Effect of drying technique and feed flow rate on bacterial survival and physicochemical properties of a non-dairy fermented probiotic juice powder

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Abstract

Drying of probiotic fruit juice can be a good alternative for new probiotic foodstuff production since most probiotic foods are dairy-based products, which are not suitable for people with galactosemia, lactose intolerance or allergy to milk protein. Thus, the aim of this study was to evaluate the influence of spray and spouted bed drying and feed flow rate on the microorganism survival and physicochemical properties of probiotic orange juice powder. Fermented probiotic orange juice containing *Lactobacillus casei* NRRL B-442 was spray-dried at 140 °C and spouted bed dried at 60 °C, respectively. Three different feed flow rates were studied for each equipment. Maltodextrin and gum Arabic were used as drying agents at 15% (w/w). The low temperature used in the spouted bed drying resulted in better probiotic bacteria viability compared to the product obtained by spray drying. Moisture and glass transition temperature were improved at the lowest feed flow rate (0.2 L/h), and faster rehydration time was achieved using maltodextrin. Thus, the spouted bed drying at low feed flow rates using maltodextrin as drying agent were the best parameters to produce powder fermented probiotic orange juice with high viability levels after drying and with good physicochemical parameters.

Key-words: Spray dryer; spouted bed; probiotics; *Lactobacillus casei*; fruit juice.

1. Introduction

Nowadays a great claim for health, well-being, and practical food consumption, leads the food industry and the researchers to the development of functional foods such as probiotics (Martins et al., 2013). Despite the growing demand for new products, the inclusion of probiotics in food matrices is still a challenging area of research in food technology (Martín et al., 2015).

A large number of probiotic products available in the marketing comprises mainly dairy products. However, a great part of the world population is affected by lactose-intolerance and cannot consume dairy-based products (Perricone et al., 2015). In addition, many people suffer from milk allergy, galactosemia and hypercholesterolemia, which also imparts restrictions to dairy products consumption (Martins et al., 2013; Mestry et al., 2011). Thus, there is a great interest in the development of probiotic products using non-dairy sources (Antunes et al., 2013). Several types of non-dairy food matrices such as fruits and vegetable juice have been successfully used to produce probiotic beverages, such as cashew juice (Pereira et al., 2011), cantaloupe melon juice (Fonteles et al., 2011) and pineapple juice (Costa, Fonteles, Jesus, & Rodrigues, 2013). However, fruit juices present a high amount of water, which increases their transport cost. Non-sterile fruit juices should be stored under refrigerate temperatures. Drying is a way to decrease the product transportation and storage cost (Krishnaiah et al., 2014), besides providing storage under ambient conditions (Rocha et al., 2011).

Fruit juices atomization is not an easy process due to the presence of a high proportion of low molecular weight sugars and short-chain organic acids in their composition (Cano-Chauca et al., 2005; Igual et al., 2014). Low molecular weight components impart low glass transition temperature (T_g) to the product that is associated with the powders tendency to forming agglomerates and become sticky (stickiness) (Cano-Chauca et al., 2005). Stickiness

phenomenon occurs when particles insufficiently dried collide with another particle or with the drier wall and become stuck. Stickiness is related to low yield, operating problems, and powder-handling difficulties (Downton et al., 1982). Thus, fruit juice atomization requires proper adjustment of the drying parameters, such as drying temperature, outlet air temperature, feed flow rate, air drying speed, atomization pressure and the use of drying agents to ensure the physicochemical quality of the powder product (Chegini, Khazaei, Ghobadian, & Goudarzi, 2008). Different high molecular weight substances as maltodextrins and gum Arabic have been reported as good alternatives to be used as drying agents in fruit juice (Fazaeli et al., 2012; Phisut, 2012). They act by **increasing** the glass transition temperature and reducing the stickiness and caking, producing free flowing powder with improved handling and physicochemical properties (Krishnaiah et al., 2014).

When the drying is performed in a product containing living cells, as probiotic juices, the viability of these microorganisms becomes one of the most important parameter to be observed (Mestry et al., 2011). Spray drying is commonly used as microencapsulation method in the food industry since it is economical and flexible (Martín et al., 2015). However, the high temperature used in spray drying might cause injuries to microbial cells (Golowczyc et al., 2011). The drying of pastes and suspensions in spouted bed is a lower cost alternative to the spray drying. Spouted bed is able to produce powders with the same quality level of the spray-drying (Bezerra, Amante, de Oliveira, Rodrigues, & da Silva, 2013; Costa, Andreola, de Andrade Mattietto, de Faria, & Taranto, 2015; Medeiros et al., 2002). In addition, the spouted bed drying uses lower temperatures than the spray drying, which can help to preserve the microorganisms and bioactive compounds of interest. To our knowledge, no studies have been performed on spouted bed drying of probiotic fruit juices containing probiotics.

Thus, the goal of this work was to evaluate the influence of spouted bed and spray drying techniques on the viability of the probiotic microorganism and on the physicochemical properties of fermented probiotic orange juice.

2. Materials and Methods

2.1. Raw Material

Frozen concentrate orange juice without added sugars and preservatives (LANJAL[®]) was obtained from a local market. *Lactobacillus casei* NRRL B-442 was donated by the United States Department of Agriculture (NRRL *Culture Collection*, Peoria Illinois, USA).

