Current knowledge about physical properties of innovative probiotic spray-dried powders produced with lactose-free milk and prebiotics

Adriana Dantas, Silvani Verruck, Maria Helena Machado Canella, Bruna Marchesan Maran, Fabio Seigi Murakami, Lindiomar Borges de Avila Junior, Carlos Eduardo Maduro de Campos, Eduard Hernandez, Elane Schwinden Prudencio

PII: S0023-6438(21)01328-1

DOI: https://doi.org/10.1016/j.lwt.2021.112175

Reference: YFSTL 112175

To appear in: *LWT - Food Science and Technology*

Received Date: 17 March 2021

Revised Date: 28 June 2021

Accepted Date: 18 July 2021

Please cite this article as: Dantas, A., Verruck, S., Machado Canella, M.H., Maran, B.M., Murakami, F.S., Borges de Avila Junior, L., Maduro de Campos, C.E., Hernandez, E., Prudencio, E.S., Current knowledge about physical properties of innovative probiotic spray-dried powders produced with lactose-free milk and prebiotics, *LWT - Food Science and Technology* (2021), doi: https://doi.org/10.1016/j.lwt.2021.112175.

Florianópolis, June 28, 2021.

CRediT author statement

The CRediT author statement of the manuscript intitled "*Current knowledge about physical properties of innovative probiotic spray-dried powders produced with lactose-free milk and prebiotics*" is described below:

Adriana Dantas: Conceptualization, Investigation, Validation, Formal analysis, Resources, Data curation, Writing – Original draft, Writing – Review and Editing, Visualization. **Silvani Verruck**: Conceptualization, Resources, Data curation, Writing – Original draft. **Maria Helena Machado Canella**: Resources, Data curation, Writing – Original draft. **Bruna Marchesan Maran**: Resources, Data curation, Writing – Original draft. **Fabio Seigi Murakami**: Resources, Data curation, Writing – Original draft. **Lindiomar Borges de Avila Junior**: Resources, Data curation, Writing – Original draft. **Carlos Eduardo Maduro de Campos**: Resources, Data curation, Writing – Original draft. **Eduard Hernandez**: Conceptualization, Validation, Formal analysis, Resources, Data curation, Writing – Original draft, Writing – Review and Editing, Visualization, Supervision. **Elane Schwinden Prudencio**: Conceptualization, Investigation, Validation, Formal analysis, Resources, Data curation, Writing – Original draft, Writing – Review and Editing, Visualization. Writing – Review & Editing. Supervision, Project administration, Funding acquisition.

MSc. Adriana Dantas
Federal University of Santa Catarina - Department of Food Science and Technology
Rodovia Admar Gonzaga, 1346, Itacorubi
88034-001- Florianópolis - Santa Catarina - Brazil
E-mail: sorrisitah@gmail.com

Current knowledge about physical properties of innovative probiotic spray-dried powders produced with lactose-free milk and prebiotics

Adriana Dantas[a], Silvani Verruck[b], Maria Helena Machado Canella[b], Bruna Marchesan Maran[a], Fabio Seigi Murakami[c], Lindiomar Borges de Avila Junior[d], Carlos Eduardo Maduro de Campos[d], Eduard Hernandez[e], & Elane Schwinden Prudencio[a,b*]

[a]*Department of Chemical and Food Engineering, Federal University of Santa Catarina, 88.040-900, Florianópolis, Santa Catarina, Brazil*

[b]*Department of Food Science and Technology, Agricultural Sciences Center, Federal University of Santa Catarina, 88.034-001, Florianópolis, Santa Catarina, Brazil*

[c]*Department of Pharmacy, Federal University of Paraná, Av. Pref. Lothário Meissner, 632, Jardim Botânico, 80210-170 Curitiba, PR, Brazil*

[d]*Department of Physics, Federal University of Santa Catarina, 88.040-970, Florianópolis, Santa Catarina, Brazil*

[e]*Department of Agri-Food Engineering and Biotechnology, Universitat Politécnica de Catalunya BarcelonaTech, 8. 08860, Castelldefels, Barcelona, Spain*

* Corresponding author: +55 48 3721 5366 (E.S. Prudencio)

E-mail address: elane.prudencio@ufsc.br

Abstract

Lactose-free probiotic powders were obtained by mixing *Bifidobacterium* BB-12 suspensions with lactose-free milk powder or lactose-free milk powder and prebiotics (inulin or oligofructose). A thorough investigation was performed to know their water sorption properties and physical and thermal characteristics. By evaluating the water sorption properties, the Peleg model fitted well to the experimental sorption data, showing that the equilibrium moisture content of powders increased as the relative humidity increased. The isotherm found for all samples was a Type III Isotherm, commonly observed in most foods. For both morphology and particle size, the use of different carrier agents affected these properties; however, all the spray-dried powders presented good size to be added in food products. X-ray diffraction and Raman spectroscopy showed us amorphous structure for all powders, and typical bands of the milk constituents and sugars, respectively. Regarding the spray-dried powders thermal properties, it was possible to confirm that the addition of prebiotics gave higher thermal stability, highlighting the sample produced with inulin. We concluded that a good quality of lactose-free milk based probiotic powder could be obtained using spray drying technique, with great potential to be applied in lactose-free dairy products.

Keywords**:** *Bifidobacterium* BB-12; microencapsulation; lactose-free microspheres; physical characterization; prebiotics.

1. Introduction

The probiotics market was valued at USD 48.88 billion in 2019 and is estimated to increase to USD 94.48 billion by 2027. This expansion is owing to consumer perception about the health benefits of probiotic-based products, in addition to the demand for immunity-boosting products amid COVID-19 (Market Research Report, 2020). In this context, the microencapsulation of probiotic cells within dairy matrices is already a widespread and accepted technology, as well as the joint use of prebiotic carbohydrates. Due to the unfavorable conditions encountered by probiotic microorganisms when present in a food matrix (for example, changes in pH, water activity, temperature, and oxygen content), the production of powdered probiotic ingredients is necessary. In addition to ensuring the arrival to the colon in adequate quantities (Verruck et al., 2017), this type of presentation allows longer storage and versatility in applications.

Milk-based probiotic powders have already ready been applied in several types of dairy products (Pinto et al., 2017; Verruck et al., 2020; Gul, 2017), including lactose-free dairy (Pinto et al., 2019). However, the addition of these microspheres in lactose-free dairy constitutes a source of product contamination. Given the world scenario of lactose intolerance (about 70% of the world's adult population is lactose-intolerant) (Lule, Garg, Tomar, Khedkar, & Nalage, 2016), products free of this disaccharide are essential to make up the diet of intolerant people. In this state, the individual cannot digest and absorb dietary lactose, due to deficiency in β-galactosidase, the enzyme that is responsible for lactose hydrolysis. This condition leads to gastrointestinal manifestations such as diarrhea, vomiting, abdominal cramps, and gas (Suri et al.,

2019). In this context, the development of a lactose-free probiotic powder would meet this specific population demand.

Probiotic powders obtained by spray drying are usually microparticles in the amorphous state (Arslan-Tontul, 2020; Verruck, de Liz, Dias, Amboni, & Prudencio, 2019; Wang, Lin, & Zhong, 2020). This amorphous structure is associated with the presence of carbohydrates in the dehydration media, and has also been related to the improved stability of spray-dried microorganisms (Chávez & Ledeboer, 2007; Passot, Cenard, Douania, Tréléa, & Fonseca, 2012; Vivek, Mishra, & Pradhan, 2020). Depending on the temperature and relative humidity at which a powder product is subjected, it becomes susceptible to interactions with water, resulting in unwanted characteristics such as agglomeration, difficulty in rehydration, and triggering Maillard reactions. These physical changes can negatively interfere with the microencapsulated bacteria, leading to partial release or mortality. In other words, the physical state of microparticles enhances or minimizes the probability of chemical changes. Zhu, Ying, Sanguansri, Tang, and Augustin (2013) employed whey protein isolate, maltodextrin, D-glucose, and L-glucose as encapsulant matrices of *Lactobacillus rhamnosus* GG. D-glucose, which can be used by probiotic for energy, clearly exerted a physico-chemical action in stabilizing the bacteria. Likewise, the authors noted that water activity (a_w) of the probiotic powders had a large effect on the cell viability during storage. Independently of the rubbery or glassy state of the powders, an increase in a_w carried a quicker reduction in the culturability of *L. rhamnosus* during storage. At a high a_w level (0.70), it was not observed effect of glucose in cell survival, but at 0.33 (a_w most commonly found in dried products), glucose incorporation greatly improved the cell preservation for 35 days compared to a glucose-free carrier. Besides, Romano, Mobili, Zuñiga-Hansen, and Gómez-Zavaglia (2018) evaluated the stability of a bacteria with

probiotic properties after it was spray-dried with amorphous inulin. The bacteria stability was considered to depend mainly on the a_w in which the powder was stored. Thus, $a_w < 0.40$ resulted in stable cell viability for up to 180 days of storage; whereas, when samples were stored at a_w above this value, microorganisms experienced a significant reduction in their viability. Given all the above, the study of the water adsorption kinetic becomes a valuable tool in the investigation of powders, since this analysis can help in the selection of suitable packaging materials and storage conditions (Rhim, Koh, & Kim, 2011). In conjunction with this examination, we also believe that the study of sorption isotherms is essential in identifying optimal storage conditions, as it predicts and models the moisture changes that occur during this period. Consequently, the shelf life of many products can be predicted. Santos and Machado (2021) produced probiotic particles based on alginate–chitosan and studied their sorption isotherms. In addition, the authors used FT-IR analyses to qualitatively examine the presence of bindings in structures generated from the mixtures. Other researchers have also studied the water sorption behavior of probiotic powders produced by spray drying from different carrier agents (Agudelo, Cano, González-Martínez, & Chiralt, 2017; Guergoletto, Busanello, & Garcia, 2017; Romano et al., 2018; Vivek et al., 2020; Ying et al., 2016).

According to Verruck, Santana, de Oliveira Müller, and Prudencio (2018), thermal analysis techniques, such as differential scanning calorimetry and thermogravimetry, can be effectively used to determine the phase transition temperatures in spray-dried powders, as well as the degradation temperatures of its components. Moreover, Pinto et al. (2015), Muhammad, Ramzan, Huo, Tian, and Bian (2017), Dias et al. (2018), and De Liz et al. (2020) observed a close relationship between the probiotic powders thermal properties and the viable cells count.

In this work, lactose-free milk, oligofructose, and inulin were carefully selected and evaluated in order to know their properties for future food applications. Thus, this study is intended to assess the physical stability of probiotic powders (based on thermal and water sorption properties analyses), as well as to characterize them in terms of morphology and structure.

2. Material and methods

2.1. Material

Lactose-free skim milk powder (Aurora®, Cooperativa Central Aurora Alimentos, Santa Catarina, Brazil) (85.51 g total solids 100 g^{-1}, 32.50 g protein 100 g^{-1}, 0.00 g fat 100 g^{-1}, 3.01 g ash 100 g^{-1}, and 50.00 g carbohydrates 100 g^{-1}) and the prebiotics inulin (Orafti® Gr, Orafti, Tienen, Belgium) (DP ≥ 10) and oligofructose (Orafti® P95, Orafti, Tienen, Belgium) (DP = 2−8) were used as microsphere carrier agents. *Bifidobacterium* BB-12 (Nu-trish® BB-12®, Chr. Hansen, Hønsholm, Denmark) was used as the active material for the microspheres, while UHT (ultra-high temperature) lactose-free milk (3.2 g 100 g^{-1} of proteins, 5.0 g 100 g^{-1} of carbohydrates and 0.40 g 100 g^{-1} of lipids) was employed to prepare the bacterial suspension. The water sorption kinetics and moisture adsorption isotherms were conducted using salts of analytical grade.

2.2. Microencapsulation of *Bifidobacterium* BB-12 and viable probiotic cell count

Three feed solutions were prepared for the production of spray-dried powders containing *Bifidobacterium* BB-12. Therefore, the feed solutions designated as 1, 2, and 3, presented lactose-free skim milk powder (200 g L^{-1}); lactose-free skim milk powder (100 g L^{-1}) and inulin (100 g L^{-1}); and lactose-free skim milk powder (100 g L^{-1}) and oligofructose (100 g L^{-1}), respectively. All of them were prepared with sterile distilled water and heat-treated for 30 min at 80 °C. After the solutions were cooled down to room temperature (25 °C), a precipitate of probiotic cells was added to each of them. A laboratory-scale spray dryer (B-290 mini spray dryer, Buchi, Flawil, Switzerland) equipped with a cyclone was used. The feed solutions containing *Bifidobacterium* BB-12 were kept under magnetic agitation (MS-3000, BioSan, Riga, Latvia) at room temperature, and spray dried under optimum conditions of 150 °C inlet temperature and 44 °C outlet temperature to obtain probiotic spray-dried powders. The mini spray dryer has an integrated standard two-fluid nozzle, where compressed air is used to disperse the liquid body into fine droplets. The nozzle consists of a 0.7 mm liquid orifice diameter, a 1.1 mm liquid outer diameter, and a 1.5 mm gas orifice diameter. This geometry results in a mixing of fluid body and gas. Therefore, the feed solution was sprayed by the nozzle in a closed cylindrical container, in which the droplets dry during their fall onto the container wall due to the hot-air flow. The powder and wet air were separated in the cyclone, and then, the sample was collected from the cyclone base. The compressor air pressure, drying airflow rate, and feed rate were set at 0.7 MPa, 35 $m^3\ h^{-1}$, and 12 mL min^{-1}, respectively. The powders were denoted as 1, 2, and 3, and were derived from feed solutions 1, 2, and 3, respectively. They were packed in aluminum pouches under vacuum (200 B, Selovac, São Paulo, Brazil).

