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Review

Potential applications of dairy whey for the production of lactic acid bacteria cultures

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ABSTRACT

Lactic acid bacteria (LAB) are applied in the food industry as fermentation agents for a variety of products. Cheese whey (CW) and second cheese whey (SCW) are the two main by-products of cheese production, and can be reused for biotechnological purposes. In this context, this review aims to summarise literature on the use of CW and SCW as culture media for the growth of LAB, as cryoprotectants for freeze-drying and as encapsulating agents for the spray-drying of these microorganisms. CW contains several nutrients that are reported to enhance the growth of LAB. As for SCW, research shows that it has potential to be applied as culture medium, although bacterial growth remains at least one or two log orders lower than CW. Regarding freeze-drying and spray-drying, reports indicate that whey-based cryoprotectants and CW as encapsulating agent offer significant protection from the stressful conditions employed in these processes.

Contents

1. Introduction

Lactic acid bacteria (LAB) are microorganisms widely used in food applications. Their probiotic features have been reported many

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times, as well as their potential for treating gastrointestinal tract (GIT)-related diseases (Caggia, De Angelis, Pitino, Pino, & Randazzo, 2015; Greifová et al., 2017; Wang et al., 2016). In the food industry, LAB are used as starter or non-starter cultures, which primarily differ in purpose, since the former promote a rapid acidification of the medium and the latter provide aromatic compounds (Motta & Gomes, 2015) that contribute to taste, texture, nutritional value, shelf life, and safety of food products (Souza & Dias, 2017).

Cheese whey (CW) is a by-product derived from the production of cheese or casein, released in the milk coagulation step. It represents around 90% of the milk volume assessed in the dairy industry, and retains 55% of its contents, e.g., most of the lactose and mineral salts, and also proteins such as β-lactoglobulin, α-lactalbumin and immunoglobulins (Dragone, Mussatto, Almeida e Silva, & Teixeira, 2011; Guimarães, Teixeira, & Domingues, 2010). CW is sometimes re-used for the production of ricotta, in which second cheese whey (SCW) is released. This material is also known as scotta or ricotta whey, and contains 60% of CW compounds, especially lactose (Carvalho, Prazeres, & Rivas, 2013). Both CW and SCW have high organic loads, and could represent an environmental threat if discarded inappropriately (Sansonetti, Curcio, Calabrò, & Iorio, 2009). Considering their nutrient contents, these materials could be used as culture media for the growth of LAB, representing an alternative to commercial medium; thus, it is possible to reduce costs and environmental impact.

To obtain a LAB culture for industrial applications, microorganisms must be submitted to concentration or drying methods that enable them to assume an accessible configuration. In other words, the culture should be able to resist storage and food processing without decreasing cell viability. Technologies such as freeze-drying (FD) and spray-drying (SD) can be applied in this case; both have been used for concentration of LAB (Aspiyanto & Susilowati, 2014; Damodharan, Palaniyandi, Yang, & Suh, 2017; Mohammadi-Gouraji, Sheikh-Zeinoddin, & Soleimanian-Zad, 2017). FD is more commonly used for concentration of starter LAB, whereas SD has been considered as a method well-suited for drying probiotic LAB. Mendoza, Pasteris, Otero, and Nader-Macías (2014) reported that the use of whey-based cryoprotectants has significantly increased survival of LAB after FD. On the other hand, SD has been reported to represent the best cost-benefit for the industry (Pérez-Chabela, Lara-Labastida, Rodriguez-Huezo, & Totosaus, 2013); in this case, the use of different materials (encapsulating agents) allows SD to form a capsule that protects bacteria from storage and food processing (Maciel, Chaves, Grosso, & Gigante, 2014). Thus, CW has been reported as an encapsulating agent for LAB (Eckert et al., 2017; Huang et al., 2017b), which indicates another use for this material that could reduce production costs.

Therefore, the aim of this study is to review literature reporting the growth of LAB in CW and SCW, preferably using scale-up approaches, and the technologies used for concentration and drying of these microorganisms in CW, with focus on SD. The paper initially discusses LAB briefly and their applications in the industry. Further, the roles of CW and SCW as growth media for LAB are discussed, while aspects such as supplementation of culture medium and cultivation parameters are highlighted. Regarding concentration and drying methods, the use of the two materials as encapsulating agents for SD and as cryoprotectants for FD are reviewed, especially considering their effects on cell viability, survival through the gastrointestinal tract and during storage time. Additionally, articles that employ the double use of CW and/or SCW for a joint procedure of growth and SD of LAB are reviewed. Finally, the challenges of using CW and SCW for these applications are discussed.

2. Cheese whey and second cheese whey

Cheese whey (CW) is the liquid by-product of cheese or casein production, and represents 85–95% of the volume of milk assessed in the dairy industry. It retains 55% of the original raw material contents (Guimarães et al., 2010), which means 4.5–5% (w/v) lactose, 0.6–0.8% (w/v) proteins, 0.4–0.5% (w/v) fats and oils, and 8–10% (w/w dried extract) mineral salts (Dragone et al., 2011). It is classified as sweet whey if the casein coagulation process is enzymatic, or acid whey if it is derived from the presence of LAB or the addition of organic acids (Ryan & Walsh, 2016).

Smithers (2015) stated that the amount of CW produced annually is approximately 180–190 million tons, of which only 50% (~90 million tons per year) is processed. Half of this volume (~45 million tons) is used directly in its liquid form, for example, for the production of ricotta cheese and other whey-based fermented beverages. Approximately 30% of the total processed volume is transformed in powdered CW, which can be used for the production of infant formula; 15% is purified and sold as lactose and its byproducts, and the rest (~5%, meaning 4.5 million tons per year) is used for the concentration or isolation of whey proteins, as shown in Fig. 1 (Mollea, Marmo, & Bosco, 2013). Annual production of CW has still been growing every year. According to the World Cheese Market 2000–2023 (PM Food & Dairy Consulting, 2016), global cheese production for 2023 is estimated at 26 million tons. Considering that approximately 9 L of CW are released for every 1 kg of cheese, the estimated global production of CW in 2023 will be 230 million tons. This reinforces the need for new alternatives for reusing this material.