2.2. Microorganism and Inoculum Preparation

Lactobacillus casei lyophilized cells were activated in MRS (Man Rogosa and Sharpe) broth (Himedia, Mumbai, India) at 37 °C for 12 h. Glycerol 50% (v/v) (Vetec Química, Brazil) was then added to the culture before storage at -20 °C in sterile screw cap tubes. For inoculum preparation, the stock culture of *L. casei* was inoculated in 100 mL of MRS broth at 37 °C until a cell concentration of approximately 8.0 log colony forming units per milliliter (log CFU/mL) was achieved.

2.3. Orange Juice Fermentation

Frozen concentrate orange juice was diluted with water (1:7) and the juice pH was adjusted to 6.0 with NaOH 12N. The diluted juice was inoculated with 2% (v/v) of the inoculum and incubated at 30 °C for 20 h in a BOD incubator (MA 415, Marconi).

2.4. Probiotic Juice Drying

Before drying, the fermented orange juice was mixed separately with each one of the following drying agents: maltodextrin dextrose equivalent (DE) 20 (Cargill, Brazil) or gum Arabic (Willy Benecke, Germany), both at 15% (w/w). A stirrer (BestCook, China) was used to homogenize the mixture, which stayed at 25 °C for 30 min before drying.

The drying of fermented probiotic orange juice was carried out using a mini spray dryer (MSD 1.0, LU-228, Labmaq do Brasil Ltd, SP, Brazil) and a mini spouted bed dryer (FBD 3.0, Labmaq do Brasil Ltd, SP, Brazil) equipped with a conical stainless steel drying chamber (h = 881 mm; D1 = 350 mm; D2 = 102 mm). During spray drying, inlet air temperature, nozzle air flow rate and hot drying air flow rate were kept at 140 °C, 30 L/min and 3.5 m³/min, respectively. In the spouted bed drying, inlet air temperature, fluidizing air flow rate and nozzle air flow rate were kept at 60 °C, 1.7 m³/min and 30 L/min, respectively. Polystyrene spheres (400 g) with 3 mm of diameter were used as inert particles. Feed flow rates of 0.2, 0.5 and 0.7 L/h were used in the spray drying and 0.2, 0.3 and 0.4 L/h in the spouted bed drying. These values were chosen according to the minimum and maximum limits for each equipment. Both equipments were allowed to reach uniform process temperature for 15 min before drying.

2.5. Microbial Viability

Before drying, dilutions (up to 10⁶) of the fermented probiotic orange juice were done in sterile peptone water. The diluted samples were spread on MRS agar (aliquots of 0.1 mL), using spread plate method. The plates were then incubated at 37 °C for 72 hours in a BOD incubator (MA 415, Marconi). After this, plates containing between 25 and 250 typical colonies of *L. casei* were counted.

After drying, the powder was reconstituted in peptone water (1:10 w/v) and the suspension was kept at 25 °C for 30 min to release the cells. Then, the amount of *L. casei* cell

was counted as described above in this section. The colony counts were expressed as log of colony forming units per gram of solid (log CFU/g).

2.6. Physicochemical Analyses

2.6.1. Moisture Content

Moisture content was determined gravimetrically in a closed system with silica gel placed in an oven (model UM 200, Memmert, Germany) at 40 °C. The sample weight was measured for 5 days, until constant weight. Samples of 1 g were used.

2.6.2. Water Activity (A_w)

The A_w was determined using a water activity meter (HygroPalm HP 23, Rotronic, Bassersdorf, Switzerland) at 20 ± 1.5 °C.

2.6.3. Glass Transition Temperature (T_g)

The T_g was determined by differential scanning calorimetry using a Netzsch DSC 200 calorimeter (Netzsch, Germany) with a nitrogen-based cooling system. Aluminum pans were filled with 16 mg of the powder product and hermetically sealed. An empty pan was used as reference. Each sample was heated at a temperature rate of 10 °C/min in a temperature range from 0 to 100 °C. The T_g was determined using the Netzsch Proteus Thermal Analysis software.

2.6.4. Particle Size

Particle mean diameter was measured using a laser diffraction particle size analyzer, Mastersizer 3000 (Malvern Instruments, Malvern, UK) equipped with a wet sample unit. A

small sample amount was suspended in ethanol under agitation, and the particle size was measured successive times. Particle size was expressed as the volume mean diameter (d_{50}).

2.6.5. *Scanning Electron Microscopy (SEM)*

Samples were deposited on a double-sided carbon adhesive tape at room temperature and coated with gold-palladium (Polaron SC7640, Thermo VG Scientific, England). The metalized samples were observed under a scanning electronic microscopy (Cambridge Stereoscan S240) at 5 kV.

2.6.6. *Rehydration Time*

A laser diffraction particle size analyzer, Mastersizer 3000 (Malvern Instruments, Malvern, UK), equipped with a wet sample cell unit was used to determine the rehydration time. The new protocol developed is based on the fact that the particle size is reduced down to a stable value after rehydration time (RT). The sensibility of the particle size analyzer is very helpful compared to classical visual observation. The measurement method simulate addition of dry powder in water for rehydration by the end user. For this original method, the objective was to evaluate the kinetic of rehydration without including the impact of powder mass added that is the major drawback of the classical methods used to measure the rehydration time.

The wet sample unit was filled with 32 mL of distilled water under mechanical agitation (1000 rpm). After equipment stabilization, a gradient of powder mass (0.05, 0.1, 0.3, 0.5 and 1.0 g) was added in a standardized way. Immediately after the sample addition, the particles size was measured every five seconds. Rehydration was considered complete when the particle size was stabilized due to solubilization of the encapsulation matrix, full separation of all the particles and release of probiotics into water. The particles size was

expressed as d₉₀, which corresponds to the diameter for which 90 % of the particles have a diameter lower than this value.