For enumeration of entrapped cells, 1 g of spray-dried powder was previously vortexed with 9 mL of sterile phosphate buffer (pH 7.0, 0.1 mol L^{-1}) for 10 min. Then,

the pour plate method described by Vinderola and Reinheimer (1999) was used. For this, mixtures and feed solutions were serially diluted in peptone water (Oxoid; 0.1 g 100 mL^{-1}), and plated on MRS agar modified with the addition of 0.3 g 100 g^{-1} sodium propionate (Fluka, Neu-Ulm, Germany) and 0.2 g 100 g^{-1} lithium chloride (Vetec, Rio de Janeiro, Brazil). The plates were incubated in anaerobic jars containing AnaeroGen® at 37 °C during 72 h. Results were expressed as log colony-forming units per gram (log CFU g^{-1}).

2.3. Water sorption properties

The kinetics of water absorption was studied by fitting the Peleg model to the experimental data. For this, the methodology proposed by Verruck et al. (2018) was used. Probiotic spray-dried powders were dehydrated at 105 °C until reaching constant weight, thus, their initial moisture contents (X_0) were measured. After drying, triplicate samples (~ 0.5 g) were placed at 25 ºC in separate desiccators that contained different saturated salt solutions. The saline solutions provided, inside the desiccators, an environment with a relative humidity that varied from 11 to 90%. The sample mass was measured periodically until reaching the hygroscopic equilibrium that was concluded when the sample showed two similar consecutive weightings. The time required to reach that thermodynamic equilibrium varied according to the different relative humidity in which the samples were exposed, that is, 11.25 h in the 11% relative humidity, reaching 6 days for the relative humidity of 80 and 90%. The moisture adsorption curves of the samples were fitted to Equation (1) (Peleg, 1988).

$$X_{(t)} = X_0 + \frac{t}{k_1 + k_2 t} \quad (1)$$

where $X_{(t)}$ is the water content of the powder for a given instant of time (g water / g dry solid), X_0 is the initial moisture of the powder (g water / g dry solid), t is the time (h), k1 is the Peleg rate constant (g dry solid h / g water), and k2 is the Peleg capacity constant (g dry solid / g water).

The moisture adsorption isotherms of the probiotic spray-dried powders previously dehydrated were determined through the static method, using saturated saline solutions to obtain different air relative humidity as described by Labuza, Kaanane, and Chen (1985). The empirical mathematical model of GAB (Equation 2) was employed to retract the experimental equilibrium data (Al-Muhtaseb, McMinn, & Magee, 2002). The parameters of the model were estimated by nonlinear regression using the STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, CA).

$$X_{eq} = \frac{M_0 \, C \, k \, a_w}{(1 - k \, a_w)(1 - k \, a_w + C \, k \, a_w)} \quad (2)$$

where X_{eq} is the equilibrium moisture (g water / g dry solid), M_0 is the moisture content in the monolayer (g water / g dry solid), a_w is the water activity, and C and k are model constants.

2.4. Physical characterization of probiotic powders

2.4.1. Morphology and particle size

The morphology and particle size of the spray-dried powders were observed with a Jeol scanning electron microscope, model JSM 6390 LV (Jeol, Tokyo, Japan), at an accelerating voltage of 5 kV. Therefore, the microparticles were fixed with carbon tape on a stub (this was carried out on the day of obtaining the powders) and covered with gold.

The diameter of the powders was estimated from the SEM micrographs in their initial magnification using ImageJ (version 1.51k; http://rsb.info.nih.gov/ij/). The diameter of 120 particles from each of the powders was measured (Fritzen-Freire et al., 2012).

2.4.2. X-Ray diffraction

X-ray powder diffraction (XRPD) analysis of the spray-dried powders was performed on an XPERT PANalytical diffractometer, equipped with an X'Celerator detector and using filter radiation of Cu Kα (λ = 1.5418 Å), the tension of 45 kV and current of 40 mA. Samples were analyzed at a scattering range of $4° < 2\theta < 60°$ with 0.12º stepsize and 30 s counting time with at least 5 scans averaged to improve data statistics.

2.4.3. Raman spectroscopic analysis

Raman spectra of spray-dried powders and prebiotics samples were obtained with a PeakSeeker PRO-785 Raman spectrometer using a 50 X objective lens at room temperature. Raman system with a diode laser of 785 nm and 300 mW at the source were employed. Raman spectra were collected at 6 cm^{-1} resolution in the range of 200–2000 cm^{-1} with a Peltier-cooled charge-coupled device CCD detector.

2.4.4. Thermal properties

For obtaining of the thermogravimetry/derivative thermogravimetry (TGA/DrTGA) curves, it was used a DTG-60 thermobalance (Shimadzu DTG-60, Kyoto, Japan) previously calibrated with calcium oxalate. Six milligrams of each spray-dried powder were placed in an aluminum pan. Then, a heating rate of 10 °C min^{-1} was employed to heat the sample from 30 to 300 °C, maintaining a dynamic synthetic air atmosphere of 50 mL min^{-1}.

The spray-dried powders and the raw materials were submitted to differential scanning calorimetry (DSC) (Shimadzu DSC-60, Kyoto, Japan). A standard reference of indium was used for preliminary calibration of equipment. So, approximately two milligrams of sample were placed in aluminum pans covered with a lid. All measurements were performed at 10 °C min^{-1} under a dynamic synthetic air atmosphere of 50 mL min^{-1}, and in a temperature range of 30 to 300 °C.

2.5. Statistical analysis

The mean and standard deviation (SD) were calculated from data obtained in triplicate. The one-way analysis of variance (ANOVA) was conducted using the STATISTICA version 13.3 software (TIBCO Software Inc., Palo Alto, CA). Differences between treatments mean values were analyzed using the Tukey test at a significance level of 0.05.

3. Results and discussion

3.1. Probiotic viability

The viable probiotic cell count in the feed solutions, and in the spray-dried powders were higher than 10.00 log CFU g^{-1}, and 9.00 log CFU g^{-1}, respectively. Therefore, all spray-dried powders are considered a probiotic product.

3.2. Water sorption properties

Peleg's constants at different relative humidity are shown in Table 1. Khazaei and Mohammadi (2009) affirm that the constant k1 is related to the initial rate of absorption, and the lower its value, the higher the initial mass transfer rate. Therefore, we can also study it through its reverse (1/k1). For all samples, the values found for this constant showed sensitive but significant changes ($P < 0.05$) when the relative humidity where the sample was conditioning increased. There was a tendency to increase the values of 1/k1 with the increase of the relative humidity, mainly from 11% for the other relative humidity. This behavior was already expected, as according to Verruck et al. (2018), the initial rate of absorption depends of the difference between the moisture content of the sample and the moisture content of the environment (saturation humidity). In other words, the higher the moisture gradient, the higher the initial rate of absorption, since the gradient is the driving force of the process. Besides, these values may be explained due to the high hydrophilicity of the components (carbohydrates and protein) (Zhang, Kim, Yokoyam, & Kim, 2018). Wang et al. (2020) affirmed that at a_w between 0 and 0.34, the numerous polar groups of proteins are known as the primary component absorbing water because they can strongly and rapidly absorb water via hydrogen bonding. The high hydration of the casein micelles in their native structure (3.7 mL H_2O g^{-1} protein) contributes to this (Schuck, 2011).

296 According to Khazaei and Mohammadi (2009), the constant k2 of the Peleg's
297 model is inversely related to the maximum water adsorption capacity, i.e., the lower the
298 k2, the higher the water absorption capacity of the product. In our work, 1/k2 values
299 increased with the increase of the relative humidity ($P < 0.05$) (Table 1), especially at
300 the highest relative humidity (80 and 90%). The adsorption of moisture is due to the
301 movement of water (water vapor available to make exchanges with the sample), given
302 the difference in water vapor pressure between the product surface and the air
303 surrounding it. Consequently, from the moment that we increase the relative humidity
304 inside the desiccator, the vapor pressure of air also increases, which explains the values
305 of 1/k2 found. Spray-dried powder 1 sample showed the highest value of 1/k2 at 90%
306 relative humidity (0.6936), followed by spray-dried powder 3 (0.5706), and finally,
307 spray-dried powder 2 (0.4860). As occurred in the work by Verruck et al. (2018), higher
308 values of 1/k2 were correlated with the highest equilibrium moistures observed during
309 the experiments, since the equilibrium moistures of the samples when subjected to the
310 environment with 90% relative humidity were 0.6551, 0.5295, and 0.4637 g water g^{-1}
311 dry solid for spray-dried powders 1, 3 and 2, respectively. Our results for all samples
312 are also in agreement with those obtained by Vivek et al. (2020), who studied probiotic
313 spray-dried powder obtained from Sohiong fruit. In both cases, the equilibrium moisture
314 content increased as the relative humidity increased.

315 The higher values of 1/k2 constant and equilibrium moisture for spray-dried
316 powder 1 may be explained by the higher amount of short-chain carbohydrates glucose
317 and galactose, they are simpler carbohydrates compared to the prebiotics used in the
318 other preparations. Saavedra-Leos et al. (2014) reported that more water molecules can
319 be readily absorbed by these carbohydrates, given a higher content of OH groups on
320 their surfaces. Moreover, Jimenez-Sánchez, Calderón-Santoyo, Ortiz-Basurto, Bautista-

Rosales, and Ragazzo-Sáncheset (2018) highlighted that the lower the degree of polymerization of a product, the more hygroscopic it will be. On the other hand, Pilatti-Riccio et al. (2019) reported that, given the high hygroscopicity, the use of short-chain carbohydrates in mixtures of wall materials is advantageous because it can result in a fast release of encapsulated compounds. Another factor that is closely related to the moisture and water activity of a sample is the glass transition temperature (T_g). Galactose and glucose present lower T_g than oligofructose and inulin (30, 31, 102, and 132 ºC, respectively) (Schuck et al., 2005; Silva, Zabot, Bargas, & Meireles, 2016; Hinrichs, Prinsen, & Frijlink, 2001). Juliano and Barbosa-Cánovas (2010) discussed that a glass transition temperature nearby to room temperature favors water absorption, and as consequence, it can bring technological problems to milk powder such as rehydration difficulty, particle agglomeration, and cacking.

Chirife, Timmermann, Iglesias, and Boquet (1992) stated that a mechanistic approach is needed for a full-proof validation, that is, a mere fitting of the sorption model to the experimental data cannot guarantee its validity. We concluded that the Peleg model fits exceptionally well to the experimental data because, in addition to showing high values of R^2 and low values of SSE (Table 1), randomness in the standardized residual plots was observed in all cases. Moreover, it was noticed a good fit in the highest, intermediate, and the lower relative humidity, providing adequate values of the initial rates of water adsorption and maximum water adsorption capacity. Given its relative simplicity, Peleg's model has been used satisfactorily by several authors to represent hydration kinetics in dairy products (Seth, Dash, Mishra, & Deka, 2018; Ruano-Uscategui, Ciro-Velásquez, & Sepúlveda-Valencia, 2018; Verruck et al., 2018; Liang, Bund, & Hartel, 2009; Varghese, Ramachandrannair, & Mishra, 2009).

345 Vivek et al. (2020) emphasized that data obtained from the isotherm study can
346 be useful to define process condition, transport, and storage, to predict desorption or
347 adsorption behavior of the powder and the shelf life of the material. It can also be used
348 to describe the energy requirements of a dehydration process. Experimental data of
349 adsorbed moisture content (equilibrium moisture) as a function of a_w was well described
350 by the Guggenheim–Anderson–Boer (GAB) model (Fig. 1). The data for all probiotic
351 powders followed a type III isotherm behavior. In addition to indicating the formation
352 of multilayer, the type III isotherm curve is related to the presence of amorphous sugars
353 and their dissolution in water (Rao & Rizvi, 1994).