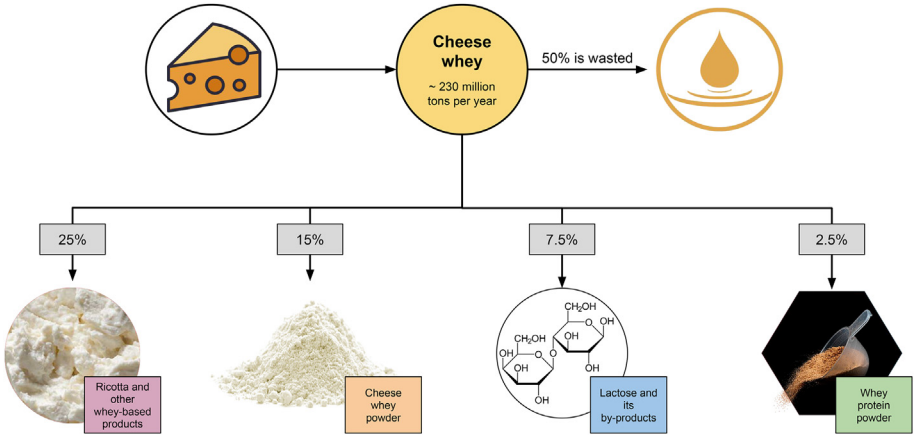

Fig. 1. Percentages of cheese whey destined for its major applications in the industry, according to Mollea et al. (2013).

As mentioned above, ricotta is described as one of the most common products of CW reuse. In this case, another residue is released: second cheese whey (SCW), also known as scotta or ricotta cheese whey. It represents 60% of CW total solids, containing most of the lactose (50 g L^{-1}) and high salinity (7–23 mS cm^{-1}), mainly due to the addition of salts in the cheese production process (Carvalho et al., 2013). Both CW and SCW have high organic loads, with biological oxygen demand (BOD) and chemical oxygen demand (COD) values in the range 30–50 g L^{-1} and 60–80 g L^{-1}, respectively (Sansonetti et al., 2009). Comparatively, the average BOD and COD of urban wastewaters is 0.202 and 0.408 g L^{-1}, respectively, which means around 1/150 of the pollution charge of both CW and SCW (Gallego-Schmid & Tarpani, 2019).

Considering CW and SCW organic loads, finding alternatives for the reuse of these materials becomes necessary. In this regard, SCW has been reported in the production of fuels such as bio-ethanol (Curcio et al., 2010; Sansonetti, Curcio, Calabrò, & Iorio, 2010; Vincenzi et al., 2014), hydrogen (Vasmara & Marchetti, 2017), biogas (Comino, Riggio, & Rosso, 2012; Curcio et al., 2010), and in the production of microbial lipids for biodiesel (Carota et al., 2017). Recent studies indicate CW hydrolysates as a source of bioactive peptides (Brandelli, Daroit, & Corrêa, 2015; Dullius, Goettert, & de Souza, 2018) and its applications in food products (Wójciak et al., 2018). Consequently, new technologies are being developed to maximise hydrolysis (Mao & Kulozik, 2018). As a matter of fact, technologies for obtaining protein hydrolysates from SCW have also been reported (Monti, Donati, Zambrini, & Contarini, 2018). Moreover, due to the presence of lactose, vitamins, and minerals, whey can be used as substrate for the production of other metabolites such as enzymes (Geiger et al., 2016; You et al., 2017) or lactic acid (Soriano-Perez, Flores-Velez, Alonso-Davila, Cervantes-Cruz, & Arriaga, 2011), for the synthesis of galacto-oligosaccharides (Golowczyc et al., 2013; Hugo, Bruno, & Golowczyc, 2016; Reinaldo Lisboa et al., 2012), and finally, for the growth of LAB (Burns, Vinderola, Molinari, & Reinheimer, 2008; Kareb, Champagne, Jean, Gomaa, & Aïder, 2018; Pescuma, Hébert, de Valdez, Mozzi, & Bru, 2012).

3. Lactic acid bacteria

Lactic acid bacteria (LAB) are Gram-positive, catalase negative, non-sporulating, microaerophilic microorganisms that naturally inhabit the human gut. The major LAB genera are *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Enterococcus*, and *Streptococcus* (Motta & Gomes, 2015). Many LAB are classified as probiotic, which means that they can provide health benefits to the host when administered in adequate amounts (FAO/WHO, 2002). Recent research has shown that these bacteria affect immunity enhancement and cancer prevention (Riaz Rajoka et al., 2017b), treatment of airway inflammation (Zhang et al., 2015) and especially diseases related to the gastrointestinal tract (GIT) (Caggia et al., 2015; Greifová et al., 2017; Wang et al., 2016). Additionally, these microorganisms can be found not only in cow milk but also in camel (Abushelaibi, Al-Mahadin, El-Tarabily, Shah, & Ayyash, 2017), goat (Ferrari et al., 2016), ewe (Settanni et al., 2013) and human milk (Riaz Rajoka et al., 2017a).

Even though they are most commonly related to milk products, LAB are applied as fermentation agents in many food commodities (Jensen, Grimmer, Naterstad, & Axelsson, 2012), and in an array of biotechnological processes. Regarding their technological properties, LAB are classified as homofermentative when lactic acid is produced 85% more than other fermentation products, or as heterofermentative, when acetate, ethanol, and carbon dioxide are also formed. Homofermentative LAB are commonly used as part of starter cultures, and their function is to promote a rapid acidification of food products. Heterofermentative LAB, on the other hand, are agents of non-starter cultures, primarily intended for increased sensorial quality (Gatti, Bottari, Lazzi, Neviani, & Mucchetti, 2013; Guarrasi et al., 2017) due to their glycolytic, lipolytic, and proteolytic activities, as well as for the production of diacetyl (Motta & Gomes, 2015). These compounds enhance taste, texture, nutritional value, shelf life, and safety (Souza & Dias, 2017) of a variety of fermented food products such as table olives (Hurtado, Reguant, Bordons, & Rozès, 2012), carrot puree (Juvonen et al., 2015), leek (Wouters et al., 2013), sauerkraut (Xiong, Li, Guan, Peng, & Xie, 2014), sourdough (Gobbetti, Minervini, Pontonio, Di Cagno, & De Angelis, 2016), meat derivatives (Belgacem et al., 2010; Nediani et al., 2017; Santa et al., 2014), and several dairy products (Domingos-Lopes, Stanton, Ross, Dapkevicius, & Silva, 2017; Mangia, Murgia, Garau, Fancello, & Deiana, 2013; Settanni et al., 2012). In addition, cultures of LAB offer protection against food-borne pathogens through competition for specific compounds, production of antimicrobial substances, or nutrient depletion (Geria & Caridi, 2014).

