 ± 0.1d	16.56 ± 0.29c
FSJ[b]	6.51 ± 0.46a	0.256 ± 0.003bc	63.74 ± 3.37ab	9.42 ± 0.42b	16.13 ± 1.19a	59.7 ± 0.73c	18.68 ± 1.24b
FWSAJ[c]	2.20 ± 0.02cd	0.201 ± 0.008d	59.66 ± 0.08b	14.97 ± 0.01a	15.35 ± 0.04ab	45.71 ± 0.04e	21.44 ± 0.04a
FSSAJ[d]	4.93 ± 0.60ab	0.288 ± 0.004b	63.37 ± 0.01ab	14.36 ± 0.23a	14.31 ± 0.27ab	44.91 ± 0.07e	20.27 ± 0.35ab
WJ[e]	1.39 ± 1.06d	0.222 ± 0.021cd	51.65 ± 0.37c	6.12 ± 0.09cd	-4.02 ± 0.04c	326.68 ± 0.12a	7.32 ± 0.1e
SJ[f]	1.90 ± 0.89cd	0.295 ± 0.009b	50.62 ± 0.16c	6.75 ± 0.04cd	-6.67 ± 0.01d	315.34 ± 0.18b	9.49 ± 0.03d
JM[g]	3.01 ± 0.29cd	0.468 ± 0.034a	52.38 ± 1.46c	5.66 ± 0.09d	-5.75 ± 0.04cd	314.52 ± 0.25b	8.06 ± 0.09de

L* = luminosity (0 = black, white = 100). a* = red (+)-green (-) color parameter. b* = blue (-)-yellow (+) color parameter. Hue = tone. Chroma = color saturation. Lowercase letters in the same column mean significant differences within samples.

[a] FWJ: fermented whole milk-*J. spicigera* extract.

[b] FSJ: fermented skimmed milk-*J. spicigera* extract.

[c] FWSAJ: fermented whole milk with subsequent addition of *J. spicigera* extract.

[d] FSSAJ: fermented skimmed milk with subsequent addition of *J. spicigera* extract.

[e] WJ: unfermented whole milk-*J. spicigera* extract.

[f] SJ: unfermented skimmed milk-*J. spicigera* extract.

[g] JM: *J. spicigera* extract-maltodextrin solution (12% w/v).
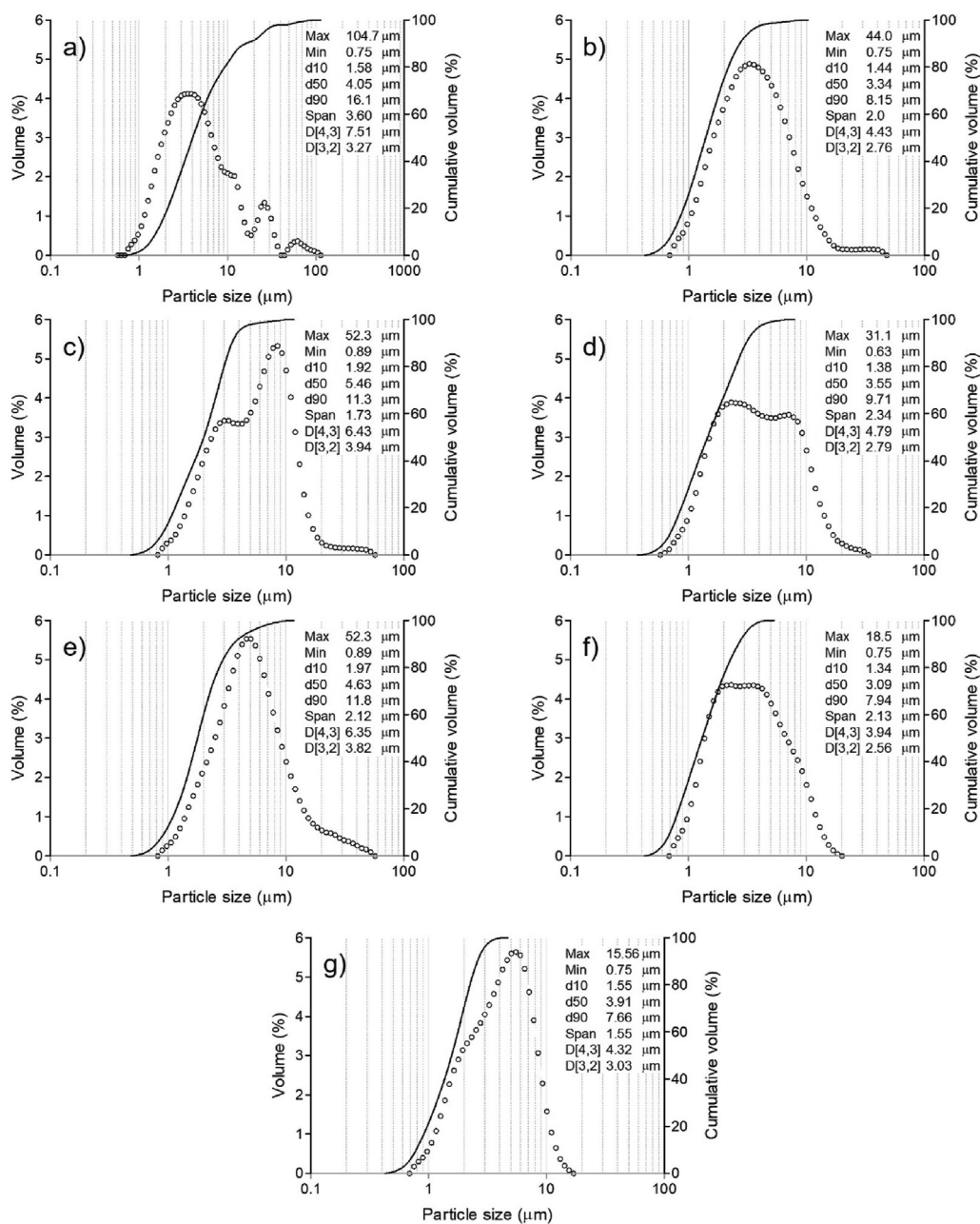
Figure 1. Particle size distribution of spray dried microencapsulates from fermented or unfermented milk containing *J. spicigera* extract. a) FWJ, b) FSJ, c) FWSAJ, d) FSSAJ, e) WJ, f) SJ, g) JM.