354 Reid and Fennema (1996) stated that the isotherms are classified into three
355 regions, as follows: first, monolayer moisture region, which includes a_w less than 0.2 to
356 0.3; second, multilayer moisture region, which comprises a_w between about 0.2 and 0.3
357 until approximately 0.8; and third, free water region, that represents the most mobile
358 and least bound water in foods. In region III, a_w are equals to 0.8–0.99, and it was in this
359 region that the samples showed a drastic increase in the adsorption of water. According
360 to Verruck et al. (2018), in this region, the product can undergo some microbiological
361 alteration, chemical, or biochemistry because of the available water.

362 The coefficients of the GAB model were presented in Table 2. The coefficient of
363 determination (R^2) and Sum of Squares Error (SSE) indicate excellent fit of the model
364 to the experimental data, due to their proximity to 1 and 0, respectively. Moreover, the
365 distribution of the residuals was randomly around zero. M_0 value means the monolayer
366 moisture content on dry basis, and it indicates a strong binding potency of water on the
367 surface of the product if moisture content remains above this value. Therefore, this
368 information is vital for the storage stability of food products. The values of parameter C
369 represent the heat of sorption of monolayer moisture while the parameter k provides the

binding potency of water in terms of heat of sorption of multilayer moisture. For all of our spray-dried powders, the values of parameter C were higher than those values of k. Seth et al. (2018) also noted similar behavior for spray dried yogurt powder. The k values were lower ($P < 0.05$) for the spray-dried powders 2 and 3 than in the spray-dried powder 1 sample. Ronkart et al. (2006) and Verruck et al. (2018) reported the same behavior, they found lower k values for samples that contained prebiotics (higher molar mass).

3.3. Physical characterization of probiotic powders

3.3.1. Morphology and particle size

Fig. 2 shows the SEM micrographs of the *Bifidobacterium* BB-12 microspheres produced with different wall materials. Micrographs revealed distinct shapes and sizes for each spray-dried powder; however, in all of them, it is evident that *Bifidobacterium* BB-12 cells were retained therein because it was possible to note the absence of cells on the outside of their surfaces. The particles produced with lactose-free milk (Fig. 2-a) showed concavities typical of materials produced by spray drying. Gul (2017) reported that the formation of concavities in the surface of atomized particles can be attributed to the shrinkage of the particles during the drying process because of the rapid evaporation of the liquid drops. Similar morphological shapes of capsules made with reconstituted skim milk were reported by Maciel, Chaves, Grosso, and Gigante (2014). The spray-dried powder 2, produced with lactose-free milk powder and inulin, showed a rough and uneven surface (Fig. 2-b), however, free of fissures or disruptions, which is fundamental for guaranteeing higher protection and lower permeability of gases (Fritzen-Freire et al.,

2012). Microspheres produced with oligofructose and lactose-free milk (Fig. 2-c) were spherical, with more aggregation than spray-dried powders 1 and 2. This aggregation of particles is due to stickiness caused by the low glass transition temperature of oligofructose (Adhikari et al., 2009). The same behavior was observed with the microspheres produced by Rajam and Anandharamakrishnan (2015), who microencapsulated *Lactobacillus plantarum* with fructooligosaccharide as wall material.

Knowledge of the bulk density is important during the processing, storage, and packaging of encapsulated microparticles. The parameters such as moisture content, particle size distribution, and morphology can affect the bulk density of spray-dried powders (Rajam & Anandharamakrishnan, 2015). In our previous work (Dantas, Verruck, De Liz, Hernandez, & Prudencio, 2021), we measured the loose bulk density of the powders. It was possible to verify that the spray-dried powder 3 showed a significant variation ($P < 0.05$) of this property (0.44 g cm^{-3} against 0.32 g cm^{-3} for both spray-dried powders 1 and 2). These results reinforce our considerations about micrographs, and we associate them with particle aggregation and less interspace between particles of the spray-dried powder 3.

The size is an important property for the microparticle because of its strong influence on product solubility, appearance, and acceptability (Parthasarathi & Anandharamakrishnan, 2016). In the present study, the probiotic spray-dried powders microparticles exhibited a wide range of average diameters: 10.62 ± 4.94, 19.60 ± 7.52, 55.66 ± 21.69 µm, for spray-dried powder 1, 2, and 3 respectively. This microparticle size is favorable for the possible incorporation of the powders in various matrices without significantly affecting the texture. According to Turchiuli, Gianfrancesco, Palzer, and Dumoulin (2011), stable solid bridges between particles can be created with the drying, leading to the formation of a bigger structure that is clustered, as observed

by SEM images (Fig. 2-b,c). The presence of clusters in the spray-dried powder 2 and 3 corroborate with results obtained for the average size of the microparticles. The cluster structures visualized in the present study could be formed by several particles bound together. Carmo et al. (2018) discussed that the difference between the particle sizes is characterized by droplet coalescence, which in turn is influenced by the carrier agent used. According to Berdnaska and Janiszewska-Turak (2020), the use of carriers' agents or their mixtures in different proportions can result in powders with different physical properties. In the present study, no changes were made in the spray drying processes; therefore, encapsulation efficiency may have been influenced by the carrier agents. Ronkart et al. (2009) highlighted that in the spray drying process, two associated factors can generate the amorphous state of inulin and oligofructose and, therefore, both would be more susceptible to the clusters formation. These factors are the temperature (such as inlet temperature) and the water present in the droplets formed.

3.3.2. X-Ray diffraction

The diffractograms of the samples (spray-dried powders, inulin, and oligofructose) are shown in Figure 3. This analysis can be utilized to characterize the type of order present in powders. X-ray amorphous materials lack long-range crystallographic order and produce a broad background pattern. The crystalline solids present long-range order and their diffractograms show a series of sharp peaks (Azároff, 1968). The diffraction pattern obtained for the spray-dried powders which contain *Bifidobacterium BB-12* microspheres is predominantly related to amorphous material, since it shows dispersed bands, indicating that the molecules are disordered. De Medeiros, Thomazini, Urbano, Pinto Correia, and Favaro-Trindade (2014) also obtained

similar results, they studied spray drying dehydration of a probiotic yogurt produced with goat's milk and *Bifidobacterium animalis* subsp. *lactis* (BI-07). According to the same authors, the crystals could damage the cells, which would reduce the viability of microorganisms, making the amorphous structure interesting. Besides, amorphous solids are in general more soluble, and the crystallization may entail a negative impact on the handling properties (Campelo et al., 2017).

Campelo et al. (2017) used oligofructose (DP = 2−10) and inulin (DP= 2−60) as wall material to microencapsulate lime essential oil by spray drying. As with our results, they also found an amorphous type structure for these types of prebiotics, revealing that the spray drying process did not influence their structures. This is probably associated with the rapidity of the drying process, which prevents easy crystallization. Other studies have also reported amorphous characteristic for inulin with different polymerization degrees (Kalaivani & Suja, 2018; Silva & Meireles, 2015), for oligofructose (Alles, Tessaro, & Norena, 2013), and spray-dried skim milk powder (Yazdanpanah & Langrish, 2016).

3.3.3. Raman spectroscopic analysis

Raman spectra of spray-dried powders and prebiotics (oligofructose and inulin) are shown in Fig. 4, and the main vibrational bands are listed in Table 3 with their respective tentative assignments based on comparisons with previously reported data. The spray-dried powder 1 showed a spectrum with typical bands associated with proteins and carbohydrates (glucose). The band at 1660 cm^{-1} was the contribution from the C=O stretching and N–H wagging modes of the group Amide I ($C{=}O{-}NH_2$)

(Almeida, Oliveira, Stephani, & de Oliveira, 2011; Li-Chan, 1996). In the spray-dried powders 2 and 3, this band shifted by 5 cm^{-1} and corresponds to the same vibrations.

A strong Raman band at 1453 cm^{-1} was noted in spray-dried powder 1, and it is related to bending vibrations in the form scissoring of the group CH_2, which was mainly due to the carbohydrate mode (Almeida et al., 2011), principally glucose (Cerchiaro, Sant'Ana, Temperini, & da Costa Ferreira, 2005). Torres et al. (2017) also comment that the region between 1500 and 1250 cm^{-1} corresponds to CH_2OH deformation modes. The same peak was observed in the spectra of the other spray-dried powders, as well as inulin and oligofructose, but with a small shift in its wavenumbers. In the prebiotics spectra, especially oligofructose, this band appeared with lower intensity because of the lower contribution of glucose in these samples.

The bands at 1259, 1263, and 1262 cm^{-1} in the spray-dried powders 1, 2, and 3, respectively, may be related to the contributions from the N–H bending and C–N stretching modes of the group Amide III (Li-Chan, 1996). As there are no proteins in oligofructose and inulin, these same bands (1263 and 1266 cm^{-1}, respectively) are assigned to the CH_2 twisting mode of carbohydrates (Almeida et al., 2011; Rodrigues Junior et al., 2016).

The region between 1121 and 1065 cm^{-1} is assigned to C–O and C–C stretching, and C–O–H bending (Rodrigues Junior et al., 2016). Note that inulin and oligofructose favored the appearance of these vibrations, since the peaks at 1122 and 1123 were observed in the spray-dried powders 2 and 3, respectively; in contrast, this same band was not observed in spray-dried powder 1. According to Almeida et al. (2011), these vibrational modes are characteristic of carbohydrates.

The spectra of spray-dried powders 2 and 3 showed two more features that were not observed in the spray-dried powder 1. The peak located at 620 cm^{-1} corresponds to

494 the deformations of groups O–C–O and O–H, this result confirms the presence of
495 prebiotics, since typical bands of inulin are in the spectral region between 833 and
496 599 cm^{-1} (Balan, Chis, Rachisan, & Baia, 2018). Similarly, the band at 536 cm^{-1} was
497 observed for spray-dried powder 2 and at 542 cm^{-1} for spray-dried powder 3, indicating
498 glucose ring deformations of the inulin. Spectra of inulin and oligofructose were very
499 similar, which was already expected since these carbohydrates have the same structure,
500 changing only the molar mass. For example, both spectra show a medium peak at
501 465 cm^{-1}, being attributed to O–H bending mode (Balan et al., 2018). According to
502 Oroian, Ropciuc, and Paduret (2018), the band at 607 cm^{-1} presents in the inulin
503 spectrum is assigned to the presence of fructose.
504
505 *3.3.4. Thermal properties*
506
507 Figure 5 shows the thermogravimetric (TGA) and derivative thermogravimetry
508 (DrTGA) curves obtained from spray-dried powders. The first weight loss (3.1%,
509 2.34%, and 2.6% for spray-dried powder 1, 2, and 3, respectively) occurred between
510 25 °C and 131 °C and it corresponds to the removal of moisture of the samples. Above
511 this temperature range, the decomposition process occurred in one or two stages,
512 according to each sample. Spray-dried powder 1 showed two stages of decomposition:
513 the first between 131 ºC and 233 ºC (mass loss of 24.84%), which is related to the
514 degradation and/or caramelization of the glucose (Saavedra-Leos et al., 2012); and the
515 second stage in the range of 233 ºC to 300 ºC (mass loss of 26.45%) associated with the
516 whey protein degradation and caseins denaturation (Barreto, Pires, & Soldi, 2003).
517 For both spray-dried powders 2 and 3, the decomposition process occurred in
518 only step (temperature range between 131 and 300 °C). However, the $DrTGA_{peak}$

519 temperature was higher for spray-dried powder 2 than for spray-dried powder 3 (209.26
520 and 199.86 °C, respectively), indicating greater stability for inulin-based microspheres.
521 This behavior was already expected since inulin has a higher degree of polymerization
522 umthan oligofructose and, therefore, tends to be more thermally stable (Verruck et al.,
523 2017). Similar results were also observed by Verruck et al. (2018) and de Liz et al.
524 (2020), who microencapsulated *Bifidobacterium* BB-12 in full-fat goat's milk,
525 cryoconcentrated whey, and prebiotics (inulin and oligofructose). These DTG_{peak}
526 temperatures may correspond to the breakdown of the fructose chains of the prebiotics
527 (Fritzen-Freire et al., 2012).

528 When investigating the DSC curve of the wall material Skim Lactose-free Milk
529 (Fig. 6-a), a change in baseline between ~80 and 150 °C indicated us an endothermic
530 event. For this event, the peak mid-temperature was found at 120 ºC. Szulc, Nazarko,
531 Ostrowska-Ligęza, and Lenart (2016) found peaks around 100 °C for dairy powders,
532 and attributed them to the caseins denaturation. Likewise, De Liz et al. (2020) observed
533 endothermic peaks around 110 °C for cryoconcentrated whey samples, which were
534 related to the denaturation of its proteins. O'Mahony, Drapala, Mulcahy, and Mulvihill
535 (2017) reported that at temperatures above 115 °C, protein denaturation peaks could not
536 be distinguished, which was credited to the Maillard reaction induced during the
537 analysis. However, for all spray-dried powders resulting from this sample (Fig. 6-b),
538 there was a well-defined endothermic event, also associated with both non-enzymatic
539 browning (advanced Maillard reaction) (Vuataz, Meunier, & Andrieux, 2010) and
540 denaturation of whey proteins (Zhou & Labuza, 2011).