2.6.7. Rheological characterization

Rheological studies were carried out using a Kinexus-pro Rheometer (Malvern Instruments, Orsay, France). The measuring system was covered with a humidity chamber to minimize the water evaporation. The rehydrated probiotic juices were allowed to rest for at least 300 s before analysis. Shear stress measurements were then determined using a cone-and-plate geometry (50 mm, 2°) in a stress range from 0.002 to 10 Pa. The shear rate versus the shear stress data were fitted using SigmaPlot software (SigmaPlot Version 11.0, Systat Software Inc., San Jose, CA, USA).

2.7. Storage Conditions

The feed flow rate influence on the microbial viability during storage was also evaluated. The sample to be studied was chosen based on the drying condition that produced the best viability, moisture, and *T_g* results. The powder samples were stored for 3 weeks in hermetically sealed in a polypropylene package at 25 °C ± 2, without vacuum.

3. Results and Discussion

3.1. Probiotic Bacteria Survival

Table 1 shows the microbial viability results, before and after spray and spouted bed drying. In the spray drying, although the droplets are subjected to high temperatures, the risk of overheating is minimal. The large surface area of the droplet and the high temperature gradient between the droplet and the hot air results in an almost instantaneous drying inside the spray-dryer chamber. This phenomenon associated with the cooling caused by evaporation

of water allows the temperature inside the droplet remains low, maintaining the nutritional and organoleptic characteristics of several food products (Phisut, 2012; Roustapour et al., 2006; Turchiuli et al., 2011). However, when a bacteria is also present in the food matrix the temperature sensitiveness might increase. In the present study, the spouted bed dried samples resulted in higher viable microbial cell counts after drying than the spray dried ones. The higher microbial survival obtained in the spouted bed drying can be attributed to the low inlet temperature used in this technique (60 °C). The lower viability achieved in the spray drying can be attributed to the high outlet temperature observed (inlet temperature of 140 °C and outlet temperature 85-95 °C), since after drying the particle remains exposed to the outlet temperature until they are recovered by the cyclone and reach the collecting glass. Anekella & Orsat (2013) reported that the microbial viability of *L. acidophilus* and *L. rhamnosus* in spray-dried raspberry juice, dropped from 9.5 to 5 log CFU/mL when the inlet temperature increased from 100 °C (outlet temperature of 67-76 °C) to 130 °C (outlet temperature of 88-97 °C). According to these authors, outlet temperatures higher than 85-90 °C are lethal for probiotics. Aside imparting microbial membrane damage, high-temperature affects proteins, DNA, RNA and ribosomes (Lapsiri et al., 2012) and the microorganism loses its functionality and survival ability (Ananta et al., 2005).

Barbosa et al. (2015) added *Lactobacillus plantarum* 299v and *Pediococcus acidilactici* HA-6111-2 in non-fermented orange juice and observed that there was no microbial viability loss after the spray drying. Although the spray dryer used by these authors was set at high inlet temperature (150 °C), the equipment allowed outlet temperature to be controlled at 70 °C. Despite the better microbial viability, the powder obtained presented an A_w value of 0.4. The A_w value reported by Barbosa et al. (2015) is higher than the values normally obtained in industrial spray dryers (approximately 0.2). In the present work, all

samples presented A_w values around 0.2, even when spouted bed was used at low inlet temperatures (70 °C). The effect of A_w on powder quality is discussed further in item 3.2.1.

The feed flow rate and the drying agent effect on the microbial survival were not significant in spouted bed drying (Table 1). This fact can be attributed the low temperature used, which was able to preserve the microbial viability. In spray drying, the microbial survival increased along with the feed flow rate increase. Mestry et al., (2011) also reported a positive correlation between the feed flow rate increase and the microbial survival in spray-dried watermelon-carrot mixed juice. The increase in feed flow rate results in larger droplet sizes and lower residence time, which reduces the product exposure to the high air temperature, preserving the microbial viability. However, higher feed flow rates also result in higher water content in the powder, which is undesirable.

3.2. Physicochemical Stability of the Probiotic Orange Juice Powder

3.2.1. Moisture and A_w

All powders presented low moisture and A_w (Table 2). The spray-dried samples showed lower values of moisture content and A_w than the ones spouted bed dried. In a drying processing, the air temperature has significant influence on the moisture content of the powder product (Costa et al., 2015). High drying temperatures, as used in this work for spray drying, normally create a strong gradient between atomized fluid and the hot air, providing a higher driving force for moisture removal (Krishnaiah et al., 2014; Mestry et al., 2011). The higher the temperature gradient the higher will be the rate of heat transfer, resulting in lower residual water content in the final product (Goula and Adamopoulos, 2010). In spray drying the temperature was substantially higher than in spouted bed, thus the moisture values of the spray-dried samples were lower. Despite this, spouted bed dried samples presented low

moisture and A_w values lower than 0.2. A_w values around 0.2 have been reported as optimal for survival of the microorganism during posterior storage period (Nuallkaekul et al., 2012). These values are within the range observed for products obtained in industrial spray dryers, which are about 0.2 (Adhikari et al., 2009). However, is necessary to observe that to scale up the spouted bed drying process is necessary an adjustment in the path of the exhaust gas to recycle it and recover its sensible heat to maintain the equipment efficiency, and therefore the quality of the product (Costa et al., 2006; Nascimento et al., 2015).