3.2.2. Color characteristics

The color of the obtained powders was one of the most remarkable characteristics since the fermentation process generated a change in the color of the powders. The color parameters ($L*$, $a*$, $b*$, Hue and Chroma) are shown in Table 2. The powders from fermented milks (FWJ, FSJ, FWSAJ and FSSAJ) were significantly ($p < 0.05$) more luminous ($L*$), compared with those that were not (WJ, SJ, and JM), which were not significant ($p > 0.05$) in lightness. On the other hand, the values of $a*$ were significantly different ($p < 0.05$), depending on the moment of addition of the *J. spicigera* extracts and the fermentation (if they were or not fermented). All values of $a*$ were in the positive segment of the color space; therefore, a paler red color was observed. Regarding the $b*$ color parameter, the encapsulates from fermented milks (FWJ, FSJ, FWSAJ and FSSAJ) were in the yellow positive segment of the color space; however, the encapsulates obtained without fermentation presented negative

values; this place them in the blue segment of the color space. The samples that were fermented with *J. spicigera* extracts (FWJ, FSJ) or with their subsequent addition (FWSAJ and FSSAJ) presented both $a*$ and $b*$ positive values, showing different hues in the 0–90° (red to yellow) of the color space: pale red-light brown for the FWJ powder; slight orange color, due to saturation, for FSJ powder; slightly coppery red or pale brick red for the FWSAJ and FSSAJ powders. Consequently, the moment of addition of the *J. spicigera* extracts, before or after fermentation, affects the final color of the powder. On the other hand, for samples WJ, SJ and JM, which were not subjected to any fermentation process, purple hues were observed in the 270–360° (blue to red) of the color space, similar to the color of ethanol-water extracts of *J. spicigera* previously reported by Baqueiro-Peña and Guerrero-Beltrán (2017). All powders presented a milky appearance due to proteins of the milk. About purity or chroma (C), powders from fermented milks had purity in the range 16.56–21.44

Table 3. Bioactive compounds of microencapsulates of *Lacticaseibacillus rhamnosus* from fermented or unfermented milks added with *J. spicigera* extract.

Sample	TPC[a]	Total anthocyanins[b]	Antioxidante capacity[c]
FWJ[d]	33.81 ± 2.61a	0.27 ± 0.05b	20.41 ± 2.64a
FSJ[e]	22.27 ± 2.65bc	0.52 ± 0.01ab	17.78 ± 1.48ab
FWSAJ[f]	21.48 ± 1.27bcd	0.29 ± 0.18b	15.46 ± 0.13abc
FSSAJ[g]	18.52 ± 0.7cd	0.62 ± 0.02a	14.13 ± 0.48bc
WJ[h]	19.76 ± 0.46bcd	0.46 ± 0.17ab	11.5 ± 2.12c
SJ[i]	23.65 ± 1.44b	0.64 ± 0.14a	13.84 ± 1.45bc
JM[j]	16.96 ± 1.41 d	0.34 ± 0.08 ab	10.18 ± 0.18 c

[a] TPC, total phenolic compounds in mg GAE/g powder in db.
[b] mg Pnd-3,5-diglu/g powder in db.
[c] mg TE/g powder in db. Lowercase letters in the same column mean significant differences within samples.
[d] FWJ: fermented whole milk-*J. spicigera* extract.
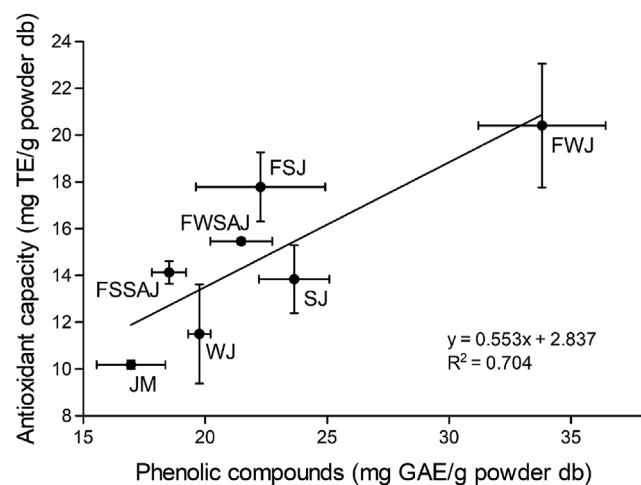[e] FSJ: fermented skimmed milk-*J. spicigera* extract.
[f] FWSAJ: fermented whole milk with subsequent addition of *J. spicigera* extract.
[g] FSSAJ: fermented skimmed milk with subsequent addition of *J. spicigera* extract.
[h] WJ: unfermented whole milk-*J. spicigera* extract.
[i] SJ: unfermented skimmed milk-*J. spicigera* extract.
[j] JM: *J. spicigera* extract-maltodextrin solution (12% w/v).