4. Cultivating LAB

Cultivating LAB to obtain biomass and to further its use as starter or probiotic culture requires the development of industrial-scale experiments, such as the operation and optimisation of processes under bioreactors. In this case, growth parameters must be maximised, since microbial cultivation is extremely sensitive to changes in pH, temperature, and dissolved O_2 in the environment.

Choosing an adequate growth medium for fermentation processes is extremely important, especially because it represents one of the major costs in the entire operation. LAB have fastidious nutritional requirements, which means that slight variations in the components of their cultivation medium can affect growth. Commercial medium such as de Man, Rogosa and Sharpe (MRS) contains glucose as carbon source for LAB; nitrogen sources are divided in peptone, beef extract, and yeast extract. Citrate, manganese, and magnesium salts are also included (Terrade, Noël, Couillaud, & de Orduña, 2009). Culture media, such as MRS, are usually very expensive; as an alternative for LAB; Dong et al. (2014) have found that maltose and lactose were suitable carbon sources for the growth of *Lactobacillus salivarius*. CW and SCW are known to be rich in lactose, both containing around 4% (w/v) of this sugar. Additionally, Aller et al. (2014) found that the branched-chain amino acids (BCAAs), L-isoleucine (Ile), L-leucine (Leu), and L-valine (Val), present in CW, are essential for the growth of *Lactococcus lactis* IL1403. It has also been found (Terrade & Orduña, 2009) that these BCAAs are essential for the growth of other LAB: *Oenococcus oeni*, *Lactobacillus buchneri*, and *Lactobacillus hilgardii*.

4.1. Cultivation of LAB using whey-based culture medium

Aiming at cost reduction, environmental sustainability, and simplification of processes, one possibility is to reuse agroindustrial residues as culture media. As mentioned above, lactose and the BCAAs present in whey are reported to affect positively the growth of several LAB. Therefore, the growth of *Propionibacterium freudenreichii* in hyper-concentrated CW has been studied (Huang et al., 2016b) regarding total solid content of the medium. *P. freudenreichii*, however, is not a lactic acid bacterium, although it can be used as adjunct culture for cheese ripening (Gagnaire, Jardin, Rabah, Briard-Bion, & Jan, 2015). Huang et al. (2016b) observed that content matter affects osmotic stress resistance of the microorganism, showing that the 30% solid (w/w) medium allowed for the best tolerance in assays with temperature (heating to 60 °C for 10 min), acidity (pH 2.0 for 1 h), and bile salts (1 g L^{-1} for 1 h). In

this sense, the authors claimed that the growth in hyper-concentrated sweet whey triggers a multi-tolerance response, which could lead to higher survival rates after concentration processes such as SD or lyophilisation.

Regarding the growth of LAB, whey-based culture media have been reported using pre-treated CW via different processes. For instance, whey permeate supplemented with yeast extract or with yeast extract plus tryptic and pancreatic peptones has been reported as cultivation broth for *Lactobacillus helveticus*. Using a 2-L laboratory-scale bioreactor, maximum biomass concentration results were of 4.0 g L^{-1} and 6.8 g L^{-1} for each medium, respectively (Amrane, 2001). Analysing these results, it is possible to conclude that the need for supplementation may be genus or even strain dependent, especially as Soriano-Perez et al. (2011) found that the addition of yeast extract does not represent enhancement on either lactic acid production or on cell growth of *Lb. helveticus*, for example, although it was not applied using a bioreactor. Regarding other LAB, ultra-filtered CW with no supplementation was evaluated as culture medium for the biomass production of *Lactobacillus casei* under batch experiments, reaching its highest yields (around 4 g L^{-1}) in 5–10 h of fermentation (Alvarez, Aguirre-Ezkauriatza, Ramírez-Medrano, & Rodríguez-Sánchez, 2010). On the other hand, comparing whey-based and commercial/in-house formulated media in the growth of *Lb. casei* (*Lactobacillus paracasei* and *Lactobacillus acidophilus* included), Burns et al. (2008) found that pure whey (unsupplemented) shows growth kinetics of at least 1 log lower than the other media evaluated in the study; a small supplementation with 0.3% of yeast extract, however, allowed yields with no significant difference ($p \leq 0.05$) when compared with MRS, as opposed to what is mentioned above. This reinforces the argument that the need for supplementation is possibly strain dependent.

Furthermore, considering the scaling-up of the biomass production, expensive pre-treatments in culture media such as filtration processes are to be avoided, focussing on cost reduction. In addition, as little supplementation as needed is highly recommended, aiming at maximum value addition to the residue used as culture medium. In this regard, Table 1 shows a number of articles that employ "pure" whey (with possible supplementation, but that has not undergone previous treatment such as concentration, e.g., whey permeate or whey protein isolate) as growth medium for biomass production of LAB. Pescuma et al. (2012) used CW as culture medium for the growth of *Lb. acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lb. casei*, *Lb. paracasei*, *Lactobacillus rhamnosus*, and *Streptococcus thermophilus*, increasing up to 3.72 log cfu mL^{-1} (*Str. thermophilus*) after 24 h of incubation in flasks. The same study shows the acidifying ability of the strains during growth, with maximum pH variation of 2.66 (*Lb. bulgaricus*). *Lb. paracasei* had the lowest growth and acidification ability among the strains tested. Another report (Lavari, Páez, Cuatrin, Reinheimer, & Vinderola, 2014) refers to growth of *Lb. rhamnosus*, *Lb. paracasei*, and *Lactobacillus gasseri* in both CW and SCW. CW and MRS were not significantly different ($p \leq 0.05$) for the growth of *Lb. gasseri*, although SCW was around 1 log order lower than the others. *Lb. rhamnosus* showed equal growth ($p \leq 0.05$) in both CW and SCW when compared with MRS. The growth of *Lb. paracasei* was satisfactory in both media, as opposed to what was mentioned above (Pescuma et al., 2012); this means that the ability to grow in dairy-based media may be related to the strain. This exemplifies not only the versatility of lactic acid-producing bacteria in adapting to different culture conditions, but also that CW is not the only by-product from the milk industry to which value can be added.