541 Zhou and Labuza (2011) mentioned that at 53 °C the proteins are barely
542 impacted by the temperature; however, the DNA of the microorganism present in the
543 milk powder matrix can be potently affected leading to damage and cell inactivation.

The same authors also commented that the denaturation temperature of whey protein isolate and the β-lactoglobulin (one of the major components of whey proteins) has a strong dependence on the water content of the sample: it decreases with increasing water content. This relation is best visualized when comparing the moisture (dry basis) of the powders on the day of their manufacture with the endothermic peaks: moisture values (g 100 g^{-1}) of 7.67, 4.45, and 4.54, and peak temperatures at 165.02 °C (ΔH = −166.94 J g^{-1}), 200.93 °C (ΔH = − 160.37 J g^{-1}), and 193.70 °C (ΔH = − 111.06 J g^{-1}) for spray-dried powders 1, 2 and 3, respectively. Furthermore, this increase of the peaks in the spray-dried powders 2 and 3 is related to the increase of the thermal stability due to the addition of the prebiotics (Pinto et al., 2015). Pilatti-Riccio et al. (2019) noted that particles prepared with oligofructose had fewer thermal events in their DSC curves in comparison to the events observed when only the core material was investigated. According to them, this thermal behavior suggests the protection and interaction of the wall material with the core extract, demonstrating that this matrix has potential for application as wall material in the food industry.

As can be seen in Fig. 6-a, the T_{peaks} found for inulin and oligofructose are 225.15 and 211.27 °C, and enthalpy changes −83.92 and −46.84 J g^{-1}, respectively. These peaks can be attributed to the inulin thermal degradation (Dan, Ghosh, & Moulik, 2009; Ronkart, Deroanne, Paquot, Fougnies, & Blecker, 2010; Leone, Colman, Schnitzler, Ellendersen, & Masson, 2014), as well as caramelization and decomposition of the oligofructose (Bersaneti, Mantovan, Magri, Mali, & Celligoi, 2016). Pilatti-Riccio et al. (2019) also observed an endothermic peak at 201.6 ºC for commercial oligofructose.

Based on these results, it can be stated that the microencapsulation process using inulin or oligofructose conferred greater stability to spray-dried powders.

Moreover, it has been found that, during the spray drying process at the air inlet temperature of 150 °C, the materials employed in the production of the microspheres did not suffer any significant degradation.

4. Conclusion

Bifidobacteria was efficiently entrapped in lactose free milk, and lactose free milk and prebiotics. Peleg model provided fair values of the initial mass transfer rate and water adsorption capacity. The isotherm found for all samples was a Type III Isotherm, it is related to the presence of amorphous sugars, as observed in the powders diffractograms. The GAB model fitted well to the experimental data, and indicated that relative humidity conditions above 33% and 43% were not efficient to maintain the storage stability of the spray-dried powders 1 and 2, and spray-dried powder 3, respectively. The Raman spectrum confirmed the incorporation of the prebiotics in the resultant spray-dried powders (that is, after microencapsulation process), ensuring their availability to the consumer. Besides, the addition of these carbohydrates conferred greater thermal stability to spray-dried powders, especially the inulin. On this approach, we concluded that a good quality of lactose-free milk based probiotic powder could be obtained using spray drying technique, with potential application in the food industry.

Acknowledgments

We are grateful to Coordination of Improvement of Higher Education Personnel (CAPES, Brazil) [CAPES-PRINT, Project number 88887.310560/2018-00]; to National Council for Scientific and Technological Development (CNPq, Brazil) by the financial support [CNPq, 405965/2016-8], and to Coordination of Improvement of Higher

Education Personnel (CAPES, Brazil) by the scholarship [001]. The authors are also grateful to FAPESC for its financial support. The XRPD and SEM/EDS measurements were performed at Laboratório de Difração de Raios-X (LDRX) and Laboratório Central de Microscopia Eletrônica (LCME) multiuser facilities at UFSC.

Declaration of competing interest

The authors declare that they have no conflict of interest.

References

Adhikari, B., Howes, T., Wood, B. J., & Bhandari, B. R. (2009). The effect of low molecular weight surfactants and proteins on surface stickiness of sucrose during powder formation through spray drying. *Journal of Food Engineering*, *94*(2), 135–143. https://doi.org/10.1016/j.jfoodeng.2009.01.022.

Agudelo, J., Cano, A., González-Martínez, C., & Chiralt, A. (2017). Disaccharide incorporation to improve survival during storage of spray dried *Lactobacillus rhamnosus* in whey protein-maltodextrin carriers. *Journal of Functional Foods*, *37*, 416–423. https://doi.org/10.1016/j.jff.2017.08.014.

Alles, M. J. L., Tessaro, I. C., & Norena, C. P. Z. (2013). Physicochemical characterization of saccharides powder obtained from Yacon Roots (*Smallanthus sonchifolius*) by membrane technology. *Brazilian Archives of Biology and Technology*, *56*(6), 1024–1033. http://dx.doi.org/10.1590/S1516-89132013000600019.

Almeida, M. R., Oliveira, K. d. S., Stephani, R., & de Oliveira, L. F. C. (2011). Fourier-transform Raman analysis of milk powder: a potential method for rapid

quality screening. *Journal of Raman Spectroscopy*, *42*(7), 1548–1552. https://doi.org/10.1002/jrs.2893.

Al-Muhtaseb, H., McMinn, W. M., & Magee, T. R. (2002). Moisture sorption isotherm characteristics of food products: A review. *Food and Bioproducts Processing*, *80*(2), 118–128. https://doi.org/10.1205/09603080252938753.

Arslan-Tontul, S. (2020). The combined usage of β-cyclodextrin and milk proteins in microencapsulation of *Bifidobacterium bifidum* BB-12. *Probiotics and Antimicrobial Proteins*, *12*, 747–755. https://doi.org/10.1007/s12602-019-09621-x.

Azároff, L. V. (1968). *Elements of x-ray crystallography* (International Student Edition). New York: McGraw Hill Co. (Chapter 6).

Balan, C., Chis, M. I., Rachisan, A. L., & Baia, M. (2018). A vibrational study of inulin by means of experimental and theoretical methods. *Journal of Molecular Structure*, 1164, 84–88. https://doi.org/10.1016/j.molstruc.2018.03.047.

Barreto, P. L. M., Pires, A. T. N., & Soldi, V. (2003). Thermal degradation of edible films based on milk proteins and gelatin in inert atmosphere. *Polymer Degradation and Stability*, *79*(1), 147–152. https://doi.org/10.1016/S0141-3910(02)00267-7.

Bednarska, M.A., & Janiszewska-Turak, E. (2020). The influence of spray drying parameters and carrier material on the physico-chemical properties and quality of chokeberry juice powder. *Journal Food Science and Technology*, *57***,** 564–577. https://doi.org/10.1007/s13197-019-04088-8.

Bersaneti, G. T., Mantovan, J., Magri, A., Mali, S., & Celligoi, M. A. P. C. (2016). Edible films based on cassava starch and fructooligosaccharides produced by

Bacillus subtilis natto CCT 7712. *Carbohydrate Polymers*, *151*, 1132–1138. https://doi.org/10.1016/j.carbpol.2016.06.081.

Campelo, P. H., Figueiredo, J. D., Domingues, R. Z., Fernandes, R. V. D., Botrel, D. A., & Borges, S. V. (2017). Use of prebiotic carbohydrate as wall material on lime essential oil microparticles. *Journal of Microencapsulation*, *34*(6). https://doi.org/10.1080/02652048.2017.1366563.

Carmo, E. L., Teodoro, R. A. R., Félix, P. H. C., Fernandes, R. V. de B., ..., & Botrel, D. A. (2018). Stability of spray-dried beetroot extract using oligosaccharides and whey proteins. *Food Chemistry*, *249*, 51–59. https://doi.org/10.1016/j.foodchem.2017.12.076.

Cerchiaro, G., Sant'Ana, A. C., Temperini, M. L. A., & da Costa Ferreira, A. M. (2005). Investigations of different carbohydrate anomers in copper(II) complexes with d-glucose, d-fructose, and d-galactose by Raman and EPR spectroscopy. *Carbohydrate Research*, *340*(15), 2352–2359. https://doi.org/10.1016/j.carres.2005.08.002.

Chávez, B. E., & Ledeboer, A. M. (2007). Drying of probiotics: optimization of formulation and process to enhance storage survival. *Drying Technology*, *25*, 1193–1201. https://doi.org/10.1080/07373930701438576.

Chirife, J., Timmermann, E. O., Iglesias, H. A., & Boquet, R. (1992). Some features of the parameter-K of the Gab equation as applied to sorption isotherms of selected food materials. *Journal of Food Engineering*, *15*, 75–82. https://doi.org/10.1016/0260-8774(92)90041-4.

Dan, A., Ghosh, S., & Moulik, S. P. (2009). Physicochemical studies on the biopolymer inulin: A critical evaluation of its self-aggregation, aggregate-morphology,

667 interaction with water, and thermal stability. *Biopolymers*, *91*, 687–699.
668 https://doi.org/10.1002/bip.21199.
669 Dantas, A., Verruck, S., de Liz, G. R., Hernandez, E., & Prudencio, E. S. (2021).
670 Lactose-free skim milk and prebiotics as carrier agents of *Bifidobacterium* BB-
671 12 microencapsulation: physicochemical properties, survival during storage and
672 in vitro gastrointestinal condition behaviour. *International Journal of Food*
673 *Science and Technology*, *56*(5), 2132–2145. https://doi.org/10.1111/ijfs.14823.
674 de Liz, G. R., Verruck, S., Canella, M. H. M., Dantas, A., Garcia, S. G., Maran, B. M.,
675 et al. (2020). Stability of bifidobacteria entrapped in goat's whey freeze
676 concentrate and inulin as wall materials and powder properties. *Food Research*
677 *International*, *127*, Article 108752.
678 https://doi.org/10.1016/j.foodres.2019.108752.
679 De Medeiros, A. C. L., Thomazini, M., Urbano, A., Pinto Correia, R. T., & Favaro-
680 Trindade, C. S. (2014). Structural characterisation and cell viability of a spray
681 dried probiotic yoghurt produced with goats' milk and *Bifidobacterium animalis*
682 subsp. *lactis* (BI-07). *International Dairy Journal*, *39*(1), 71–77.
683 https://doi.org/10.1016/j.idairyj.2014.05.008.
684 Dias, C. O., Almeida, J. S. O., Pinto, S. S., Santana, F. C. O., Verruck, S., Müller, C. M.
685 O., ... Amboni, R. D. M. C. (2018). Development and physico-chemical
686 characterization of microencapsulated bifidobacteria in passion fruit juice: A
687 functional non-dairy product for probiotic delivery. *Food Bioscience*, *24*, 26–36.
688 https://doi.org/10.1016/j.fbio.2018.05.006.
689 Fritzen-Freire, C. B., Prudêncio, E. S., Amboni, R. D. M. C., Pinto, S. S., Negrão-
690 Murakami, A. N., & Murakami, F. S. (2012). Microencapsulation of

691 bifidobacteria by spray drying in the presence of prebiotics. *Food Research*
692 *International*, *45*(1), 306–312. https://doi.org/10.1016/j.foodres.2011.09.020.
693 Guergoletto, K. B., Busanello, M., & Garcia, S. (2017). Influence of carrier agents on
694 the survival of *Lactobacillus reuteri* LR92 and the physicochemical properties of
695 fermented juçara pulp produced by spray drying. *LWT - Food Science and*
696 *Technology*, *80*, 321–327. https://doi.org/10.1016/j.lwt.2017.02.038.
697 Gul, O. (2017). Microencapsulation of *Lactobacillus casei Shirota* by spray drying
698 using different combinations of wall materials and application for probiotic dairy
699 dessert. *Journal of Food Processing and Preservation*, *41*(5), Article e13198.
700 https://doi.org/10.1111/jfpp.13198.
701 Hinrichs, W. L. J., Prinsen, M. G., & Frijlink, H. W. (2001). Inulin glasses for the
702 stabilization of therapeutic proteins. *International Journal of Pharmaceutics*,
703 *215*, 163–174. https://doi.org/10.1016/S0378-5173(00)00677-3.
704 Jimenez-Sánchez, D. E., Calderón-Santoyo, M., Ortiz-Basurto, R. I., Bautista-Rosales,
705 P. U., & Ragazzo-Sánches, J. A. (2018). Effect of maltodextrin reduction and
706 native agave fructans addition on the physicochemical properties of spray-dried
707 mango and pineapple juices. *Food Science and Technology International*, *24*(6),
708 519–532. https://doi.org/10.1177/1082013218769168.
709 Juliano, P., & Barbosa-Cánovas, G. V. (2010). Food powders flowability
710 characterization: theory, methods, and applications. *Annual Review of Food*
711 *Science and Technology*, *1*, 211–239.
712 https://doi.org/10.1146/annurev.food.102308.124155.
713 Kalaivani, G. J., & Suja, S. K. (2018). Electroenzymatic sensing of urea using
714 CP/MWCNT-inulin-TiO2 bioelectrode. *Applied Surface Science*, *449*, 266–276.
715 https://doi.org/10.1016/j.apsusc.2017.12.115.