Figure 2. Correlation between antioxidant activity and phenolic compounds of microencapsulated from fermented or unfermented milk containing *J. spicigera* extract.

(purest) than powders from unfermented milks (WJ, SJ) or JM: purity in the range (7.32–9.49), which depends on the lightness (0–100 of the sample (Table 2)).

3.2.3. Particle size

Figure 1 shows the particle size distribution, the maximum and minimum diameter, and the values d_{10}, d_{50}, d_{90}, corresponding to 10, 50 and 90%, respectively, of the volumetric mean diameter, and the accumulated particles. The D[4, 3] and D[3, 2] diameters correspond to the volume-average diameter (De Brouckere mean diameter) and the surface-volume mean diameter (Sauter mean diameter), respectively. To indicate the homogeneity of the particles, the Span value was used.

The minimum and maximum diameters were between 0.63 and 104.7 µm for all powders, values corresponding to FSSAJ (Figure 1d) and FWJ (Figure 1a) powders, respectively. The powder FWJ presented a multimodal distribution, showing a peak between 40 and 100 µm (Figure 1a), characteristic of particles that agglomerates (Ferrari et al., 2012; Zotarelli

et al., 2017). The powders FWSAJ (Figure 1c) and FSSAJ (Figure 1d) showed bimodal distributions. In the FWSAJ powder, the highest peak was observed between 8-9 µm. In the FSSAJ powder, the highest peak was observed around 2 µm. The rest of the encapsulates showed a unimodal distribution. Regarding the d_{10}, d_{50}, d_{90}, values, the encapsulates obtained from whole milk had higher values compared to the encapsulates from skimmed milk; the former being similar to the JM encapsulate (without proteins of milk). Elversson et al. (2003) mentions that the particle size is influenced by the diameter of the nozzle, the concentration of the solution, the flow of atomizing air, the speed of feeding, and the size of the droplet. Since most of these parameters were kept constant, it can be inferred that both the fermentation process and the fat content of the milk influenced the particle size; this was observed in the D[4, 3] and D[3, 2] diameters, where the encapsulates from skimmed milk presented smaller sizes. In powders from skimmed milk, fermentation generated encapsulates with larger particles. On the other hand, in general, the span values were between 1.55 and 2.34, except for the FWJ encapsulate which value was higher (3.60), indicating that the encapsulation was more homogeneous in particle size (da Costa et al., 2015).

3.3. Bioactive compounds of powders

Table 3 shows antioxidant capacity, total phenolic compounds and anthocyanins contents for all powders.

3.3.1. Total phenolic compounds

The amount of total phenolic compounds in powders from fermented milks (Table 3) was in the range 18.52–33.81 mg GAE/g powder. The TPC in powders comes from *J. spicigera* extracts mainly; however, the presence or generation of other compounds during fermentation might appear; for example, compounds with one or more rings such as phenolic acids (Curiel et al., 2015). The same has been observed by other researchers in the fermentation of anthocyanins from different sources using lactic acid bacteria (Curiel et al., 2015; Yan et al., 2019). In this study, significant differences ($p < 0.05$) in total phenolic compounds were observed within powders from distinct types of milks due to the fermentation process. The quantity of phenolic compounds was slightly higher in all fermented milk powders (FWJ, FSJ, FWSAJ, FSSAJ), especially in the powders obtained with whole milk (FWJ, FWSAJ). Whole milk contains a slightly higher amounts of phenolic compounds compared to skimmed milk (Tsen et al., 2014). On the other hand, powders from unfermented milk such as WJ (19.76 ± 0.46 mg GAE/g powder) have lower amounts of phenolic compounds compared to SJ (23.65 ± 1.44 mg GAE/g powder), although the difference was not significant ($p > 0.05$). This can be attributed to the thermal degradation and less protection of the compounds. However, the phenolic compounds content was higher in all the powders compared to those of the powder JM.