As to SCW alone, very few authors have studied the process of biomass production of LAB using it as culture medium. Maragkoudakis, Nardi, Bovo, Corich, and Giacomini (2010) showed that scotta is suitable for the growth of LAB attaining growth of 8.0–9.0 log cfu mL^{-1} for probiotic *Lb. acidophilus* LA-5 together with *St. thermophilus* and *Lb. bulgaricus*. Secchi et al. (2012) employed SCW, either supplemented (SCY) or not (SC) with yeast extract and manganese sulphate, for the growth of *Lb. casei*, *Lb. helveticus*, and *Str. thermophilus* using a 3-L stirred-tank reactor. The pH (5.5) and temperature (37 °C for lactobacilli and 42 °C for streptococci) were constant, while stirring was kept at 30 rpm. Fermentation time was 20 h. The highest bacterial growth was observed for *Str. thermophilus* in SCY (around 2.5 log cfu mL^{-1}), although it reached almost 2 log cfu mL^{-1} in SC. The lowest growing capacity was observed in *Lb. casei*, with increase in viability of approximately 1.5 in SCY and 1.0 in SC, although its bacterial counts were higher than the others (between 9 and 9.5 log cfu mL^{-1}). None of the strains completely consumed lactose in SC medium, while increased consumption was observed in SCY. *Str. thermophilus* and *Lb. helveticus* consumed 100% of the lactose in the referred medium, although *Lb. casei* showed equal or higher cell viable counts when compared with both microorganisms in

Table 1
Production of LAB biomass using CW and SCW as culture media.

Bacterial strain	Culture media	Growth parameters	Inoculum	Bacterial growth	Reference
Lb. acidophilus, *Lb. paracasei* and *Lb. casei*	Whey supplemented or not with glucose and yeast extract	In flasks, possibly (data not shown) 37 °C, 10 h	2% (v/v)	Between 8 and 9 log cfu mL^{-1}	Burns et al. (2008)
Lb. casei	Cheese whey	Batch: in flasks, at 30 °C and 200 rpm Fed-batch: in 6-L fermenter at 30 °C, pH 7.0, 200 rpm	2% (v/v)	Batch: between 0.2 and 0.33 g L^{-1} Fed-batch: from 0.55 to 1.70 g L^{-1}	Bernárdez et al. (2008)
Lb. helveticus	Cheese whey	In 1-L flasks 45 °C, pH 5.9, 200 rpm	Initial cell concentration: 7.48 log	8.58 log cfu mL^{-1} (final − initial = 1.1 log cfu mL^{-1})	Soriano-Perez et al. (2011)
Lb. acidophilus, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. casei*, *Lb. paracasei*, *Lb. rhamnosus* and *Str. thermophilus*	Cheese whey	In 200-mL bottles at 37 °C, statically for 24 h	2% (v/v)	Between 1.35 and 3.72 log cfu mL^{-1} (final − initial)	Pescuma et al. (2012)
Lb. paracasei, *Lb. rhamnosus* and *Lb. gasseri*	Cheese and ricotta whey	In flasks, possibly (data not shown) 37 °C, 18 h	1% (v/v)	Between 1 and 2.5 log cfu mL^{-1} (final − initial)	Lavari et al. (2014)
Lb. casei, *Lb. helveticus*, and *Str. thermophilus*	Ovine ricotta whey supplemented or not	In 3-L bioreactor pH 5.5, 30 rpm (data for temperature not shown)	10% (v/v)	Between 0.9 and 2 log cfu mL^{-1} (final − initial)	Secchi et al. (2012)

either media. Since the initial lactose concentration in ricotta whey evaluated in this study was around 4.98%, very similar to what has been reported for CW (Dragone et al., 2011; Guimarães et al., 2010), it is expected to allow for good bacterial growth, as much as the original by-product.

Furthermore, Bernárdez, Amado, Castro, and Guerra (2008) have produced highly concentrated cultures of *Lb. casei* both with batch and fed-batch fermentation protocols, using either CW or diluted CW (DW) as culture media. In this case, batch cultures were carried out at 200 rpm, aeration of 0.5 L h^{-1}, pH 7.00, and at 30 °C in a 6-L bench top bioreactor, with fermentation time of 36 h. Other batch cultures (with DW, in flasks: 12 h, 200 rpm) without pH control were alkalised to pH 7.0 and proceeded to fed-batch cultivation, maintaining the same parameters as batch cultures alone. Alkalisation and feeding (CW and lactose) were repeated every 12 h until the microorganism was unable to continue decreasing the pH. Secchi et al. (2012) observed maximum biomass productivity of 0.20 g L^{-1} and 0.33 g L ¯1 in batch experiments (CW and DW, respectively), in which *Lb. casei* was also unable to consume all the available lactose in either media, similar to what is mentioned above. However, in this particular experiment, results are not expressed in terms of log cfu, which makes it harder to have a proper comparison when it comes to cell viability. Enhanced biomass productivity (from 0.33 to 0.75 g L^{-1}) was observed in the fed-batch experiments. Maximum viable counts occurred at 48 h of cultivation, resulting in 9.69 log cfu mL^{-1}; when *Lb. casei* finally lost its ability to decrease pH (after 156 h), cell counts were of 9.28 log cfu mL^{-1}. These results are similar to what has been mentioned before (Secchi et al., 2012), considering that both strains of *Lb. casei* grew almost in the same order in unsupplemented CW, although under different conditions. Furthermore, to be used industrially, a LAB culture must have a configuration that is accessible, easy to use, and most importantly, that enables storage for a certain period of time.

5. Concentration methods of LAB

As mentioned, microbial cultures require an operational form to be used in the industry, which means that they must be easy to obtain and apply in food products, and must enable some storage time. If possible, the culture should not have a major decrease in viability, either during food processing or after a storage period. Many methods of LAB concentration have been implemented in large scale; freeze-drying and spray-drying, however, are the most common. This part of our paper aims at explaining and comparing these methods according to the requirements aforementioned.