It was also observed that the decrease in feed flow rate resulted in lower moisture for both drying techniques (Table 2). Chegini & Ghobadian (2005) reported that the increase in feed flow rate increased the residual moisture of spray-dried non-probiotic orange juice. According to Tan, Ibrahim, Kamil, & Taip (2011), the increase of feed flow rate increases the atomized liquid into the drying chamber producing larger liquid droplets. Larger droplets increase the distance that the moisture has to travel from the center of the droplets to the surface, resulting in higher moisture content.

3.2.2. Glass Transition Temperature (T_g)

T_g values ranged from 52.3 to 70.4 °C for spray-dried samples, while spouted bed dried samples showed T_g values from 35.5 to 50.9 °C (Table 2). Saavedra-Leos et al. (2014) reported a T_g of 46 °C for orange juice powder spray-dried with inulin ($A_w = 0.210$). This value is near to that found for spouted bed dried powders in the present study. Lower values were found by Fang & Bhandari (2011) for spray-dried bayberry juice. They reported T_g values from 18.84 to 30.54 °C, using as drying agent 10 and 30% of maltodextrin (DE 10).

Higher T_g values were obtained for the samples dried with gum Arabic in both equipments. Similar results were found by Fazaeli et al. (2012) for spray-dried black mulberry juice powder. The T_g values dropped from 63.3 to 40.2 °C when gum Arabic (8%) was

replaced by 8% of maltodextrin DE 20. Yousefi, Emam-Djomeh, & Mousavi, (2011) reported a T_g of 52.8 °C for spray-dried pomegranate juice with 12% of gum Arabic, and 40 °C for samples obtained using 12% of maltodextrin DE 20. Krishnaiah et al. (2014) reported that gum Arabic is able to reduce the powder hygroscopicity more efficiently than maltodextrin. Yousefi et al. (2011) explained that gum Arabic has higher T_g due to its larger molecule size comparing with other carriers. However, gum Arabic is more expensive than maltodextrin and has availability issues that have motivated researchers to search alternative drying agents (Krishnaiah et al., 2014).

It also was observed that T_g is related with the feed flow rate. An increase in the T_g was obtained at low feed flow rates (Table 2). As the same, low feed flow rates caused a decrease in the powders moisture and A_w (as already discussed) indicating that T_g is linked with the water content. Goula & Adamopoulos (2010) also reported that the T_g increased by decreasing the moisture content in non-probiotic orange juice. The increase in T_g with the decrease in the moisture and A_w occurs because the lower the water content, the less it can act as a plasticizer, which decreases the T_g (Krishnaiah et al., 2014).

High T_g values improved the powder stability. It has been recommended that the T_g of a stable formulated powder product should be higher than 40 °C to ensure stability and transportation resistance (Mestry et al., 2011). As long as the T_g is higher than the temperature where the powder is stored, it remains in a glassy state, and the molecular mobility is restricted controlling the rate of physical, chemical and biological changes (Semyonov et al., 2011). Most of the samples obtained herein, spray-dried or spouted bed dried, presented acceptable T_g values to assure powder quality when stored at temperatures below 30 °C.

3.2.3. *Particles Size*

Table 2 shows that the spouted bed dried samples presented d50 lower than 9.5 μm , which means that 50% of the particles had a diameter below 9.5 μm . Similar values were reported by Yousefi, Emam-Djomeh, Mousavi, Kobarfard, & Zbicinski (2014) for spray-dried black raspberry juice powders (mean particles size ranging from 6.79 to 8.76 μm). Particles with small sizes are desirable in food formulations to ensure homogeneity and better quality (Arslan et al., 2015).

Spray-dried samples presented slightly higher particle size ($9.60 \mu\text{m} < d50 < 13.3 \mu\text{m}$). The difference can be attributed to the higher temperature used in spray drying compared to spouted bed drying. According to Phisut (2012), high temperatures promote the formation of vapor-impermeable films on the droplet surface, followed by the formation of vapor bubbles. It causes skinning over on the droplets during drying and results in the droplet expansion.

Table 2 also shows that spouted bed dried samples with gum Arabic presented higher particle size compared to maltodextrin. The droplet size is a function of several factors, primarily the nozzle vibration frequency, but also of the liquid solution viscosity (Semyonov et al., 2011). The higher viscosity obtained with gum Arabic could also explain the difference in the probiotic juice powders particles size. The viscosity is discussed further (see item 3.2.5).

3.2.4. Powder Morphology

Since moisture, A_w and T_g results were better at the lowest feed flow rates tested, these conditions were chosen to study the powder morphology, rehydration, and the rheological behavior.

The morphological characteristics of spray-dried probiotic orange powders obtained at the lowest feed flow rates are shown in Fig. 1. Samples presented spherical particles with irregular size distribution. Organic materials, such as instant drinks, coffee, eggs, fruit juice,

dairy products including yogurt, show these morphological characteristics (Cervantes-Martínez et al., 2014), which are typical of materials produced by a spray dryer (Ferrari et al., 2013).