3.3.2. Anthocyanins content

The anthocyanin content in microencapsulates were in the range 0.27–0.64 mg Pnd-3,5-diglu/g of powder (Table 3). The highest concentration of anthocyanins was observed in the SJ and FSSAJ powders. The powders with the lowest anthocyanin content were FWJ and FWSAJ; so that, the type of milk affected the anthocyanins content to a greater extent, not the fermentation process. In this study, slight significant differences ($p < 0.05$) were observed in the concentration of anthocyanins within all powders, especially depending on the type of milk, whole or skimmed. It is observed that the powders from whole milk had higher degradation of anthocyanins which could be due to different reasons: a) a higher heat flux during dehydration of the droplet and/or differences in specific heat (Cp) since skimmed milk does not require latent heat for phase change (Hu et al ., 2009); b) the same polarity of the compounds since anthocyanins are highly polar and milk with high fat content would give them less stability. In addition, it has been reported that casein proteins can interact with anthocyanins through hydrogen bonding or
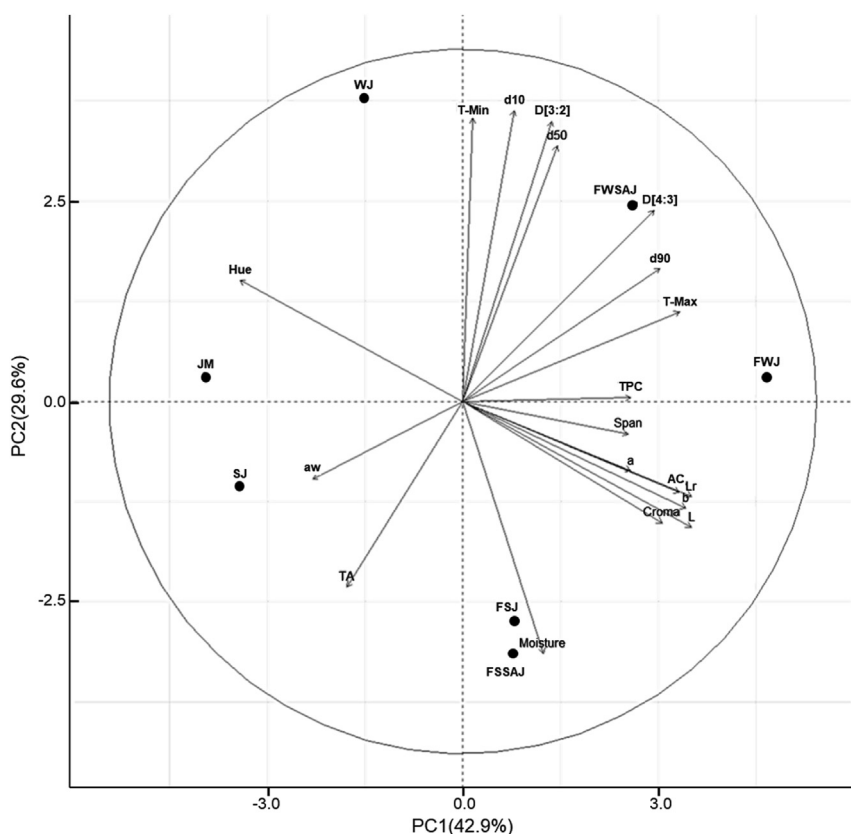
Figure 3. Biplot of principal component analysis (PCA) for FWJ, FSJ, FWSAJ, FSSAJ, WJ, SJ, JM powders. The variables include physical (moisture content (Moisture), water activity (aw), luminosity (L), red-green (a); yellow-blue (b), tone (Hue), color saturation (Chroma)), particle (minimum particle size (T-min), maximum particle size (T-max), d10, d50 and d90, Span, particle size distributions (D[4, 3] and D[3, 2])), antioxidant (antioxidant capacity (AC), total phenolics content (TPC), total anthocyanins (TA)), and microbiological (*Lactobacillus rhamnosus* (Lr)) characteristics. Each line represents the intensity or magnitude of each component.

hydrophobic reactions, forming complexes that make them more stable to temperature, oxidation, and light (He et al., 2016). Awad et al. (2015) reported several types of anthocyanins in extracts of *J. spicigera* obtained with ethyl acetate, the highest concentration being peonidin 3,5-diglucoside (Pnd-3,5-diglu), followed by malvidin 3,5-diglucoside. However, the concentration of the different anthocyanins in plant parts can vary depending on the polarity of the solvents (Bosiljkov et al., 2017; Bubalo et al., 2016) used for extractions.

3.3.3. Antioxidant capacity

The antioxidant capacity for powders from fermented milks was in the range 14.13–20.41 mg TE/g powder. For unfermented powders and JM was in the range 10.18–13.84 mg GAE/g powder (Table 3). Significant differences (p < 0.05) were observed in antioxidant capacity within all powders. The fermentation process increased the antioxidant capacity of the powders; this has been observed by other authors, where fermentation with lactic acid bacteria increases the antioxidant capacity (Curiel et al., 2015; Hunaefi et al., 2013; Yan et al., 2019). Changes in antioxidant capacity could be related to different ways: i) the formation of bioactive peptides from proteins, which has related to metal ion chelation, free radical scavenging and singlet oxygen quenching (Pihlanto, 2013; Tadesse and Emire, 2020), ii) the influenced by other phenolic compounds, in addition to anthocyanins (Figure 2), iii) presence of milk caseins (Sharma et al., 2008), and iv) some reactions that occurred during drying, such as Maillard reactions (Perusko et al., 2021; Wang et al., 2011), due to the formation of melanoidins (Wang et al., 2011), influence the antioxidant capacity of powders. Which becomes evident compared to the JM powder.