5.1. Freeze-drying of LAB

Freeze-drying (FD), also known as lyophilisation, is a method for concentrating compounds based on water sublimation. The product of interest is previously frozen to low temperatures, which enables the formation of ice crystals. Subsequently, applying low pressures and temperatures, ice sublimates and vapour is removed by diffusion and convection (primary drying). Approximately 10–35% of bound water still remains on the formed cake; for that reason, in secondary drying, the sample is slightly heated causing vapour to be removed by desorption (Lim et al., 2016; Morais et al., 2016; Qian & Zhang, 2011). This process is described in Fig. 2. FD is largely employed in the industry especially because it preserves the original properties in the dried product, which is useful for aromas, flavours, pharmaceuticals, and also for biotechnological products. The issue, however, is that this process requires expensive equipment and it consumes large amounts of energy, due to extensive drying time (Luo & Shu, 2017; Nakagawa & Ochiai, 2015).

Therefore, FD is reported for the drying of nanoparticles (Sigehuzi, 2017; Umerska et al., 2018), pharmaceuticals (Bjelošević et al., 2018), human milk (Castro-Albarrán et al., 2016), food products (Djekic et al., 2018), and proteins (Tu, Zhong, & Wang, 2017). FD is also the conventional method for drying LAB cultures in the food industry. However, stressful FD conditions could be harmful for these microorganisms, decreasing cell viability, and consequently, decreasing process efficacy. In fact, Santivarangkna, Kulozik, and Foerst (2008) affirm that there is a two-factor hypothesis for cell damage during FD, including dehydration and mechanical pressure injuries. Dehydration occurs because the cytoplasm of bacterial cells freezes at lower temperatures than its surroundings, and frozen solutes are concentrated, causing an osmotic unbalance that forces water to be removed from inside the cell. Mechanical pressure is driven by the formation of extra and/or intracellular crystals, which can damage cell membrane and internal structures. Thus, the optimisation of cooling rates is highly necessary, as well as employing other materials as cryoprotectants, which means they will serve as a protection for frozen bacteria, diminishing the injuries mentioned above (Bravo-Ferrada et al., 2018).

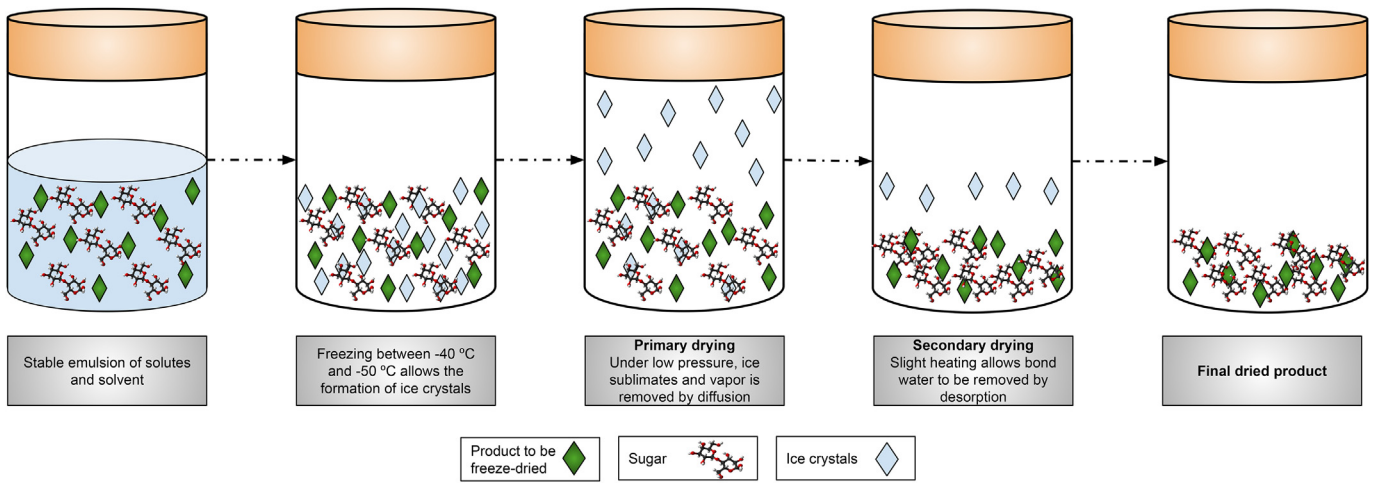

Fig. 2. Representative illustration describing the steps involved in the freeze-drying process, using sugar-based cryoprotectants.

5.1.1. Freeze-drying of LAB using whey-based cryoprotectants

Mendoza et al. (2014) freeze-dried *Lc. lactis*, *Lactococcus garvieae*, and *Lactobacillus plantarum* and showed that survival after the process was significantly ($p \leq 0.05$) higher when cryoprotectants (lactose, sucrose, skim milk, whey protein concentrate (WPC), and combinations of the former) were used, compared with cells dried in water matrixes. The studies also showed higher values ($p \leq 0.05$) of cell viability after storage at 4 °C when compared with 25 °C, meaning dried material must be kept under refrigeration. In this situation (4 °C storage), there were no significant differences in cell viability between milk, milk + sugars, and WPC + sugars as cryoprotectants for either *Lc. lactis* or *Lb. plantarum*. While the former LAB is used as a starter in many fermented food products, the latter is associated with probiotic benefits, which indicates that FD with whey-based cryoprotectants could be suitable for drying both types of LAB.

Probiotic *Bifidobacterium longum* 1941 was freeze-dried on milk and whey protein sources (casein and WPC) with the addition of different sugars as cryoprotectants (Dianawati, Mishra, & Shah, 2013). Results show that the highest viability after FD was observed in WPC + glycerol, WPC + casein + glycerol, and casein + mannitol (over 95% of survival for all of them). As to starter LAB, FD of *Str. thermophilus* was also performed in milk/whey proteins (Sharma et al., 2014). WPC resulted in a survival rate of 65%, whereas sweet whey powder provided protection that enabled 58% of the microorganisms to survive through the process. Sodium caseinate, however, had the highest viability of all the media tested (81%). To the best of our knowledge, there are no reports exploiting SCW for this purpose, nor there have been any studies using CW alone (unsupplemented and without concentration processes) as cryoprotectant for LAB.