The drying agent and the technique presented a substantial influence on the particle shape. Samples produced with maltodextrin were more spherical, less rough than samples produced with gum Arabic. These samples also presented some fissures, and when produced at spouted bed, fused particles were observed (Fig 1). Morphological images of spray-dried date fruit (*Phoenix dactylifera* L.) using maltodextrin as drying agent were similar in roughness, sphericity and fused particles (Manickavasagan et al. 2015). Ferrari et al. (2013) also reported that spray-dried blackberry powder produced with maltodextrin were less rough than the sample produced with gum Arabic, which showed shriveled surfaces. These differences can be attributed to the fact that different constituents of the fluid used, as the drying agent, may migrate within the particle during the drying, changing the dried particles characteristics. Also, during the drying, particles may inflate, distort, shrink or fracture depending on the rheological properties, porosity degree of the particle skin or crust formed (Walton, 2000).

The microstructures of fruit powders also depend on the drying method applied (Caparino et al., 2013). The morphology of spray-dried powders is related to the inlet temperature, the type and the concentration of the carriers added (Du et al., 2014). Particles produced using the spray dryer were rougher and less spherical than the produced in the spouted bed. During the non-saturated surface drying period, the moisture movement may inflate, distort or shrink the particles (Walton, 2000). At low air inlet temperatures, as the used in the spouted bed drying, the particles have more time to shrink, since the water diffusion occurs at a low rate (Vergara et al., 2014). As temperature can influence the water evaporation

rate during the drying process, the temperatures used in each equipment may also be related to the particle morphology differences.

3.2.5. Rehydration Time

Table 3 shows a summary of rehydration times (RT) obtained for 1 g of probiotic orange juice spray and spouted bed dried, using gum Arabic and maltodextrin as drying agents at the lowest feed flow rate tested for each equipment. The powders produced with gum Arabic presented rehydration time up to 9 times higher than that obtained using maltodextrin. Fontes, Silva, Sampaio-Neta, da Costa, & Rodrigues (2014) reported that prebiotic pineapple, melon and orange juice powders spray-dried with gum Arabic (10 and 20%) presented rehydration times five times higher than that obtained using maltodextrin. Maltodextrin is more soluble in water than gum Arabic (Goula and Adamopoulos, 2010; Nguyen and Pham, 2011), which can contribute to lower rehydration time.

Most of traditional rehydration analysis consists of adding the powder product in a system containing water under agitation. The powder sample is added to the system and from visual observation the time required for the complete disappearance of the powder particles is considered as the rehydration time. This type of analysis is subjective and low repeatable. Therefore, in the methodology used in this article, an equipment (Mastersizer), able to accurately measure particle sizes, was used to replace the human observation.

Fig. 2a displays the particle size variation versus the rehydration time of the probiotic orange juice powder obtained by spray drying, using maltodextrin as the drying agent. At the beginning of the analysis, the sample was placed in contact with the water forming agglomerates, which were detected by the equipment as big particles with 2500 μm of size. After submersion, the agglomerates gradually solubilized decreasing the particles size until

the complete hydration (dissolution) in water. After this, the particles size stabilized (Fig. 2a). The time from which the particles size has stabilized is the time of rehydration, expressed as RT (min). For this sample, the RT is 2.7 min, which is the time from which the agglomerates disappear and the particle size stabilized (Fig. 2a). The reydration time is visually confirmed by the pictures showed at the top of Fig. 2a., and by the particle size distribution showed in Fig. 2b. The solids remaining after RT are the probiotic microorganism suspended cells and some solids coming from the juice pulp.

Samples produced with gum Arabic showed a different rehydration profile as presented in Fig. 3a. Peaks were observed during all assay. According to Fig. 3a, the product remained in powder form on the water surface during the rehydration assay, suggesting that this product is not easily wettable. From time to time, some powder submerged in the equipment cell in an agglomerated form. The equipment detected the agglomerates as large particles, which resulted in the peaks (Fig 3a). Fig. 3b shows the particle size distribution for the first peak of the graph shown in Fig. 3a. Particle size distribution shows that this agglomerate takes around 15 s to submerge and be detected by the instrument. However, it is not necessarily the time required to rehydrate the agglomerate, since it can submerge, be detected by the instrument and return to the cell surface of the Mastersizer, submerge and being detected as another peak, until complete rehydration. Thus, the same agglomerate can be detected several times resulting in an oscillatory profile until the complete rehydration.

The profile of the samples dried in the spouted bed with gum Arabic and maltodextrin followed identical patterns of the samples spray-dried with gum Arabic and maltodextrin, respectively, but with different rehydration times (see supplementary material).

The effect of the sample amount in the rehydration time was also studied. Thus, aside the use of only 1 g of product, 0.5, 0.3, 0.1 and 0.05 g of powder product was also used. For all sample the relation between the powder quantity and rehydration time was linear.

Equations 1, 2, 3 and 4 are the fitted linear regression models obtained for the experimental results presented in Fig 4.

$$RT_{SDG} = 14.56Q + 3.83 \quad (1)$$

$$RT_{SDM} = 1.36Q + 1.39 \quad (2)$$

$$RT_{SBG} = 11.85Q + 3.27 \quad (3)$$

$$RT_{SBM} = 0.68Q + 1.39 \quad (4)$$

Where RT_{SDG} is the time required to rehydrate the powders obtained from spray drying with gum Arabic; RT_{SDM} is the time required to rehydrate the powders obtained from spray drying with maltodextrin; RT_{SBG} is the time required to rehydrate the powders obtained from spouted bed with gum Arabic; RT_{SBM} is the time required to rehydrate the powders obtained from spouted bed drying with maltodextrin; while Q is the amount of sample. As can be seen in Fig 4, the amount of sample strongly affects the rehydration time when gum Arabic is used as drying agent. When maltodextrin is the drying agent the effect of the sample size is lower in the rehydration time.