3.4. Principal components analysis (PCA)

All data described above were analyzed with statistical tools to observe differences due to the formulation in the microcapsules (Figure 3). The PCA was carried out to establish a relationship between

the type of milk, fermentation and the moment of addition of *J. spicigera* extract (anthocyanins) and the relationship of the microbiological, physical and antioxidant characteristics of the microencapsulates obtained by spray drying. Based on the microbiological, physical and antioxidant characteristics, data of powders can be placed in 4 groups according to the cluster analysis. In the first group are found the data of the FWSAJ and FWJ powders with 52.73% similitude, in the second group the data of the FSJ and FSSAJ powders (89.45% similitude), in the third group are found the data of the JM and SJ powders (80.79% similitude), and data of WJ are in the fourth group. The results obtained by the PCA showed that the variables could be reduced to two components, which explained 72.5% of the variability of experimental data. The first component (PC1) represents 42.9% of the variability, while the second component (PC2) represents 29.6%. PC1 is represented by some color parameters (*L*, b**, Hue, Chroma), particle size (maximum diameter, d_{90}, D[4,3]), and antioxidant capacity. PC2 showed a correlation with particle size (minimum diameter, d_{10}, d_{50}, D[3, 2]) and moisture.

Figure 3 also shows that the antioxidant capacity is correlated with the *a** color parameter and the content of *L. rhamnosus* (or the fermentation process). The foregoing may be due to the fact that the greater the color saturation, the greater the number of compounds with antioxidant capacity or changes in the structure of the molecules due to the pH of the medium: at acid pHs (resulting from fermentation) the flavylium form predominates and is the one that is related to antioxidant capacity (Tena et al., 2020). In addition, the fermentation process generates other compounds with antioxidant capacity.

On the other hand, an inverse relationship between particle size (especially small particles) and moisture content was observed. A higher number of small particles represents a higher surface area and higher humid surface (Hann and Stražišar, 2007). The a_w was inversely correlated with particle size (especially large particles). This can be explained due to the fact that fine particles tend to join together by means of van der Waals or cohesive forces, which could limit the available water on the surface of the particle. It is also observed that the FSJ and FSSAJ powders

have high moisture content values, low a_w and smaller particle sizes than the other powders. FWSAJ and FWJ powders were characterized by larger particle sizes and lower moisture contents than the FSJ and FSSAJ powders.

Meanwhile, as mentioned above, the antioxidant capacity was influenced by the total content of phenols (Figure 2) and not only by anthocyanins. One of the powders that presented large content of phenols was the FWJ powder (Figure 3).

Finally, the powders from fermented milks were found on the opposite side were unfermented or JM were, indicating that they presented different characteristics, specially in the Hue parameter, since WJ, SJ and JM had values around 320°. FWSAJ, FWJ, FSJ, FSSAJ powders from fermented samples had Hue values close to 55°.

4. Conclusions

All powders added with extracts that were fermented (FWJ and FSJ) and non-fermented (FWSAJ and FSSAJ) retained more than 4 Log_{10} of *L. rhamnosus* (CFU/g) at the end of the process. The addition of *J. spicigera* extracts after fermentation increased the growth of *L. rhamnosus*. The fermentation of milk changed the hue of powders from purple (without fermentation) to a reddish hue because the pH modified the anthocyanins structures. All powders (except JM) could be highly stable due to the low water activity ($a_w < 0.30$). The particle size of powder showed low values in powders from low fat in milk. The low fat content in milk increased the anthocyanin content in powders. The FWJ powder had the highest total phenolic compounds content. The Principal Component Analysis showed that the type of milk and the fermentation process divided the powders into four groups due to their physical and antioxidant properties and between total phenolic compounds and antioxidant capacity. It was observed that there is a direct correlation between color and antioxidant capacity and an inverse correlation between particle size and moisture and a_w.

Declarations

Author contribution statement

Jiménez-Gonzalez: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data, Wrote the paper.

Guerrero-Beltrán: Analyzed and interpreted the data; Contributed reagents, materials analysis tools or data; Wrote the paper.

Funding statement

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Alvarez-Poblano, L., Roman-Guerrero, A., Vernon-Carter, E.J., Alvarez-Ramirez, J., 2020. Exogenous addition of muicle (*Justicia spicigera* Schechtendal) extract to white maize tortillas affects the antioxidant activity, texture, color, and in vitro starch digestibility. LWT 133, 110120.

Anekella, K., Orsat, V., 2013. Optimization of microencapsulation of probiotics in raspberry juice by spray drying. LWT–Food Sci. Technol. 50 (1), 17–24.

Arberet, L., Pottier, F., Michelin, A., Nowik, W., Bellot-Gurlet, L., Andraud, C., 2021. Spectral characterisation of a traditional Mesoamerican dye: relationship between in situ identification on the 16th century Codex Borbonicus manuscript and composition of *Justicia spicigera* plant extract. Analyst 146 (8), 2520–2530.

Awad, N.E., Abdelkawy, M.A., Hamed, M.A., Souleman, A.M.A., Abdelrahman, E.H., Ramadan, N.S., 2015. Antioxidant and hepatoprotective effects of *Justicia spicigera* ethyl acetate fraction and characterization of its anthocyanin content. Int. J. Pharm. Pharmaceut. Sci. 7 (8), 91–96.