Khazaei, J., & Mohammadi, N. (2009). Effect of temperature on hydration kinetics of sesame seeds (*Sesamum indicum L.*). *Journal of Food Engineering*, *91*(4), 542–552. https://doi.org/10.1016/j.jfoodeng.2008.10.010.

Labuza, T. P., Kaanane, A., & Chen, J. Y. (1985). Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. *Journal of Food Science*, *50*(2), 385–392. https://doi.org/10.1111/j.1365-2621.1985.tb13409.x.

Leone, R. D. S., Colman, T. A. D., Schnitzler, E., Ellendersen, L. N., & Masson, M. L. (2014). Evolved gas analysis (coupled TG-DSC-FTIR) applied to the thermal behaviour of inulin. *Journal of Analytical and Applied Pyrolysis*, *108*, 323–326. https://doi.org/10.1016/j.jaap.2014.04.009.

Liang, B., Bundi, R. K., & Hartel, R. W. (2009). Effect of composition on moisture sorption of delactosed permeate. *International Dairy Journal*, *19*(10), 630–636. https://doi.org/10.1016/j.idairyj.2009.04.010.

Li-Chan, E. C. Y. (1996). The applications of Raman spectroscopy in food science. *Trends in Food Science & Technology*, *7*(11), 361–370. https://doi.org/10.1016/S0924-2244(96)10037-6

Lule, V., Garg, S., Tomar, S., Khedkar, C. D., & Nalage, D. N. (2016). Food intolerance: lactose intolerance. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), Encyclopedia of food and health (pp. 43–48). Oxford, UK: Elsevier Ltd. https://doi.org/10.1016/B978-0-12-384947-2.00312-3.

Maciel, G. M., Chaves, K. S., Grosso, C. R. F., & Gigante, M. L. (2014). Microencapsulation of *Lactobacillus acidophilus* La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. *Journal of Dairy Science*, *97*(4), 1991–1998. https://doi.org/10.3168/jds.2013-7463.

Market Research Report. (2020). Food Supplements — Probiotics Market (2020–2027). Available online: https://www.fortunebusinessinsights.com/industry-reports/probiotics-market-100083 (accessed on 6 June 2021).

McGoverin, C. M., Clark, A. S. S., Holroyd, S. E., & Gordon, K. C. (2010). Raman spectroscopic quantification of milk powder constituents. *Analytica Chimica Acta*, *673*(1), 26–32. https://doi.org/10.1016/j.aca.2010.05.014.

Muhammad, Z., Ramzan, R., Huo, G.-S., Tian, H., Bian, X. (2017) Integration of polysaccharide-thermoprotectant formulations for microencapsulation of *Lactobacillus plantarum*, appraisal of survivability and physico-biochemical properties during storage of spray dried powders. *Food Hydrocolloids*, *66*, 286–295. https://doi.org/10.1016/j.foodhyd.2016.11.040.

O'Mahony, J. A., Drapala, K. P., Mulcahy, E. M., & Mulvihill, D. M. (2017). Controlled glycation of milk proteins and peptides: Functional properties. *International Dairy Journal*, *67*, 16–34. https://doi.org/10.1016/j.idairyj.2016.09.012.

Oroian, M., Ropciuc, S., & Paduret, S. (2018). Honey adulteration detection using raman spectroscopy. *Food Analytical Methods*, *11*(4), 959–968. https://doi.org/10.1007/s12161-017-1072-2.

Paquet-Durand, O., Zettel, V., & Hitzmann, B. (2015). Optimal experimental design for parameter estimation of the Peleg model. *Chemometrics and Intelligent Laboratory Systems*, *140*, 36–42. https://doi.org/10.1016/j.chemolab.2014.10.006.

Parthasarathi, S., & Anandharamakrishnan, C. (2016). Enhancement of oral bioavailability of vitamin E by spray-freeze drying of whey protein

microcapsules. *Food and Bioproducts Processing*, *100*, 469–476. https://doi.org/10.1016/j.fbp.2016.09.004.

Passot, S., Cenard, S., Douania, I., Tréléa, I. C., & Fonseca, F. (2012). Critical water activity and amorphous state for optimal preservation of lyophilised lactic acid bacteria. *Food Chemistry*, *132*, 1699–1705. https://doi.org/10.1016/j.foodchem.2011.06.012.

Peleg, M. (1988). An empirical model for the description of moisture sorption curves. *Journal of Food Science*, *53*(4), 1216–1217. https://doi.org/10.1111/j.1365-2621.1988.tb13565.x.

Pilatti-Riccio, D., dos Santos, D. F., Meinhart, A. D., Knapp, M. A., Hackbart, H. C. dos S., Pinto, V. Z. (2019). Impact of the use of saccharides in the encapsulation of *Ilex paraguariensis* extract. *Food Research International*, *125*, article 108600. https://doi.org/10.1016/j.foodres.2019.108600.

Pinto, S. S., Cavalcante, B. D. M., Verruck, S., Alves, L. F., Prudêncio, E. S., & Amboni, R. D. M. C. (2017). Effect of the incorporation of *Bifidobacterium* BB-12 microencapsulated with sweet whey and inulin on the properties of Greek-style yogurt. *Journal of Food Science & Technology*, *54*, 2804–2813. https://doi.org/10.1007/s13197-017-2717-2.

Pinto, S. S., Fritzen-Freire, C. B., Benedetti, S., Murakami, F. S., Petrus, J. C. C., Prudêncio, E. S., & Amboni, R. D. M. C. (2015). Potential use of whey concentrate and prebiotics as carrier agents to protect *Bifidobacterium* BB-12 microencapsulated by spray drying. *Food Research International*, *67*, 400–408. https://doi.org/10.1016/j.foodres.2014.11.038.

Pinto, S. S., Fritzen-Freire, C. B., Dias, C. O., & Amboni, R. D. M. C. (2019). A potential technological application of probiotic microcapsules in lactose-free

Greek-style yoghurt. *International Dairy Journal*, *97*, 131–138. https://doi.org/10.1016/j.idairyj.2019.05.009.

Rajam, R., & Anandharamakrishnan, C. (2015). Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharide as wall material by spray drying. *LWT - Food Science and Technology*, *60*(2, Part 1), 773–780. https://doi.org/10.1016/j.lwt.2014.09.062.

Rao, M. A., & Rizvi, S. S. H. (1994). *Engineering properties of foods* (2th ed.). Marcel Dekker Inc, New York.

Reid, D. S., & Fennema, O. R. (1996). Water and ice. In O. R. Damodaran, S. Parkin, & K. L. Fennema (Eds.), *Fennema's food chemistry* (pp. 17–77) (4th ed.). CRC Press.

Rhim, J.-W., Koh, S., & Kim, J.-M. (2011). Effect of freezing temperature on rehydration and water vapor adsorption characteristics of freeze-dried rice porridge. *Journal of Food Engineering*, *104*(4), 484–491. https://doi.org/10.1016/j.jfoodeng.2010.08.010.

Rodrigues Junior, P. H., de Sa Oliveira, K., de Almeida, C. E., De Oliveira, L. F., Stephani, R., Pinto M. S., et al. (2016). FT-Raman and chemometric tools for rapid determination of quality parameters in milk powder: Classification of samples for the presence of lactose and fraud detection by addition of maltodextrin. *Food Chemistry*, *196*, 584–588. https://doi.org/10.1016/j.foodchem.2015.09.055.

Romano, N., Mobili, P., Zuñiga-Hansen, M. E., & Gómez-Zavaglia, A. (2018). Physico-chemical and structural properties of crystalline inulin explain the stability of *Lactobacillus plantarum* during spray-drying and storage. *Food*

Research International, *113*, 167–174. https://doi.org/10.1016/j.foodres.2018.07.007.

Ronkart, S., Blecker, C., Fougnies, C., Van Herck, J. C., Wouters, J., & Paquot, M. (2006). Determination of physical changes of inulin related to sorption isotherms: An X-ray diffraction, modulated differential scanning calorimetry and environmental scanning electron microscopy study. *Carbohydrate Polymers*, *63*(2), 210–217. https://doi.org/10.1016/j.carbpol.2005.08.030.

Ronkart, S. N., Deroanne, C., Paquot, M., Fougnies, C., & Blecker, C. S. (2010). Impact of the crystallisation pathway of inulin on its mono-hydrate to hemi-hydrate thermal transition. *Food Chemistry*, *119*, 317–322. https://doi.org/10.1016/j.foodchem.2009.06.035.

Ronkart, S. N., Paquot, M., Blecker, C. S., Fougnies, C., Doran, L., Lambrechts, J. C., Norberg, B., & Deroanne, C. (2009). Impact of the crystallinity on the physical properties of inulin during water sorption. *Food Biophysics*, *4*, 49–58. https://doi.org/10.1007/s11483-008-9097-0.

Ruano-Uscategui, D. C., Ciro-Velásquez, H., & Sepúlveda-Valencia, U. (2018). Estudio cinético de algunas características de calidad de un producto en polvo obtenido mediante secado por aspersión a partir de jugo de caña panelera y concentrado proteico de lactosuero dulce. *Revista UIS Ingenierías*, *17*(2), 127–140. https://doi.org/10.18273/revuin.v17n2-2018012.

Saavedra-Leos, M. Z., Alvarez-Salas, C., Esneider-Alcalá, M. A., Toxqui-Terán, A., Pérez-García, S. A., & Ruiz-Cabrera, M. A. (2012). Towards an improved calorimetric methodology for glass transition temperature determination in amorphous sugars. *CyTA - Journal of Food*, *10*(4), 258–267. https://doi.org/10.1080/19476337.2011.639960.

Saavedra-Leos, M. Z., Leyva-Porras, C., Martínez-Guerra, E., Pérez-García, S. A., Aguilar-Martínez, J. A., & Álvarez-Salas, C. (2014). Physical properties of inulin and inulin-orange juice: Physical characterization and technological application. *Carbohydrate Polymers*, *105*(1), 10–19. https://doi.org/10.1016/j.carbpol.2013.12.079.

Santos, M. A. S., Machado, M. T. C. (2021). Coated alginate–chitosan particles to improve the stability of probiotic yeast. *International Journal of Food Science and Technology*, *56*(5), 2122–2131. https://doi.org/10.1111/ijfs.14829.

Schuck, P., Blanchard, E., Dolivet, A., Méjean, S., Onillon, E., & Jeantet, R. (2005). Water activity and glass transition in dairy ingredients. *Lait*, *85*, 295–304. https://doi.org/10.1051/lait:2005020.

Schuck, P. (2011). Dehydrated dairy products. Milk powder: Physical and functional properties of milk powders. In J. W. Fuquay, P. F. Fox, & P. L. H. McSweeney (Eds.). *Encyclopedia of dairy sciences* (2th ed.). London, UK: Elsevier Ltd 2:117-2:124. https://doi.org/10.1016/B978-0-12-374407-4.00122-9.

Szulc, K., Nazarko, J., Ostrowska-Ligęza, E., & Lenart, A. (2016). Effect of fat replacement on flow and thermal properties of dairy powders. *LWT - Food Science and Technology, 68,* 653–658. https://doi.org/10.1016/j.lwt.2015.12.060.

Silva, E. K., & Meireles, M. A. A. (2015). Influence of the degree of inulin polymerization on the ultrasound-assisted encapsulation of annatto seed oil. *Carbohydrate Polymers*, *133*, 578–586. https://doi.org/10.1016/j.carbpol.2015.07.025.

Silva, E. K., Zabot, G. L., Bargas, M. A., & Meireles, A. A. (2016). Microencapsulation of lipophilic bioactive compounds using prebiotic carbohydrates: effect of the

degree of inulin polymerization. *Carbohydrate Polymers*, *152*, 775–783. https://doi.org/10.1016/j.carbpol.2016.07.066

Seth, D., Dash, K. K., Mishra, H. N., & Deka, S. C. (2018). Thermodynamics of sorption isotherms and storage stability of spray dried sweetened yoghurt powder. *Journal of Food Science & Technology*, *55*(10), 4139–4147. https://doi.org/10.1007/s13197-018-3340-6.

Soukoulis, C., Behboudi-Jobbehdar, S., Yonekura, L., Parmenter, C., & Fisk, I. (2014). Impact of milk protein type on the viability and storage stability of microencapsulated *Lactobacillus acidophilus* NCIMB 701748 using spray drying. *Food and Bioprocess Technology*, *7*(5), 1255–1268. https://doi.org/10.1007/s11947-013-1120-x.