5.2. Spray-drying of LAB

Spray-drying (SD) was invented in the 1920s, primarily for the production of milk and soap powders. It involves the atomisation of a liquid feedstock into particles (small droplets) that get in contact with hot air circulating inside a drying chamber, as seen in Fig. 3. Thus, evaporation of water content and formation of capsules occur simultaneously, as the ready-to-use material is collected in a specific recipient. The fact that this process is fast and the possibility of working on a continuous system have made SD useful to assess large amounts of LAB cultures; moreover, it represents 12% of operational costs and 24% of maintenance costs compared with other methods such as FD (Peighambardoust, Golshan Tafti, & Hesari, 2011).

Nonetheless, the contact with hot air may cause a great loss in viability of LAB cells (Gong et al., 2014). For that reason, many authors have reported suitable wall materials and optimal thermal conditions for the protection of bacteria through the process of SD. Huang et al. (2017a), for example, defined a table of inlet and outlet temperatures and respective wall materials of many sources that help enhance cell viability throughout SD. The mean air inlet temperature (IT) is around 150 °C, whereas mean outlet temperature is around 80 °C (Huang et al., 2017a). Behboudi-Jobbehdar, Soukoulis, Yonekura, and Fisk (2013) studied the optimisation of the SD process for *Lb. acidophilus*, obtaining the best results (total viability counts of 8.59 log cfu g^{-1}) with mean air inlet and outlet temperatures of 134 °C and 73–74 °C, respectively. The wall material evaluated in this study was a combination of maltodextrin, whey concentrate, and glucose (Behboudi-Jobbehdar et al., 2013).

5.2.1. Encapsulation of LAB via spray-drying

Along with concentrating LAB cultures for further use in the industry, techniques that provide protection are also welcome. Microorganisms and other substances undergo many types of stress in food processing. Encapsulation is a method for the bioprotection of bioactive compounds, in which pigments (Bucurescu, Blaga, Estevinho, & Rocha, 2018), nutrients (Troya, Tupuna-Yerovi, & Ruales, 2018), enzymes (Tetter & Hilvert, 2017), conservatives (Istenič et al., 2015; Matos, Gutiérrez, Martínez-Rey, Iglesias, & Pazos, 2018), or microorganisms (Burgain, Gaiani, Linder, &

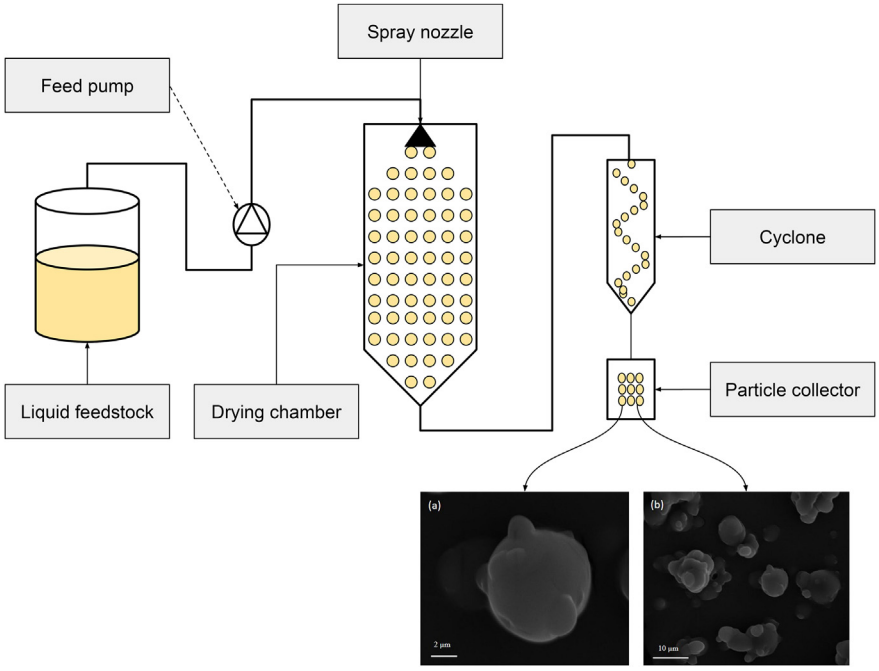

Fig. 3. Representative schematics of the spray-drying process, illustrating system steps and the formation of the microcapsules. The picture of a *Lactobacillus plantarum* ATCC8042 encapsulated with cheese whey as wall material using scanning electron microscopy in this figure is adapted from Eckert et al. (2017).

Table 2
Encapsulation of LAB by spray-drying using CW as protecting agent.[a]

Bacterial strain	Encapsulating agent	Spray-drying parameters	Viability after the process	Reference
B. animalis	Cheese whey	Laboratory scale IT: 150 °C OT: 50–60 °C. FF: 6 mL min^{-1}	>9 log cfu g^{-1}	De Castro-Cislaghi et al. (2012)
*Lb. plantarum**	Whey supplemented or not (malto-dextrin, pectin, Arabic gum)	Laboratory-scale IT: 180 °C OT: 70 °C. FF: data not shown.	Around 9 log cfu g^{-1}	Bustos and Bórquez (2013)
Lb. acidophilus	Sweet whey	Laboratory scale IT: 180 °C OT: 85–95 °C. FF: 8 mL min^{-1}	Around 7 log cfu g^{-1} (~76%)	Maciel et al. (2014)
*Lb. paracasei**	Cheese whey	Laboratory scale IT: 160 °C OT: 55–60 °C. FF: 6 mL min^{-1}	9.49 log cfu g^{-1} (~93%)	Ilha et al. (2015)
Lb. plantarum	Cheese whey	Laboratory scale IT: 90 °C, OT: 75 °C. FF: ~4.17 mL min^{-1}	10.16 log cfu g^{-1} (reduction of 0.42 log)	Eckert et al. (2017)
Lb. rhamnosus	Cheese whey and starch	Laboratory scale IT: 140 °C OT: 83 °C. FF: 4.5 mL min^{-1}	9.4 log cfu mL^{-1} (reduction of 0.5 log)	Lavari et al. (2017)

[a] Abbreviations are: IT, air inlet temperature; OT, air outlet temperature; FF, feed flow. An asterisk indicates endogenous bacteria.