Baqueiro-Peña, I., Guerrero-Beltrán, J.A., 2014. Uses of *Justicia spicigera* in medicine and as a source of pigments. Funct. Foods Health Dis. 4 (9), 401.

Baqueiro-Peña, I., Guerrero-Beltrán, J.Á., 2017. Physicochemical and antioxidant characterization of *Justicia spicigera*. Food Chem. 218, 305–312.

Beristain, C.I., Azuara, E., Vernon-Carter, E.J., 2002. Effect of water activity on the stability to oxidation of spray-dried encapsulated orange peel oil using mesquite gum (*Prosopis Juliflora*) as wall material. J. Food Sci. 67 (1), 206–211.

Bernardo-Mazariegos, E., Valdez-Salas, B., González-Mendoza, D., Abdelmoteleb, A., Tzintzun Camacho, O., Ceceña Duran, C., Gutiérrez-Miceli, F., 2019. Silver nanoparticles from *Justicia spicigera* and their antimicrobial potentialities in the biocontrol of foodborne bacteria and phytopathogenic fungi. Rev. Argent. Microbiol. 51 (2), 103–109.

Bolea, C.-A., Cotârleţ, M., Enachi, E., Barbu, V., Stănciuc, N., 2021. Co-Microencapsulated black rice anthocyanins and lactic acid bacteria: evidence on powders profile and in vitro digestion. Molecules 26 (9), 2579.

Bosiljkov, T., Dujmić, F., Cvjetko Bubalo, M., Hribar, J., Vidrih, R., Brnčić, M., Zlatic, E., Radojčić Redovniković, I., Jokić, S., 2017. Natural deep eutectic solvents and ultrasound-assisted extraction: green approaches for extraction of wine lees anthocyanins. Food Bioprod. Process. 102, 195–203.

Bubalo, M.C., Ćurko, N., Tomašević, M., Kovačević Ganić, K., Radojčić Redovniković, I., 2016. Green extraction of grape skin phenolics by using deep eutectic solvents. Food Chem. 200, 159–166.

Burgain, J., Corgneau, M., Scher, J., Gaiani, C., 2015. Encapsulation of probiotics in milk protein microcapsules. In: Sagis, L.M.C. (Ed.), Microencapsulation and Microspheres for Food Applications. Elsevier, The Netherlands, pp. 391–406.

Casanova-González, E., García-Bucio, A., Ruvalcaba-Sil, J.L., Santos-Vasquez, V., Esquivel, B., Falcón, T., Arroyo, E., Zetina, S., Roldán, M.L., Domingo, C., 2012. Surface-enhanced Raman spectroscopy spectra of Mexican dyestuffs. J. Raman Spectrosc. 43 (11), 1551–1559.

Chan-Bacab, M.J., Sanmartín, P., Camacho-Chab, J.C., Palomo-Ascanio, K.B., Huitz-Quimé, H.E., Ortega-Morales, B.O., 2015. Characterization and dyeing potential of colorant-bearing plants of the Mayan area in Yucatan Peninsula, Mexico. J. Clean. Prod. 91, 191–200.

Coultate, T., Blackburn, R.S., 2018. Food colorants: their past, present and future. Color. Technol. 134 (3), 165–186.

Curiel, J.A., Pinto, D., Marzani, B., Filannino, P., Farris, G.A., Gobbetti, M., Rizzello, C.G., 2015. Lactic acid fermentation as a tool to enhance the antioxidant properties of *Myrtus communis* berries. Microb. Cell Factories 14, e67.

da Costa, J.M.G., Silva, E.K., Toledo, A.A.C., Azevedo, V.M., Malta, M.R., Ferreira Alves, J.G.L., Borges, S.V., 2015. Microencapsulation of Swiss cheese bioaroma by spray-drying: process optimization and characterization of particles. Powder Technol. 274, 296–304.

Elversson, J., Millqvist-Fureby, A., Alderborn, G., Elofsson, U., 2003. Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying. J. Pharm. Sci. 92 (4), 900–910.

Fan, G., Han, Y., Gu, Z., Gu, F., 2008. Composition and colour stability of anthocyanins extracted from fermented purple sweet potato culture. LWT–Food Sci. Technol. 41 (8), 1412–1416.

Filannino, P., Di Cagno, R., Gobbetti, M., 2018. Metabolic and functional paths of lactic acid bacteria in plant foods: get out of the labyrinth. Curr. Opin. Biotechnol. 49, 64–72.

Ferrari, C.C., Germer, S.P.M., Alvim, I.D., Vissotto, F.Z., de Aguirre, J.M., 2012. Influence of carrier agents on the physicochemical properties of blackberry powder produced by spray drying: spray drying of blackberry pulp. Int. Food Sci. Technol. 47 (6), 1237–1245.

García-Ríos, R.I., Mora-Pérez, A., González-Torres, D., Carpio-Reyes, R. de J., Soria-Fregozo, C., 2019. Anxiolytic-like effect of the aqueous extract of *Justicia spicigera* leaves on female rats: a comparison to diazepam. Phytomedicine 55, 9–13.