Equations 1 to 4 were used to estimate the minimal rehydration time (Table 3). The minimal rehydration time can be considered the time needed to rehydrate a single powder particle and it shows the single powder particle resistance to interacting with water and solubilize. As the mass of a single powder particle is very small ($Q \rightarrow 0$), the linear coefficient of equations 1 to 4 is the minimum rehydration time. The results confirmed the difficulty to rehydrate samples with gum Arabic even in minimal amounts, which requires rehydration times up to 2.8 times higher than the ones obtained for samples dried with maltodextrin.

3.2.6. Rheological Properties

The study of the rheological behavior of beverages provides information about the fluid structure and the interactions between resting particles at rest and during flow. The rheological properties of the rehydrated probiotic juices were investigated at refrigerated and room temperatures of 4, 20 and 30 °C, respectively.

Figure 5 shows the flow curves of the probiotic orange juice samples obtained with Gum Arabic and maltodextrin. The solid lines represented the fits to the power law model (Vitali and Rao, 1984) given in equation 5.

$$\sigma = k\gamma^n \quad (5)$$

Where σ is the shear stress, k is the consistency index, γ is the shear rate, and n is the flow behavior index. The consistency index k is numerically equal to the viscosity at 1s^{-1} and could be useful as a general measure of viscosity for comparative purposes.

The experimental results of shear stress and shear rate showed that the used drying agent had a substantial impact on the juice rheological behavior. In fact, the viscosity is represented by the slope of the rheological curves. They show that gum Arabic resulted in the higher viscous fluid than maltodextrin.

Equation 5 was used to fit the experimental data of different samples. The rheological parameters are shown in Table 4. The fitted model presented correlation coefficients higher than 0.9980.

The n and k values ranged from 0.93 to 1.36 and from 0.0003 to 0.0351 Pa.s, respectively. The use of gum Arabic as a drying agent resulted in a nearly Newtonian behavior ($n \approx 1$) with low shear dependency of the dynamic viscosity. This rheological behavior is interesting and is generally observed for clarified and depectinated orange juices (Ibarz et al., 1994).

In the case of maltodextrin, a shear thickening behavior was observed ($n > 1$). In fact, the flow curves of maltodextrin showed a Newtonian behavior at low shear rates (linear slope)

followed by a shear thickening character (shear rate $> 500 \text{ s}^{-1}$) (Fig. 5). This rheological behavior change can be related to maltodextrin molecules aggregation due to orthokinetic flocculation. The aggregates would present sufficient cohesive forces to withstand the shear stress leading to a greater flow resistance (Jiménez et al., 2014).

The consistency index (k) of probiotic orange juice containing gum Arabic was 14, 29 and 64 times higher than maltodextrin at 4, 20 and 30 °C, respectively. The significant difference of viscosity at a shear rate of 1 s^{-1} could be related to the drying agent molecular weight. In fact, maltodextrin is defined as low converted products of starch hydrolysis with therefore low molecular weight compared to gum Arabic, which is a heteropolysaccharide with three different fractions (arabinogalactan, arabinogalactan protein and glycoprotein) (Dokic et al., 2004; Renard et al., 2006).

From these results, we can conclude that, despite the differences, the use of both drying agents could be interesting to prepare a probiotic orange juice with an acceptable viscosity. However, due the difficulties to rehydrate the samples produced with gum Arabic, maltodextrin is adequate to produce probiotic orange juice in powder by the techniques tested.

3.3. Influence of Feed Flow Rate in Probiotic Survival During Storage

Since the spouted bed drying was better to microbial survival after the drying, and the objective of this work was to produce a probiotic orange juice powder with easy reconstitution, the survival analysis during storage were performed for the probiotic orange juice spouted bed dried with maltodextrin, that showed considerable better rehydration time than gum Arabic.

The powders were stored for 3 weeks in sealed polypropylene bags at $25 \text{ °C} \pm 2$, without vacuum. The powder samples were not stored under controlled A_w to simulate the

real storage. This kind of storage was chosen to study the stability of the microorganism because the ambient condition of juice powder storage is usually not controlled.

During storage, spouted bed powder produced at low flow rates showed better microbial survival (Fig. 6). The viability improvement with the decreasing of feed flow rate can be related to the water content that also decreased with the increase in feed flow rate, as already discussed in section 3.2.1. Powders produced at the lowest feed flow rate (0.2 L/h) showed the highest level of probiotic bacteria after three weeks of storage (7.79 log CFU/g). Some studies have associated the microbial viability loss to the water content (Martín et al., 2015; Nualkaekul et al., 2012; Vesterlund et al., 2012). The higher the water content in the powder, or the higher the A_w of the ambient where the powder is stored, the higher is the microbial viability loss (Barbosa et al., 2015; Nualkaekul et al., 2012). It can be related to the fact that in fruit powders with high A_w the migration of acids or antibacterial compounds occurs, damaging the cells and reducing their survival during subsequent storage (Nualkaekul et al., 2012).

It is recommended that a probiotic product should contain an adequate microbial counts (at least 10^6 CFU/g) after processing and during the storage to reach the intestine in enough levels to perform its beneficial actions in the body (Nag and Das, 2013). In this case, the powder product produced at the lowest feed flow rate showed good viability at the end of the storage period, ensuring its beneficial effects.