Suri, S., Kumar, V., Prasad, R., Tanwar, B., Goyal, A., Kauar, S., et al. (2019). Considerations for development of lactose-free food. *Journal of Nutrition & Intermediary Metabolism*, *15*, 27–34. https://doi.org/10.1016/j.jnim.2018.11.003.

Torres, J. K. F., Stephani, R., Tavares, G. M., de Carvalho, A. F., Costa, R. G. B., de Almeida, et al. (2017). Technological aspects of lactose-hydrolyzed milk powder. *Food Research International*, *101*, 45–53. https://doi.org/10.1016/j.foodres.2017.08.043.

Turchiuli, C., Gianfrancesco, A., Palzer, S., & Dumoulin, E. (2011). Evolution of particle properties during spray drying in relation with stickiness and agglomeration control. *Powder Technology*, *208*(2), 433–440. https://doi.org /10.1016/j.powtec.2010.08.040.

Varghese, S., Ramachandrannair, S. V., & Mishra, H. N. (2009). Moisture sorption characteristics of curd (Indian yogurt) powder. *International Journal of Dairy Technology*, *62*, 85–92. https://doi.org/10.1111/j.1471-0307.2008.00441.x.

Verruck, S., Barretta, C., Miotto, M., Canella, M. H. M., de Liz, G. R., Maran, B. M., et al. (2020). Evaluation of the interaction between microencapsulated *Bifidobacterium* BB-12 added in goat's milk Frozen Yogurt and *Escherichia coli* in the large intestine. *Food Research International*, *127*, Article 108690. https://doi.org/10.1016/j.foodres.2019.108690.

Verruck, S., de Carvalho, M. W., de Liz, G. R., Amante, E. R., Vieira, C. R. W., Amboni, R. D. D., et al. (2017). Survival of *Bifidobacterium* BB-12 microencapsulated with full-fat goat's milk and prebiotics when exposed to simulated gastrointestinal conditions and thermal treatments. *Small Ruminant Research*, *153*, 48–56. https://doi.org/10.1016/j.smallrumres.2017.05.008.

Verruck, S., de Liz, G. R., Dias, C. O., Amboni, R. D. M. C., & Prudencio, E. S. (2019). Effect of full-fat goat's milk and prebiotics use on *Bifidobacterium* BB-12 survival and on the physical properties of spray-dried powders under storage conditions. *Food Research International*, *119*, 643–652 https://doi.org/10.1016/j.foodres.2018.10.042.

Verruck, S., Santana, F., de Olivera Müller, C., & Prudencio, E. S. (2018). Thermal and water sorption properties of *Bifidobacterium* BB-12 microcapsules obtained from goat's milk and prebiotics. *LWT - Food Science and Technology*, *98*, 314–321. https://doi.org/10.1016/j.lwt.2018.08.060.

Vinderola, C. G., & Reinheimer, J. A. (1999). Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of

yoghurt bacteria. *International Dairy Journal*, *9*(8), 497–505. https://doi.org/10.1016/S0958-6946(99)00120-X.

Vivek, K., Mishra, S., & Pradhan, R. C. (2020). Characterization of spray dried probiotic Sohiong fruit powder with *Lactobacillus plantarum*. *LWT - Food Science and Technology*, *117*, Article 108699. https://doi.org/10.1016/j.lwt.2019.108699.

Vuataz, G., Meunier, V., & Andrieux, J. C. (2010). TG-DTA approach for designing reference methods for moisture content determination in food powders. *Food Chemistry*, *122*(2), 436–442. https://doi.org/10.1016/j.foodchem.2009.05.066.

Wang, A., Lin, J., & Zhong, Q. (2020). Physical and microbiological properties of powdered *Lactobacillus salivarius* NRRL B-30514 as affected by relative amounts of dairy proteins and lactose. *LWT - Food Science and Technology*, *121*, Article 109044. https://doi.org/10.1016/j.lwt.2020.109044.

Yazdanpanah, N., & Langrish, T. A. G. (2016). Heterogeneous particle structure formation during post-crystallization of spray-dried powder. *Particuology*, *27*, 72–79. https://doi.org/10.1016/j.partic.2015.09.007.

Ying, DY., Sanguansri, L., Weerakkody, R., Bull, M., Singh, T. J., & Augustin, M. A. (2016). Effect of encapsulant matrix on stability of microencapsulated probiotics. *Journal of Functional Foods*, *25*, 447–458. https://doi.org/10.1016/j.jff.2016.06.020.

Zhang, S., Kim, N., Yokoyam, W., & Kim, Y. (2018). Effects of moisture content on mechanical properties, transparency, and thermal stability of yuba film. *Food Chemistry, 243*, 202–207. https://doi.org/10.1016/j.foodchem.2017.09.127.

933 Zhou, P., & Labuza, T. P. (2011). Differential scanning calorimetry. In J. W. Fuquay, P.
934 F. Fox, & P. L. H. Mcsweeney (Eds.). *Encyclopedia of dairy sciences* (2th ed.).
935 London, UK: Elsevier Ltd 1:256-1:263.
936 Zhu, S. C., Ying, D. Y., Sanguansri, L., Tang, J. W., & Augustin, M. A. (2013). Both
937 stereo-isomers of glucose enhance the survival rate of microencapsulated
938 *Lactobacillus rhamnosus* GG during storage in the dry state. *Journal of Food*
939 *Engineering*, *116*, 809–813. http://dx.doi.org/10.1016/j.jfoodeng.2013.01.028.

Table 1
Peleg model coefficients and fit parameters. 1/k1 = g water. (g dry solid. h) $^{-1}$; 1/k2 = g water. (g dry solid) $^{-1}$.

Samples	% RH	1/k1	1/k2	R^2	SSE
Spray-dried powder 1	11	0.0186 ± 0.0057^{bA}	0.0266 ± 0.0007^{fA}	0.9676 ± 0.0061^{cB}	4.02×10^{-5}
	33	0.0451 ± 0.0074^{aA}	0.0741 ± 0.0018^{eAB}	0.9840 ± 0.0041^{bB}	1.46×10^{-4}
	43	0.0408 ± 0.0042^{abA}	0.0952 ± 0.0010^{eA}	0.9917 ± 0.0020^{abA}	1.69×10^{-4}
	58	0.0556 ± 0.0039^{aA}	0.1487 ± 0.0018^{dA}	0.9934 ± 0.0003^{abA}	3.49×10^{-4}
	75	0.0478 ± 0.0031^{aA}	0.2787 ± 0.0074^{cA}	0.9975 ± 0.0005^{aA}	4.29×10^{-4}
	80	0.0583 ± 0.0089^{aA}	0.3802 ± 0.0206^{bA}	0.9965 ± 0.0008^{aA}	1.78×10^{-3}
	90	0.0427 ± 0.0064^{aA}	0.6936 ± 0.0106^{aA}	0.9920 ± 0.0014^{abA}	1.46×10^{-2}
Spray-dried powder 2	11	0.0144 ± 0.0005^{bA}	0.0262 ± 0.0019^{gA}	0.9892 ± 0.0019^{aA}	1.18×10^{-5}
	33	0.0356 ± 0.0091^{abA}	0.0674 ± 0.0025^{fB}	0.9927 ± 0.0025^{aAB}	5.22×10^{-5}
	43	0.0359 ± 0.0061^{abA}	0.0921 ± 0.0003^{eA}	0.9895 ± 0.0033^{aA}	2.03×10^{-4}
	58	0.0348 ± 0.0022^{abB}	0.1431 ± 0.0002^{dA}	0.9954 ± 0.0003^{aA}	2.27×10^{-4}
	75	0.0524 ± 0.0071^{aA}	0.2390 ± 0.0019^{cB}	0.9960 ± 0.0029^{aA}	5.38×10^{-4}
	80	0.0500 ± 0.0057^{aA}	0.2850 ± 0.0012^{bB}	0.9964 ± 0.0002^{aA}	1.06×10^{-3}
	90	0.0403 ± 0.0023^{aA}	0.4860 ± 0.0074^{aC}	0.9933 ± 0.0020^{aA}	5.93×10^{-3}
Spray-dried powder 3	11	0.0100 ± 0.0001^{cA}	0.0241 ± 0.0004^{fA}	0.9877 ± 0.0034^{aA}	1.09×10^{-5}

33	0.0182 ± 0.0024^{bcA}	0.0766 ± 0.0003^{eA}	0.9981 ± 0.0008^{aA}	1.46×10^{-5}
43	0.0279 ± 0.0005^{abA}	0.0963 ± 0.0024^{eA}	0.9910 ± 0.0012^{aA}	1.92×10^{-4}
58	0.0315 ± 0.0045^{abB}	0.1543 ± 0.0094^{dA}	0.9908 ± 0.0075^{aA}	5.42×10^{-4}
75	0.0387 ± 0.0030^{aA}	0.2767 ± 0.0022^{cA}	0.9983 ± 0.0004^{aA}	2.75×10^{-4}
80	0.0336 ± 0.0054^{aA}	0.3497 ± 0.0032^{bA}	0.9983 ± 0.0010^{aA}	7.56×10^{-4}
90	0.0306 ± 0.0056^{abA}	0.5706 ± 0.0088^{aB}	0.9954 ± 0.0004^{aA}	5.57×10^{-3}

$^{a–g}$ Means ± standard deviation with different superscript lowercase letters in the same column indicate significant differences ($P < 0.05$) among the different relative humidity (RH) for each sample. $^{A–C}$Means ± standard deviation with different superscript uppercase letters in the same column indicate significant differences ($P < 0.05$) among the samples on the same relative humidity (RH). Means found in triplicate.

Table 2
GAB model coefficients and fit parameters. M_0 = g water. (g dry matter) $^{-1}$.

	Spray-dried powder 1	Spray-dried powder 2	Spray-dried powder 3
M_0	0.084 ± 0.005^{ab}	0.073 ± 0.004^{b}	0.092 ± 0.001^{a}
C	2.573 ± 0.583^{a}	3.786 ± 0.775^{a}	1.994 ± 0.018^{a}
k	0.977 ± 0.005^{a}	0.944 ± 0.008^{b}	0.935 ± 0.005^{b}
R^2	0.998 ± 0.002^{a}	0.999 ± 0.001^{a}	0.998 ± 0.001^{a}
SSE	0.00143	0.00016	0.00072

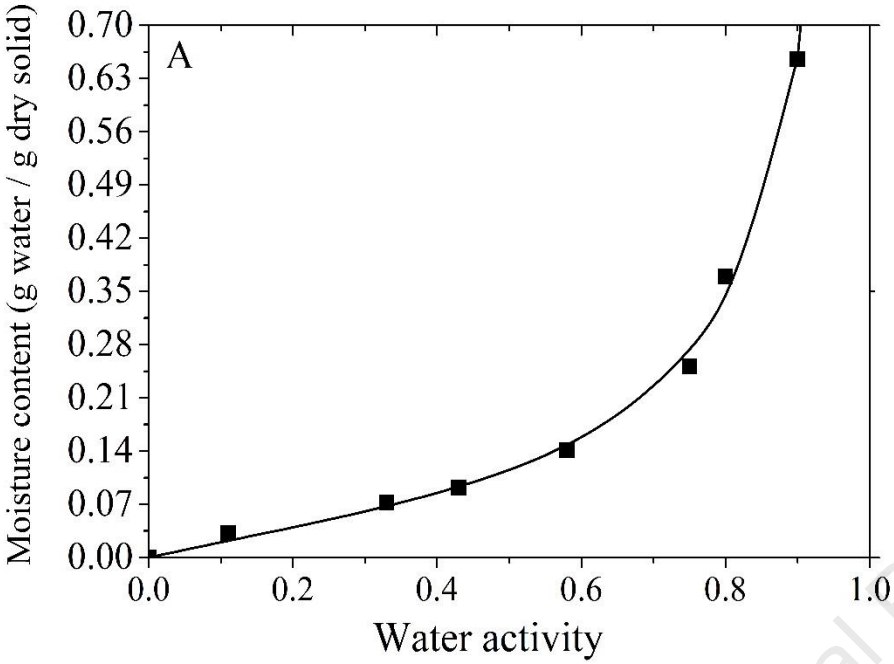
Means ± standard deviation with different superscript lowercase letters in the same line indicate significant differences ($P < 0.05$) between samples. Means found in triplicate.