Scher, 2011) are trapped inside a capsule made of variable substances, called wall material or encapsulating agent. This method produces particles (capsules) in a range of millimetres to nanometres (Đorđević et al., 2014). Many techniques have been recently reported for the encapsulation of LAB, such as SD (Pérez-Chabela et al., 2013), emulsification with ionic or enzymatic gelification (Qu et al., 2016), and extrusion with vibrational technology, also known as vibrating-jet or prilling (Eckert et al., 2018). As mentioned, SD is a technique that combines drying and encapsulation of bioactive compounds. The study of wall materials for bacteria is particularly important since their viability is significantly reduced because of the temperature employed in the process.

5.2.2. CW and SCW as encapsulating agents for the spray-drying of LAB

CW and SCW contain nutrients that could protect LAB from the SD process. Lactose, for instance, interacts with the polar section of phospholipids in bacterial cell membrane, minimising the damage caused by SD and increasing viability in storage time (Maciel et al., 2014). In addition, the temperature used in this encapsulation method helps to unfold the protein structure. Denatured proteins have free carboxylic and amino groups available for reactions, which increases hydrophobic forces, inasmuch as hydrogen and sulphide bonds, resulting in aggregation, coagulation, and even precipitation. This creates a microcapsule capable of protecting LAB from harsh drying and storage conditions, and it also increases survival when passing through the GIT (Khem, Small, & May, 2016). Researchers have employed whey protein isolate (Khem, Bansal, Small, & May, 2016) or concentrate (Behboudi-Jobbehdar et al., 2013) as encapsulating agents for LAB. Considering industrial scale production, cost reduction and fewer steps of previous treatment in CW are important goals.

Following this reasoning, encapsulation using non-concentrated CW as wall material has been reported by some authors (Table 2). The SD of *Lb. plantarum* using whey has shown reduction of 0.42 log cfu g^{-1} in viability after the process (Eckert et al., 2017). Likewise, an endogenous strain of *Lb. plantarum* was also spray-dried in pure whey (W) and in whey supplemented with maltodextrin (WM) plus pectin (WMP) or Arabic gum (WMG). In this case, the microorganism showed a viability loss of 0.27 log cycles when spray-dried in W, while WMP medium provided lower protection, showing a reduction of 0.86 log cycles in cell viability (Bustos & Bórquez, 2013). Hence, supplementation of CW with different carbohydrate sources does not always lead to lower reduction in survival rates, especially because whey alone has shown higher protection in the latter studies mentioned. Similarly, Lavari, Burns, Páez, Reinheimer, and Vinderola (2017) also supplemented whey with an extra carbohydrate source, in this case starch, to analyse the SD of *Lb. rhamnosus*. The resulting reduction was of 0.5 log cfu mL^{-1}, very similar to that obtained with unsupplemented whey, which corroborates the argument exposed above. In fact, as seen in Table 2, supplementation does not mean enhanced viability, since bacterial counts after SD were very similar in almost all cases. In addition, and to the best of the authors' knowledge, there are no papers reporting the use of SCW as an encapsulating agent for the SD of LAB.

Moreover, storage of encapsulated bacteria is highly important in an industrial environment, mainly because the dried product may remain in stock for several weeks. Therefore, results such as 0% of reduction in viability (Eckert et al., 2017) represent a microbial resistance that is very useful in food processing. In comparison, the viability of spray-dried *Bifidobacterium animalis* BB-12 using pure CW as wall material remained high and steady (>9 log cfu g^{-1}) for 12 weeks of storage at 4 °C (De Castro-Cislaghi, Silva, Fritzen-Freire, Lorenz, & Sant'Anna, 2012). These two reports also evaluated the viability of encapsulated bacteria when stored in milk sources, such as raw milk (Eckert et al., 2017) or a milk-based dessert (De Castro-Cislaghi et al., 2012), both at 4 °C for 42 days. Similarly, *Lb. plantarum* and *B. animalis* were able to maintain cell viability of over 9 and 7 log cfu g^{-1}, respectively, and reduction was of approximately 1 log cfu g^{-1} after the 42 days. Maciel et al. (2014) spray-dried *Lb. acidophilus* using unsupplemented CW and have observed similar bacterial counts after 90 days of storage at 4 °C, with final microbial population of over 6 log cfu g^{-1}, and reduction of 0.43 log cfu g^{-1}. On the other hand, *Lb. plantarum* showed bacterial counts of 9.98 log cfu g^{-1} when stored for 70 days with whey alone as encapsulating agent, which provided significantly higher protection than

Table 3
Joint procedure of using CW/SCW as culture media and as encapsulating agents.[a]