4. Conclusion

Spouted bed drying was better than spray drying regarding the microbial survival of the probiotic microorganism due to the low temperature used in this process. The spray-dried powders produced with gum Arabic presented better physicochemical characteristics compared to the ones obtained with maltodextrin. However, the physicochemical

characteristics of the spouted bed dried powders produced with maltodextrin were enough to ensure powder quality. Because of the remarkable higher rehydration capacity of the powders produced with maltodextrin, this drying agent proved to be adequate to produce fermented probiotic orange juice in powder, since it will be easier for the consumers to reconstitute the product. Storage during three weeks showed that the lowest feed flow rate resulted in better microbial viability due the lower water content. Thus, spouted bed drying at the smallest feed flow rate (0.2 L/h), using maltodextrin as drying agent were found as the best choice to produce probiotic orange juice powder with high cell viability counts, with good physicochemical characteristics that assure the powder quality. However, for industrial application, the processing scale up must take into account economical aspects. Increasing the size of the spouted bed chamber, the residence time will also increase allowing a higher feed-flow rate. The scale up should search for a configuration where the product quality is not decreased, which is possible using CFD tools.

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Figure captions

Figure captions

Fig. 1. SEM images of probiotic orange juice powders containing *L. casei* spray-dried with Arabic gum at a feed flow rate of 0.3 (SDG03); spray-dried with maltodextrin at a feed flow rate of 0.3 (SDM03); spouted bed dried with Arabic gum at a feed flow rates of 0.2 (SBG02); and spouted bed dried with maltodextrin at a feed flow rate of 0.2 (SBM02).

Fig. 2. Particle size over time variation (a) and size distribution profile (b) obtained during rehydration of probiotic orange juice powder spray-dried using maltodextrin as drying agent. RT means rehydration time.

Fig. 3. Particle size over time variation (a) and size distribution profile (b) obtained during rehydration of probiotic orange juice powder spray-dried using Arabic gum as drying agent. RT means rehydration time.

Fig. 4. Influence of the powder amount on rehydration time of probiotic orange juice powder spray-dried with Arabic gum at a feed flow rate of 0.3 (SDG03); spray-dried with maltodextrin at a feed flow rate of 0.3 (SDM03); spouted bed dried with Arabic gum at a feed flow rates of 0.2 (SBG02); and spouted bed dried with maltodextrin at a feed flow rate of 0.2 (SBM02).

Fig. 5. Rheological behavior of the probiotic orange juice samples: Shear stress vs shear stress. The solids lines are the fit to power law model. Sample spray-dried with Arabic gum at a feed flow rate of 0.3 (SDG03); spray-dried with maltodextrin at a feed flow rate of 0.3

(SDM03); spouted bed dried with Arabic gum at a feed flow rates of 0.2 (SBG02); and
spouted bed dried with maltodextrin at a feed flow rate of 0.2 (SBM02).

Fig. 6. Influence of the feed flow rate on the *L. casei* survival during the storage of fermented
probiotic orange juice powders samples spray-dried with Arabic gum at a feed flow rate of 0.3
(SDG03); spray-dried with maltodextrin at a feed flow rate of 0.3 (SDM03); spouted bed-
dried with Arabic gum at a feed flow rates of 0.2 (SBG02); and spouted bed-dried with
maltodextrin at a feed flow rate of 0.2 (SBM02). Survival values were calculated in relation to
viability values before drying.

Fig.1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Table 1 – Viable cell counts of *Lactobacillus casei* NRRL B-442 in fermented orange juice spray-dried with gum Arabic at feed flow rates of 0.3, 0.5 and 0.7 L/h (SDG03, SDG05 and SDG07, respectively); spray-dried with maltodextrin at feed flow rates of 0.3, 0.5 and 0.7L/h (SDM03, SDM05 and SDM07, respectively); spouted bed dried with gum Arabic at feed flow rates of 0.2, 0.3 and 0.4 L/h (SBG02, SBG03 and SBG04, respectively) and spouted bed dried with maltodextrin at feed flow rates of 0.2, 0.3 and 0.4 L/h (SBM02, SBM03 and SBM04, respectively).

Sample	Viable cell counts (log CFU/g)		
	Before Drying	After Drying	Log reduction
SDG03	9.97 ± 0.01	5.22 ± 0.03	4.75 ± 0.04 ^b
SDG05	9.92 ± 0.01	5.33 ± 0.01	4.59 ± 0.01 ^{bc}
SDG07	9.92 ± 0.01	5.49 ± 0.01	4.43 ± 0.01 ^d
SDM03	9.98 ± 0.01	3.42 ± 0.06	6.56 ± 0.07 ^a
SDM05	9.95 ± 0.02	5.45 ± 0.01	4.50 ± 0.05 ^{cd}
SDM07	9.95 ± 0.02	5.88 ± 0.02	4.07 ± 0.07 ^e
SBG02	9.88 ± 0.01	9.66 ± 0.03	0.22 ± 0.03 ^f
SBG03	9.93 ± 0.02	9.61 ± 0.01	0.32 ± 0.02 ^f
SBG04	9.94 ± 0.03	9.63 ± 0.07	0.31 ± 0.10 ^f
SBM02	10.02 ± 0.04	9.72 ± 0.02	0.30 ± 0.01 ^f
SBM03	10.02 ± 0.04	9.70 ± 0,10	0.32 ± 0.06 ^f
SBM04	10.02 ± 0.04	9.77 ± 0,06	0.25 ± 0.09 ^f

Values are mean ± standard deviation. Values with different letters in the same column means significant differences according to the Tukey

test (p<0.05).