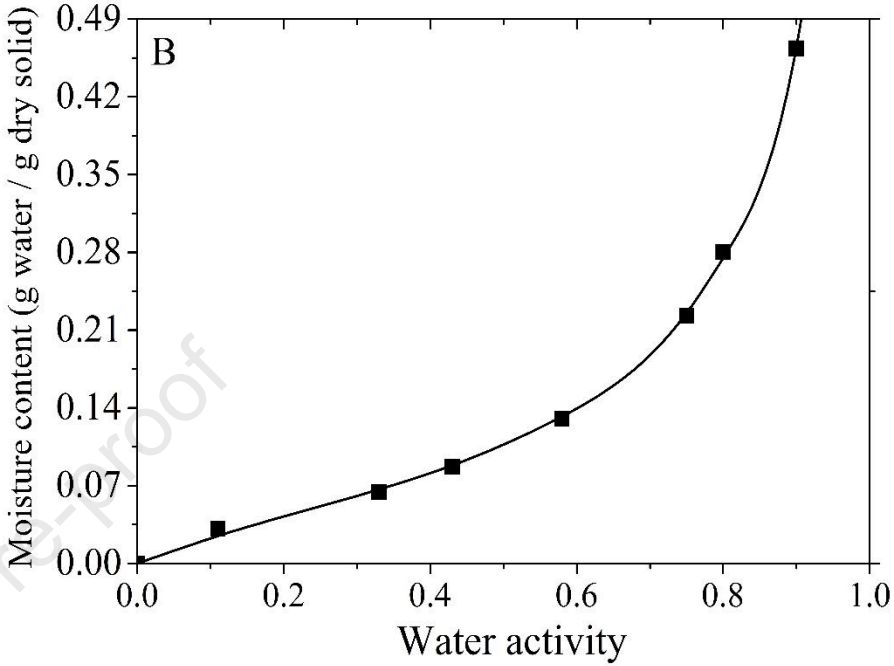
Table 3
Main Raman wavenumbers, in cm^{-1}, and their respective tentative assignments.

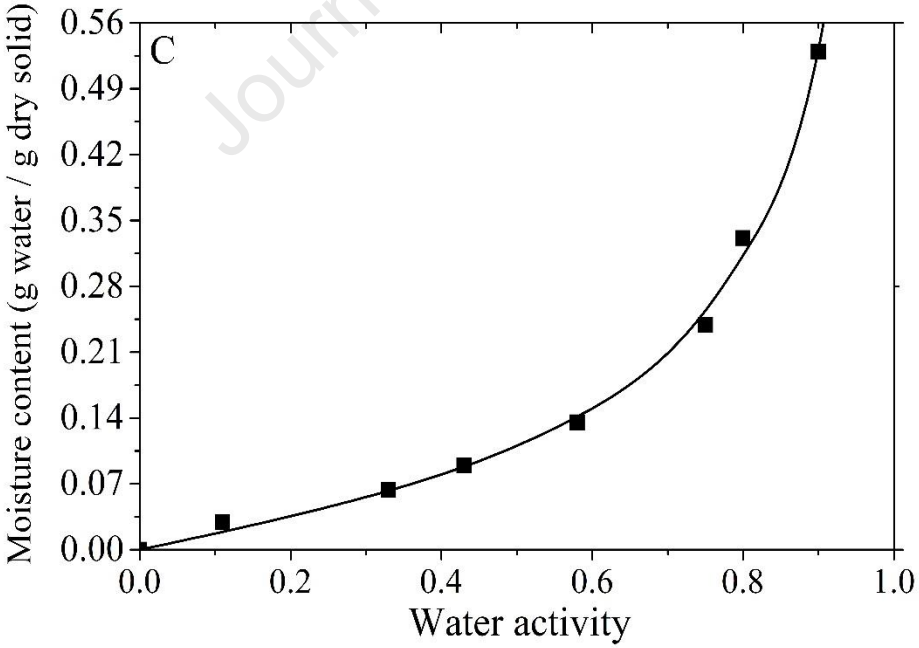
Sample	Raman shift (cm^{-1})	Assignment	Reference
Spray-dried powder 1	1259.15	δ (N–H) Amide III; ν (C–N) Amide III; and/or γ $(CH_2)_{twisting}$	(Li-Chan, 1996; Rodrigues Junior et al., 2016)
	1352.03	Glucose	(Torres et al., 2017)
	1453.83	δ $(CH_2)_{scissoring}$	(McGoverin, Clark, Holroyd, & Gordon, 2010)
	1660.4 m	ν (C=O) Amide I; γ $(N–H)_{wagging}$ Amide I	(Almeida et al., 2011; Li-Chan, 1996)
Spray-dried powder 2	536.127 vs	glucose ring def	(Balan et al., 2018)
	620.835 s	def (O–C–O); def (O–H)	(Balan et al., 2018)
	1122.97 m	ν (C–O); ν (C–C); δ (C–O–H)	(Rodrigues Junior et al., 2016)
	1263.39 m	δ (N–H) Amide III; ν (C–N) Amide III; and/or γ $(CH_2)_{twisting}$	(Li-Chan, 1996; Rodrigues Junior et al., 2016)
	1665.1	ν (C=O) Amide I; γ $(N–H)_{wagging}$ Amide I	(Almeida et al., 2011; Li-Chan, 1996)
Spray-dried powder 3	621.938 s	def (O–C–O); def (O–H)	(Balan et al., 2018)
	1064.11 m	ν (C–O); ν (C–C); δ (C–O–H)	(Rodrigues Junior et al., 2016)
	1123.69 m	ν (C–O); ν (C–C); δ (C–O–H)	(Rodrigues Junior et al., 2016)

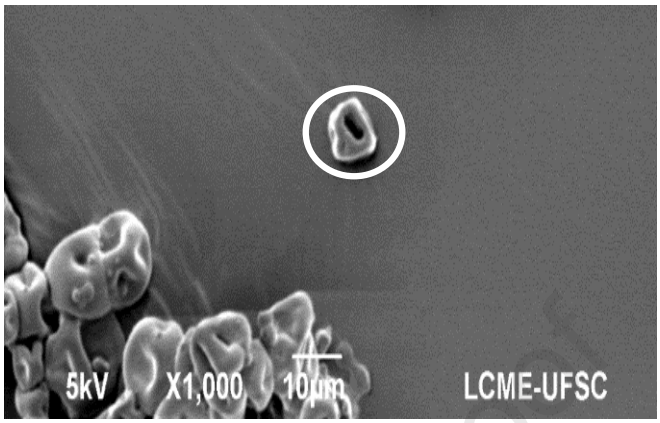
	1262.77 m	δ (N–H) Amide III; ν (C–N) Amide III; and/or γ $(CH_2)_{twisting}$	(Li-Chan, 1996; Rodrigues Junior et al., 2016)
	1665.8	ν (C=O) Amide I; γ $(N–H)_{wagging}$ Amide I	(Almeida et al., 2011; Li-Chan, 1996)
Oligofructose	465.421	δ (O–H)	(Balan et al., 2018)
	531.073 vs	glucose ring def	(Balan et al., 2018)
	616.226 s	def (O–C–O); def (O–H)	(Balan et al., 2018)
	674.193 m	def (O–C–C); def (O–H); def (C–H)	(Balan et al., 2018)
	823.398 s	δ (O–H); def (C–H)	(Balan et al., 2018)
	1458.65	δ $(CH_2)_{scissoring}$	(McGoverin et al., 2010)
Inulin	464.955	δ (O–H)	(Balan et al., 2018)
	532.485 vs	glucose ring def	(Balan et al., 2018)
	607.886	Fructose	(Oroian et al., 2018)
	663.164 m	def (O–C–C); def (O–H); def (C–H)	(Balan et al., 2018)
	707.939 w	def (O–H)	(Balan et al., 2018)
	816.277 vs	def (C–C–O); def (O–C–C); δ (C–H)	(Balan et al., 2018)
	830.212 s	δ (O–H); def (C–H)	(Balan et al., 2018)
	1461.17	δ $(CH_2)_{scissoring}$	(McGoverin et al., 2010)

Vs – very strong; s – strong; m – medium; w – weak.

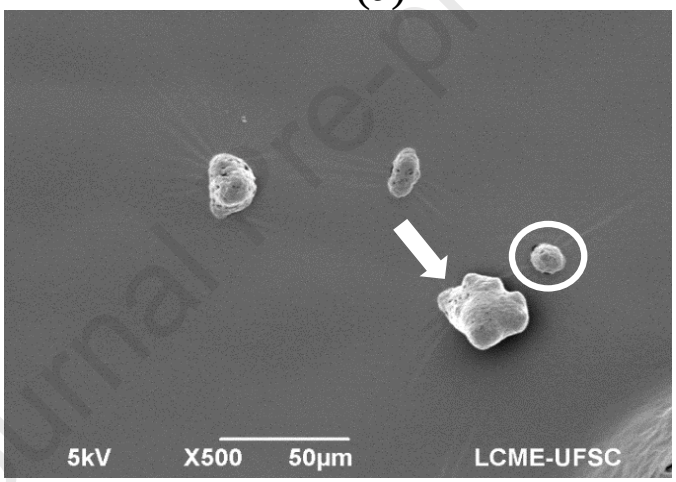




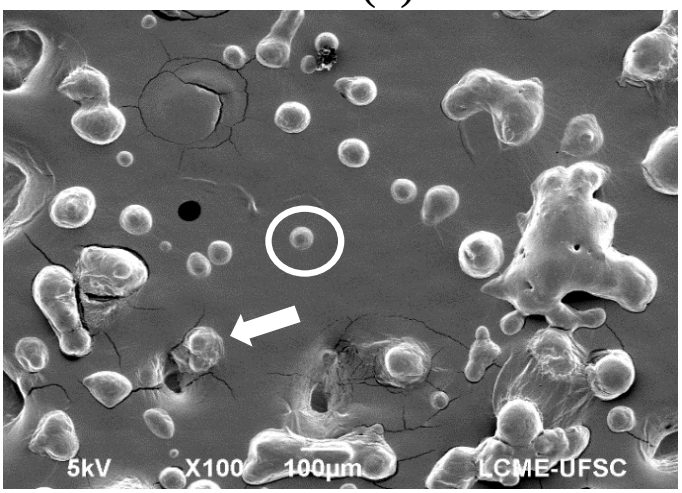




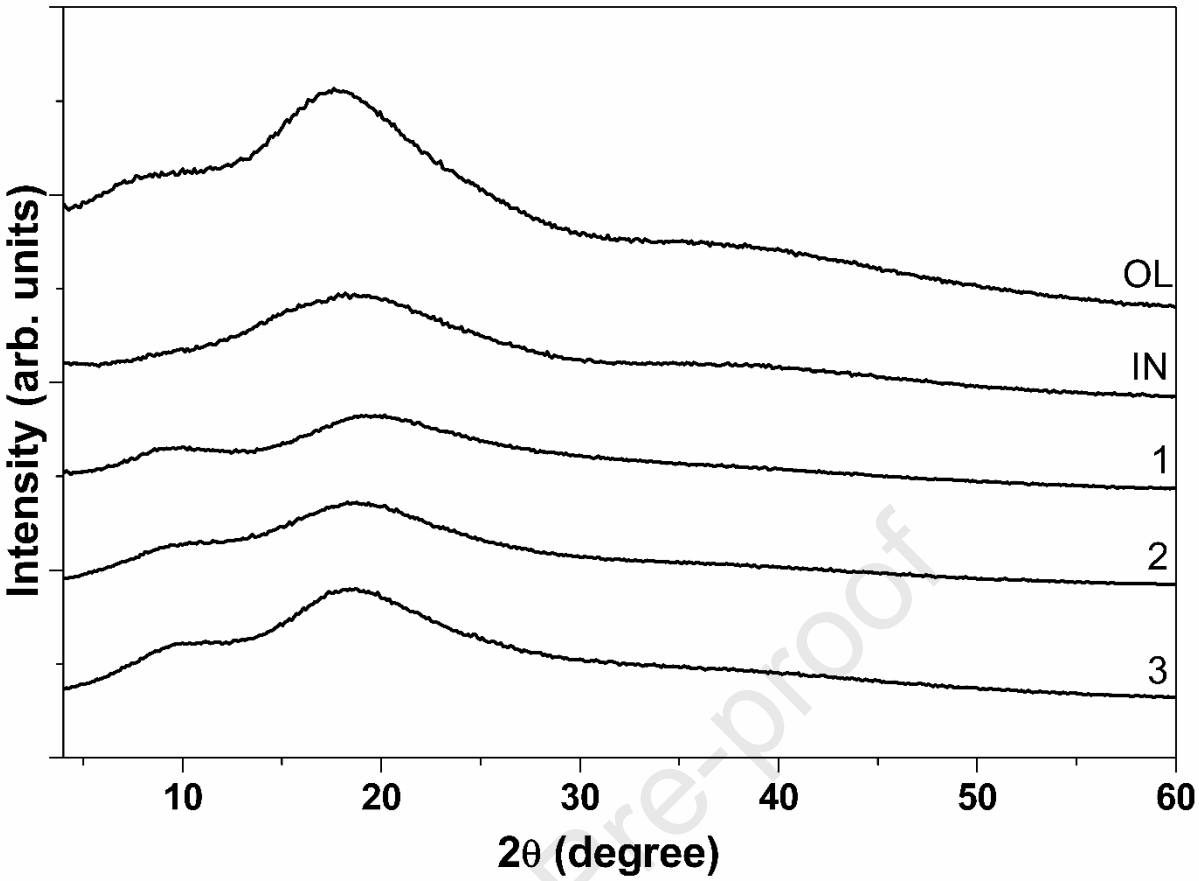
(a)