Giuliani, A., Cerretani, L., Cichelli, A., 2016. Colors: properties and determination of natural pigments. In: Encyclopedia of Food and Health. Elsevier, The Netherlands, pp. 273–283.

Hann, D., Stražišar, J., 2007. Influence of particle size distribution, moisture content, and particle shape on the flow properties of bulk solids. Instrum. Sci. Technol. 35 (5), 571–584.

He, Z., Xu, M., Zeng, M., Qin, F., Chen, J., 2016. Interactions of milk α- and β-casein with malvidin-3-*O*-glucoside and their effects on the stability of grape skin anthocyanin extracts. Food Chem. 199, 314–322.

Horbowicz, M., Kosson, R., Grzesiuk, A., Dębski, H., 2008. Anthocyanins of fruits and vegetables—their occurrence, analysis and role in human nutrition. Veg. Crops Res. Bull. 68 (1), 5–22.

Hu, J., Sari, O., Eicher, S., Rija Rakotozanakajy, A., 2009. Determination of specific heat of milk at different fat content between 1°C and 59°C using micro DSC. J. Food Eng. 90 (3), 395–399.

Hunaefi, D., Gruda, N., Riedel, H., Akumo, D.N., Saw, N.M.M.T., Smetanska, I., 2013. Improvement of antioxidant activities in red cabbage sprouts by lactic acid bacterial fermentation. Food Biotechnol. 27 (4), 279–302.

Jacobo-Salcedo, M.R., Alonso-Castro, A.J., Salazar-Olivo, L.A., Carranza-Alvarez, C., González-Espíndola, L.Á., Domínguez, F., Maciel-Torres, S.P., García-Lujan, C., González-Martínez, M.R., Gómez-Sánchez, M., Estrada-Castillón, E., Zapata-Bustos, R., Medellin-Milán, P., García-Carrancá, A., 2011. Antimicrobial and cytotoxic effects of Mexican medicinal plants. Nat. Prod. Commun. 6 (12), 1934578X1100601.

Jiménez-González, O., Guerrero-Beltrán, J.Á., 2021. Extraction, microencapsulation, color properties, and experimental design of natural pigments obtained by spray drying. Food Eng. Rev. 13, 769–811.

Kallam, K., Appelhagen, I., Luo, J., Albert, N., Zhang, H., Deroles, S., Hill, L., Findlay, K., Andersen, Ø.M., Davies, K., Martin, C., 2017. Aromatic decoration determines the formation of anthocyanic vacuolar inclusions. Curr. Biol. 27 (7), 945–957.

Khwanpruk, K., Akkaraphenphan, C., Wattananukit, P., Kaewket, W., Chusai, S., 2018. Effect of drying air condition and feed composition on the properties of orange juice spray dried powder. Matec. Web Conf. 192, 03013.

Kil, B.J., Yoon, S.J., Yun, C.-H., Huh, C.-S., 2020. The effect of milk protein on the biological and rheological properties of probiotic capsules. J. Microbiol. Biotechnol. 30 (12), 1870–1875.

Koc, B., Yilmazer, M.S., Balkır, P., Ertekin, F.K., 2010. Spray drying of yogurt: optimization of process conditions for improving viability and other quality attributes. Dry. Technol. 28 (4), 495–507.

Langrish, T.A.G., Marquez, N., Kota, K., 2006. An investigation and quantitative assessment of particle shape in milk powders from a laboratory-scale spray dryer. Dry. Technol. 24 (12), 1619–1630.

Lee, J., Durst, R.W., Wrolstad, R.E., Eisele, T., Giusti, M.M., Hach, J., Hofsommer, H., Koswig, S., Krueger, D.A., Kupina, S., Martin, S.K., Martinsen, B.K., Miller, T.C., Paquette, F., Ryabkova, A., Skrede, G., Trenn, U., Wightman, J.D., 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J. AOAC Internat. 88 (5), 1269–1278.

Lima, E.M.F., Madalão, M.C.M., dos Santos, W.C., Bernardes, P.C., Saraiva, S.H., Silva, P.I., 2019. Spray-dried microcapsules of antioxidant-rich extracts from *Euterpe edulis* M. as an alternative for maintaining color and bioactive compounds in dairy beverages. J. Food Sci. Technol. 56 (9), 4147–4157.

Muhammad, Z., Ramzan, R., Zhang, R., Zhang, M., 2021. Resistant starch-based edible coating composites for spray-dried microencapsulation of *Lactobacillus acidophilus*, comparative assessment of thermal protection, in vitro digestion and physicochemical characteristics. Coatings 11 (5), 587.

Perusko, M., Ghnimi, S., Simovic, A., Stevanovic, N., Radomirovic, M., Gharsallaoui, A., Smiljanic, K., Van Haute, S., Stanic-Vucinic, D., Cirkovic Velickovic, T., 2021. Maillard reaction products formation and antioxidative power of spray dried camel milk powders increases with the inlet temperature of drying. LWT 143, e111091.

Pihlanto, A., 2013. Lactic fermentation and bioactive peptides. In: Kongo, J.M. (Ed.), Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes. InTechOpen, London. Ch. 13.