Table 2 – Influence of the drying technique and the drying agent in the A_w , moisture, T_g and particles size of fermented probiotic orange juice powder spray-dried with gum Arabic at feed flow rates of 0.3, 0.5 and 0.7 L/h (SDG03, SDG05 and SDG07, respectively); spray-dried with maltodextrin at feed flow rates of 0.3, 0.5 and 0.7 L/h (SDM03, SDM05 and SDM07, respectively); spouted bed dried with gum Arabic at feed flow rates of 0.2, 0.3 and 0.4 L/h (SBG02, SBG03 and SBG04, respectively) and spouted bed dried with maltodextrin at feed flow rates of 0.2, 0.3 and 0.4 L/h (SBM02, SBM03 and SBM04, respectively).

Sample	Moisture (%)	A_w	T_g (°C)	Particle size (d50) (µm)
SDG03	0.70 ± 0.07^a	0.092 ± 0.002^a	70.4 ± 2.6^a	9.61 ± 0.11^c
SDG05	1.02 ± 0.00^{bd}	0.103 ± 0.005^a	62.2 ± 0.8^b	11.70 ± 0.10^b
SDG07	1.49 ± 0.06^c	0.152 ± 0.006^b	55.9 ± 0.6^c	12.57 ± 0.06^b
SDM03	0.89 ± 0.04^b	0.174 ± 0.001^c	55.3 ± 1.1^{cd}	12.80 ± 0.62^{ba}
SDM05	1.13 ± 0.04^{de}	0.174 ± 0.010^c	53.3 ± 0.3^{cde}	12.50 ± 0.35^{ba}
SDM07	1.20 ± 0.04^e	0.179 ± 0.002^{cd}	52.3 ± 0.2^{de}	13.30 ± 0.01^a
SBG02	2.44 ± 0.08^f	0.221 ± 0.008^f	50.9 ± 0.6^{ef}	9.23 ± 0.11^{cd}
SBG03	2.72 ± 0.02^g	0.212 ± 0.004^{ef}	49.1 ± 0.5^{fg}	8.96 ± 0.11^d
SBG04	2.78 ± 0.03^g	0.215 ± 0.004^{ef}	47.7 ± 0.7^{gh}	9.49 ± 0.02^d
SBM02	2.03 ± 0.01^h	0.197 ± 0.006^{de}	45.8 ± 0.7^h	7.84 ± 0.03^e
SBM03	2.43 ± 0.04^f	0.223 ± 0.001^f	39.0 ± 1.0^i	7.62 ± 0.09^e
SBM04	3.23 ± 0.05^i	0.264 ± 0.006^g	35.5 ± 0.9^j	7.89 ± 0.06^e

Values are mean $\pm$ standard deviation. Values with different letters in the same column means significant differences according to the Tukey test ($p < 0.05$).

Table 3 – Influence of the drying technique and the drying agent in the reconstitution time of probiotic orange juice powder spray-dried with gum Arabic at a feed flow rate of 0.3 L/h (SDG03); spray-dried with maltodextrin at a feed flow rate of 0.3 L/h (SDM03); spouted bed dried with gum Arabic at a feed flow rates of 0.2 L/h (SBG02); and spouted bed dried with maltodextrin at a feed flow rate of 0.2 L/h (SBM02).

Sample	Rehydration time for 1 g of product (min)	Minimal rehydration time (min)
SDG03	18.33 ± 0.44 ^a	3.84
SDM03	2.67 ± 0.08 ^c	1.39
SBG02	15.47 ± 1.02 ^b	3.27
SBM02	1.96 ± 0.14 ^c	1.39

Values are mean ± standard deviation. Values with different letters in the same column means significant differences according to the Tukey test (p<0.05).

Table 4 – Rheological properties of the fermented probiotic orange juice powder spray-dried with gum Arabic at a feed flow rate of 0.3 L/h (SDG03); spray-dried with maltodextrin at a feed flow rate of 0.3 L/h (SDM03); spouted bed dried with gum Arabic at a feed flow rates of 0.2 L/h (SBG02); and spouted bed dried with maltodextrin at a feed flow rate of 0.2 L/h (SBM02).

Power law model			
Samples	k (Pa.s)	η	R²
4 °C			
SDG03	0.0334 ± 0.0004	0.9347 ± 0.0022	0.9999
SDM03	0.0026 ± 0.0002	1.1241 ± 0.0102	0.9991
SBG02	0.0351 ± 0.0007	0.9324 ± 0.0035	0.9998
SBM02	0.0025 ± 0.0002	1.1347 ± 0.0110	0.9990
20 °C			
SDG03	0.0200 ± 0.0003	0.9748 ± 0.0021	0.9999
SDM03	0.0006 ± 0.00008	1.2899 ± 0.0173	0.9981
SBG02	0.0191 ± 0.0009	0.9500 ± 0.0079	0.9992
SBM02	0.0007 ± 0.00009	1.2684 ± 0.0174	0.9980
30 °C			
SDG03	0.0191 ± 0.0006	0.9430 ± 0.0051	0.9997
SDM03	0.0003 ± 0.00004	1.3602 ± 0.0176	0.9982
SBG02	0.0140 ± 0.0002	0.9597 ± 0.0020	0.9999
SBM02	0.0004 ± 0.00005	1.3343 ± 0.0184	0.9980