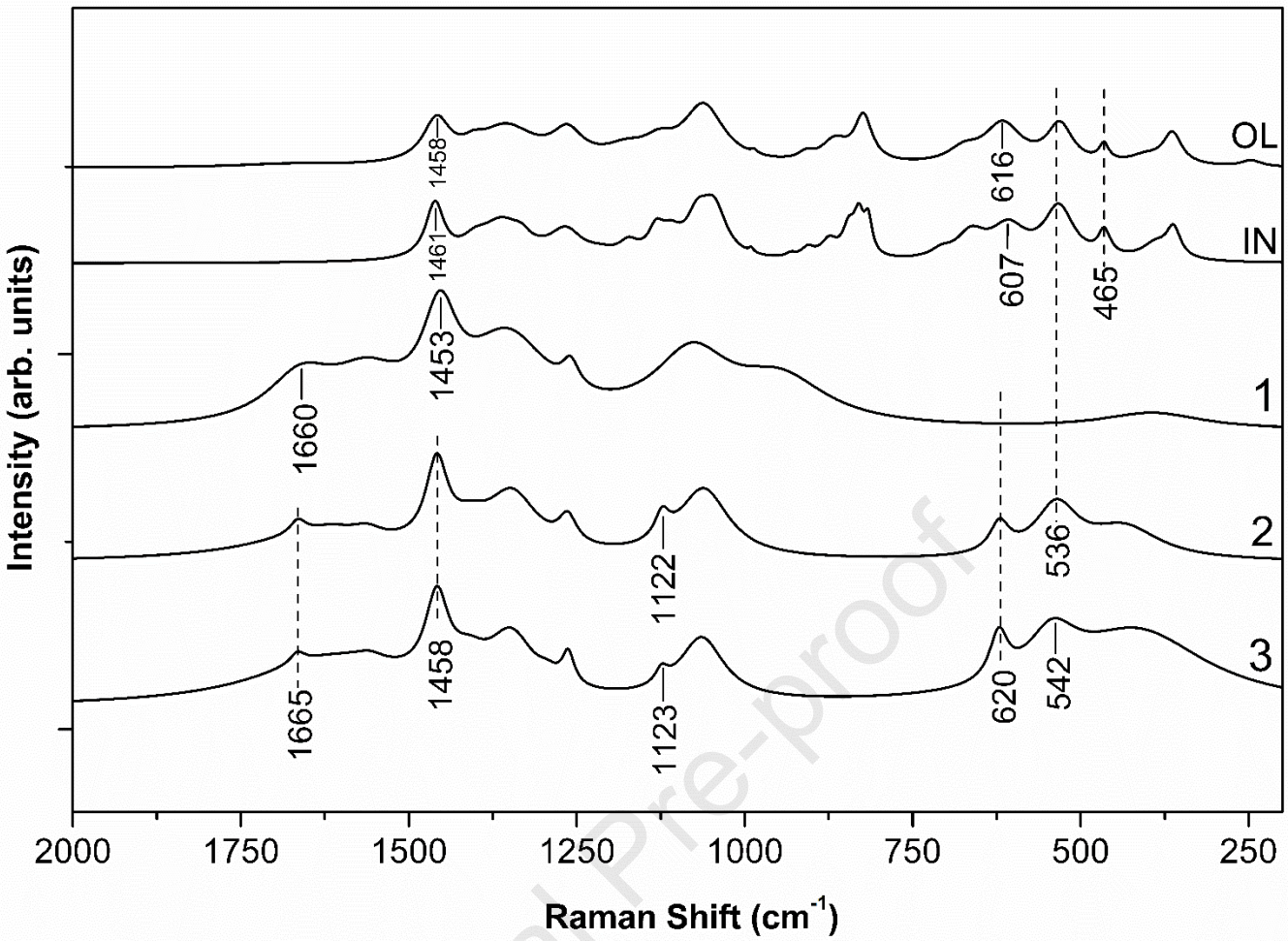


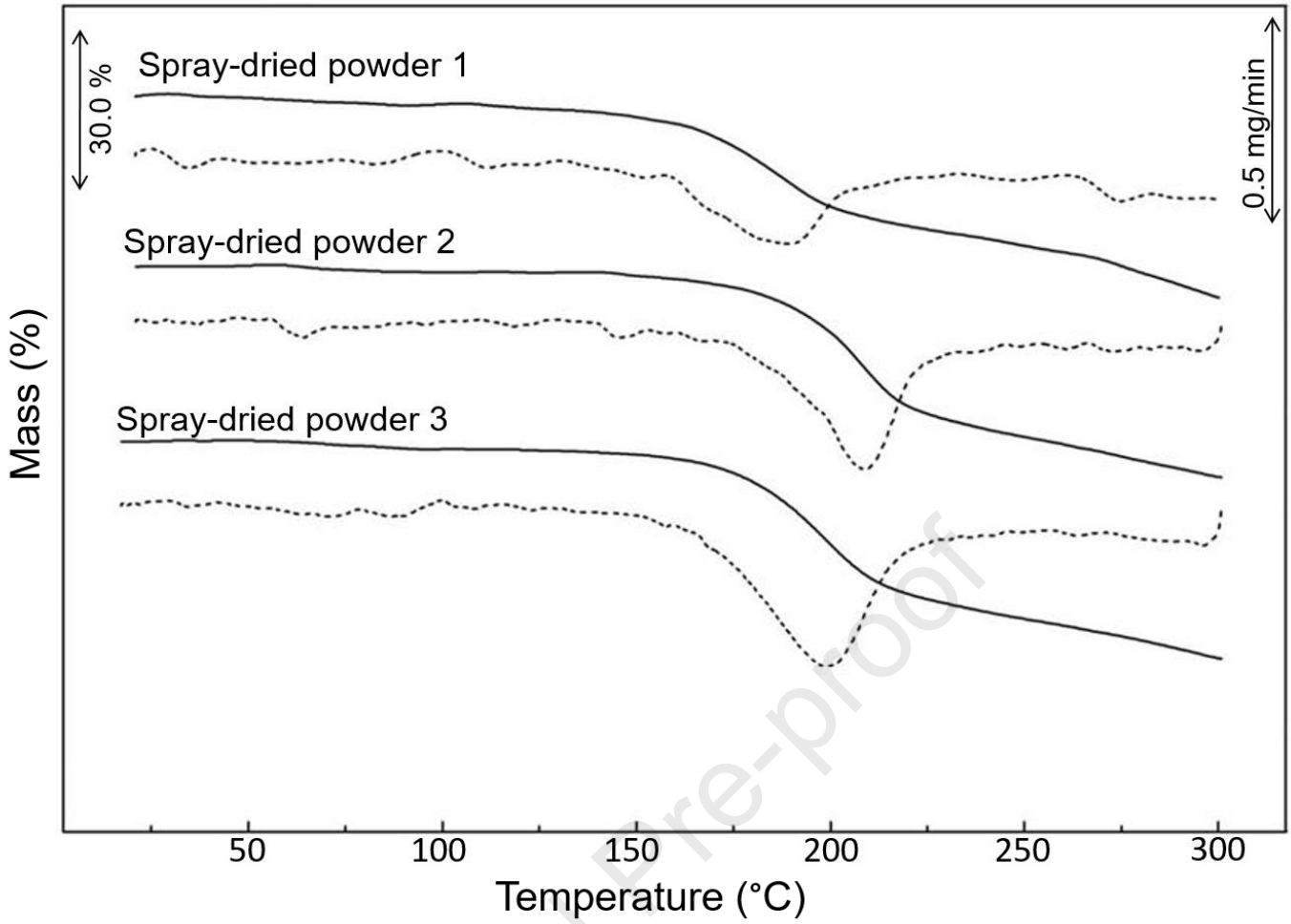
(b)

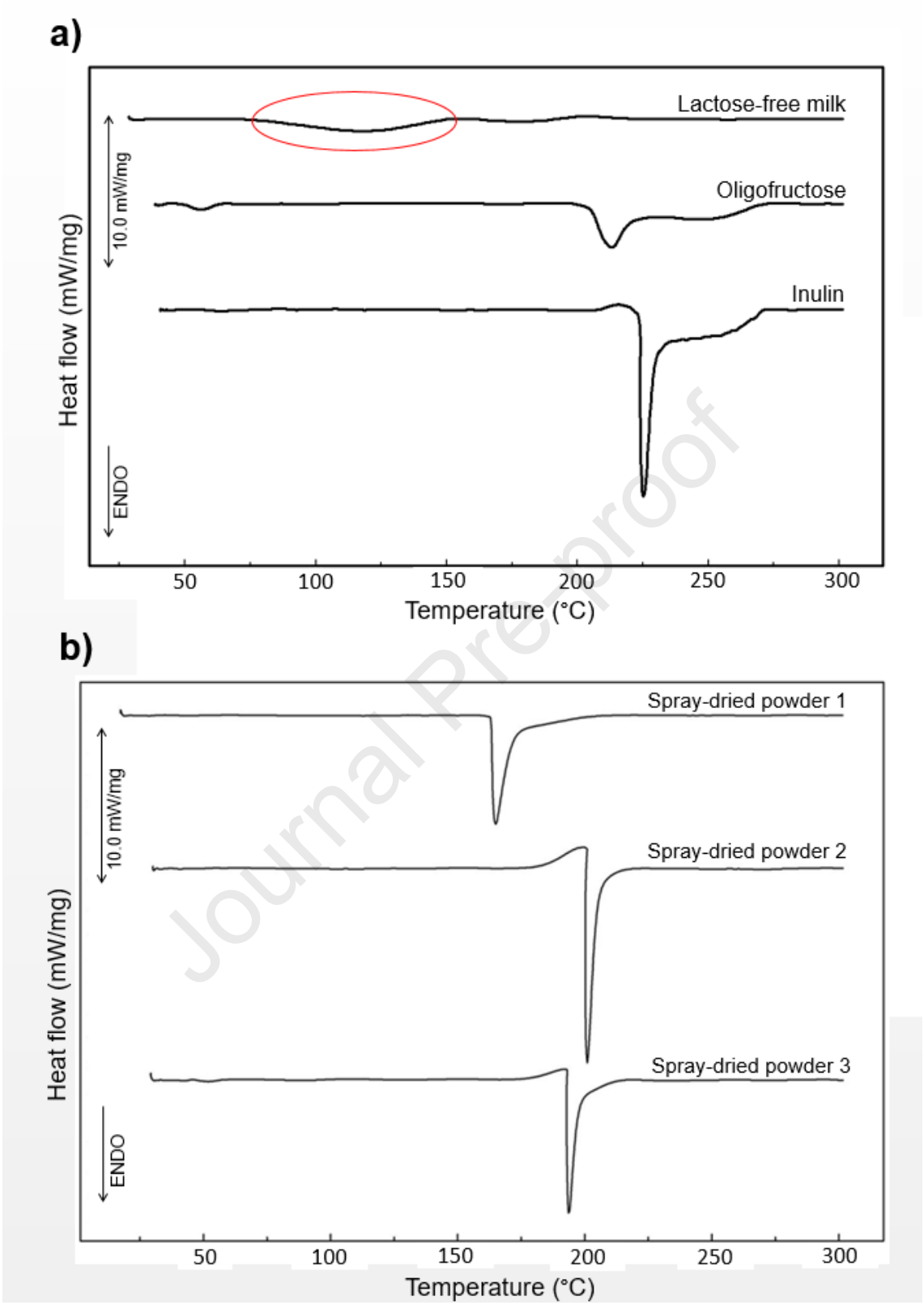


(c)









- Probiotic stability was found for the microspheres with lactose-free milk.
- Probiotic stability was found for the microspheres with prebiotics.
- The amorphous sugars were responsible to obtain a Type III isotherm.
- RH > 33 and 43% were not able to maintain the spray-dried powders stability.
- The inulin conferred greater thermal stability to spray-dried powder.

Federal University of Santa Catarina / UFSC
Department of Food Science and Technology / CAL
Laboratory of Milk and Dairy Products

Florianópolis, March 17, 2021

Rakesh K. Singh, Ph.D.

Editor-in-Chief of *LWT – Food Science and Technology*

Dear Editor,

The authors declare that they have no conflict of interest.

Best regards,

MSc. Adriana Dantas
Federal University of Santa Catarina - Department of Food Science and Technology
Rodovia Admar Gonzaga, 1346, Itacorubi
88034-001- Florianópolis - Santa Catarina - Brazil
E-mail: sorrisitah@gmail.com