Real-Sandoval, S.A., Gutiérrez-López, G.F., Domínguez-López, A., Paniagua-Castro, N., Michicotl-Meneses, M.M., Jaramillo-Flores, M.E., 2020. Downregulation of proinflammatory liver gene expression by *Justicia spicigera* and kaempferitrin in a murine model of obesity-induced by a high-fat diet. J. Funct.Foods 65, 103781.

Sarı, A., Şahin, H., Özsoy, N., Özbek Çelik, B., 2019. Phenolic compounds and in vitro antioxidant, anti-inflammatory, antimicrobial activities of *Scorzonera hieraciifolia* Hayek roots. South Afr. J. Bot. 125, 116–119.

Sharma, V., Vijay Kumar, H., Jagan Mohan Rao, L., 2008. Influence of milk and sugar on antioxidant potential of black tea. Food Res. Int. 41 (2), 124–129.

Shori, A.B., 2015. The potential applications of probiotics on dairy and non-dairy foods focusing on viability during storage. Biocatal. Agric. Biotechnol. 4 (4), 423–431.

Shrestha, A.K., Howes, T., Adhikari, B.P., Bhandari, B.R., 2008. Spray drying of skim milk mixed with milk permeate: effect on drying behavior, physicochemical properties, and storage stability of powder. Dry. Technol. 26 (2), 239–247.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16 (3), 144–158.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. ume 299, 152–178. Academic Press, New York.

Sun, H., Zhang, P., Zhu, Y., Lou, Q., He, S., 2018. Antioxidant and prebiotic activity of five peonidin-based anthocyanins extracted from purple sweet potato (*Ipomoea batatas* (L.) Lam.). Sci. Rep. 8 (1), 5018.

Sun, X., Shokri, S., Gao, B., Xu, Z., Li, B., Zhu, T., Wang, Y., Zhu, J., 2022. Improving effects of three selected co-pigments on fermentation, color stability, and anthocyanins content of blueberry wine. LWT–Food Sci. Technol. 156, 113070.

Sunitha, B., Bhavya, J.N., Arvind, G., Farzana, Sk., Anusha, K., 2016. Production of powdered yoghurt by spray drying. Int. J. Eng. Res. Technol. 5 (5), 668–678.

Syngai, G.G., Gopi, R., Bharali, R., Dey, S., Lakshmanan, G.M.A., Ahmed, G., 2016. Probiotics—the versatile functional food ingredients. J. Food Sci. Technol. 53 (2), 921–933.

Tadesse, S.A., Emire, S.A., 2020. Production and processing of antioxidant bioactive peptides: a driving force for the functional food market. Heliyon 6 (8), e04765.

Tena, N., Martín, J., Asuero, A.G., 2020. State of the art of anthocyanins: antioxidant activity, sources, bioavailability, and therapeutic effect in human health. Antioxidants 9 (5), 451.

Tsen, S.Y., Siew, J., Lau, E.K.L., Afiqah bte Roslee, F., Chan, H.M., Loke, W.M., 2014. Cow's milk as a dietary source of equol and phenolic antioxidants: differential distribution in the milk aqueous and lipid fractions. Dairy Sci. Technol. 94 (6), 625–632.

Varzakas, T., Kandylis, P., Dimitrellou, D., Salamoura, C., Zakynthinos, G., Proestos, C., 2018. Innovative and fortified food: probiotics, prebiotics, GMOs, and superfood. In: Eaqub Ali, Md., Ahmad Nizar, Nina Naquiah (Eds.), Preparation and Processing of Religious and Cultural Foods. Elsevier, The Netherlands, pp. 67–129.

Wang, H.-Y., Qian, H., Yao, W.-R., 2011. Melanoidins produced by the Maillard reaction: structure and biological activity. Food Chem. 128 (3), 573–584.

Yan, Y., Zhang, F., Chai, Z., Liu, M., Battino, M., Meng, X., 2019. Mixed fermentation of blueberry pomace with *L. rhamnosus* GG and L. plantarum-1: enhance the active ingredient, antioxidant activity and health-promoting benefits. Food Chem. Toxicol. 131, 110541.

Zapata-Morales, J.R., Alonso-Castro, A.J., Domínguez, F., Carranza-Álvarez, C., Castellanos, L.M.O., Martínez-Medina, R.M., Pérez-Urizar, J., 2016. Antinociceptive activity of an ethanol extract of *Justicia spicigera*. Drug Dev. Res. 77 (4), 180–186.

Zhang, Q., Gonzalez de Mejia, E., Luna-Vital, D., Tao, T., Chandrasekaran, S., Chatham, L., Juvik, J., Singh, V., Kumar, D., 2019. Relationship of phenolic composition of selected purple maize (*Zea mays* L.) genotypes with their anti-inflammatory, anti-adipogenic and anti-diabetic potential. Food Chem. 289, 739–750.

Zhang, Y., Li, B., Han, L., 2017. Microencapsulation of *Lactobacillus acidophilus* KLDS 1.0391 by electrostatic spray increases viability after *In vitro* digestibility. J. Food Process. Eng. 40 (2), e12416.

Zotarelli, M.F., da Silva, V.M., Durigon, A., Hubinger, M.D., Laurindo, J.B., 2017. Production of mango powder by spray drying and cast-tape drying. Powder Technol. 305, 447–454.