Bacterial strain	Culture media	Growth device and parameters	Bacterial growth	Encapsulating agent	Spray-drying parameters	Viability after the process	Reference
Lb. reuteri	Cheese whey ± yeast extract	1-L fermentor 37 °C, pH 5.0, 200 rpm Inoculum: 10^4 cfu mL^{-1}	9.23 log cfu mL^{-1}	Whey (from fermentation broth)	Laboratory scale IT: 55–65 °C OT: 89–100 °C. FF: 2–4 mL min^{-1}.	7.4 log cfu g^{-1}	Jantzen et al. (2013)
Lb. casei	Sweet whey ± casein peptone	37 °C for 48 h 1% (v/v) inoculum	9.3 log cfu mL^{-1}	Whey (from fermentation broth)	Pilot scale IT: 140 °C OT: 63 °C. FF: not shown.	*Lb. casei*: 40%	Huang et al. (2016a)
Lb. paracasei	Cheese whey supplemented with sucrose	In 200 mL-flasks 6 h, 37 °C 1% (v/v) inoculum	8.64 log cfu mL^{-1}	Whey from fermentation + whey protein concentrate	Laboratory scale IT: 150 °C OT: 70–75 °C. FF: 4.5 mL min^{-1}.	7.09 log cfu mL^{-1}	Ardanareswari, Utami, and Rahayu (2017)
Lb. casei	Cheese whey	500-L bioreactor 37 °C for 48 h 1% (v/v) inoculum	9.2 log cfu mL^{-1}	Whey (from fermentation broth)	Industrial scale IT: 140 °C OT: 60 °C. FF: not shown.	Around 9.65 log cfu g^{-1}	Huang et al. (2017a)
Lb. casei	Cheese whey	1 to 2-L bioreactor, probably (specific data not shown) 37 °C for 48 h 1% (v/v) inoculum	8.48 log cfu mL^{-1}	Whey (from fermentation broth)	Laboratory scale IT: 185 °C OT: 75 °C. FF: not shown.	Around 9 log cfu g^{-1}	Huang et al. (2018)
Lb. plantarum and *Lb. casei*	Cheese whey + yeast extract, glucose and sucrose	Data for device and parameters not shown 3% (v/v) inoculum	*Lb. casei*: 7.47 log cfu mL^{-1} *Lb. plantarum*: 8.07 log cfu mL^{-1}	Whey (from fermentation broth)	Laboratory scale IT: 150 °C OT: 100 °C. FF: not shown.	*Lb. casei*: 7.34 log cfu g^{-1} *Lb. plantarum*: 8.14 log cfu g^{-1}	Bartkiene et al. (2018)
Lb. fermentum	Whey + yeast extract	1-L bioreactor 37 °C, pH 5.5, for 10 h.	10.29 log cfu mL^{-1}	Whey from fermentation broth + maltodextrin, Arabic gum, and sweet whey	Laboratory scale IT: 190–200 °C OT: 90.8 °C. FF: not shown.	Reduction of 0.96 log cfu g^{-1}	Aragón-Rojas et al. (2018)

[a] Abbreviations are: IT, air inlet temperature; OT, air outlet temperature; FF, feed flow.

whey supplemented with maltodextrin, Arabic gum, or pectin (Bustos & Bórquez, 2013). Hence, supplementation does not necessarily enhance viability during storage, and it apparently does not represent better protection to the SD process itself.

Another important factor, especially when considering probiotic bacteria, is to evaluate survival when passing through simulated GIT. In this regard, SD could represent a way to increase viability of these microorganisms in the harsh conditions of low pH and in the presence of bile salts. An endogenous strain of *Lb. paracasei* was spray-dried with CW and showed lower reduction in viability (1 log cfu g^{-1}) compared with free cells (4.5 log cfu g^{-1}), especially in the lowest value of pH (2.0), which corresponds to the stomach environment. In the presence of bile salts, encapsulated *Lb. paracasei* also had higher counts (reduction of 2.17 log cfu g^{-1}) and stability for a longer period of time (12 h) than free cells (3.34 log cfu mL^{-1}, 9 h) (Ilha, da Silva, Lorenz, de Oliveira Rocha, & Sant'Anna, 2015). Similarly, *B. animalis* showed high survival rates (reduction of 0.73 log cfu g^{-1}) in the most extreme condition of the GIT; pH 2. In the same conditions, non-encapsulated bacteria showed reduction of 1.51 log cfu g^{-1} (De Castro-Cislaghi et al., 2012). Hence, encapsulating LAB with CW leads to a better maintenance of the survival of these microorganisms in the human gut, playing an important role in increased health, since many of these bacteria are probiotic.

Ferreira et al. (2017) freeze- and spray-dried *Lb. plantarum*, using sweet whey as encapsulating agent/cryoprotectant. No differences ($p \leq 0.05$) were observed in survival after both processes, meaning CW is a suitable medium for protecting this microorganism. After 60 days of storage at 4 °C, FD samples had a 2 log reduction in viability, while SD samples remained stable. Spray-dried *Lb. plantarum* was also more efficient in reducing pH of skimmed milk, compared with freeze-dried microorganisms. SD samples were also more resistant under stressful conditions of acid and bile salts.

Therefore, SD is considered a suitable technique for the encapsulation of probiotic LAB. In fact, the majority of reports on SD concern these type of microorganisms, unlike what is observed with FD. In the dairy industry, CW is used for several uses that add value to this material, and protein concentration (for the production of whey protein concentrate and isolate) is one of the most profitable (Smithers, 2015). On the other hand, with the market of probiotics reaching USD$43 billion in 2017 and with estimated growth of up to USD$59 billion in 2022 (Market Research, 2018), new alternatives for the development of dried cultures of these microorganisms are required. Under this perspective, and considering that CW is an ideal culture medium and encapsulating agent for the SD of probiotics, the demand for this by-product will increase and so will the interest in selling this material for these functions.

6. Double use of CW: culture medium and encapsulating agent for spray-drying of LAB

Few researchers have explored the use of CW as culture medium for the growth of LAB, and subsequently, as encapsulating agent for SD (Table 3). Studies reporting the use of SCW in these procedures have not been found, which may be due to the lack of proteins in this medium, resulting in poor protective properties considering the SD process.

Jantzen, Göpel, and Beermann (2013) encapsulated *Lactobacillus reuteri* using CW as culture medium and encapsulating agent in a direct process. The biomass was obtained via fermentation in a 1 L-stirred tank reactor using whey (20%, w/v) supplemented with yeast extract (0.5%, w/v) under pH of 5.0, at 37 °C and 200 rpm. The culture reached stationary phase (9.23 log cfu mL^{-1}) after 24 h. Maximum bacterial counts were approximately 1 log order higher in supplemented whey compared with growth in pure medium, without yeast extract, which also corroborates the findings in section 4.1 of this paper. After that, 200 mL of the fermented whey were submitted directly to the spray-dryer. Inlet and outlet temperatures in the laboratory-scale equipment were 55–65 °C and 89–100 °C, respectively, resulting in a dried product that contained 7.40 log cfu g^{-1}. Survival under simulated GIT conditions increased by 34% compared with non-encapsulated *Lb. reuteri*. During the 4 weeks of storage at 4 °C, cells lost viability of around 1 log cycle.

Later, Huang et al. (2016a) studied the influence of CW total solids (TS) on its efficiency as a culture medium and encapsulating agent for *Lb. casei*. Medium concentration varied in TS (w/v) of 5, 10, 20, 30 and 40%, either with or without the supplementation of casein peptone (0.5%, w/v). Results showed maximum biomass production of 9.30 log cfu mL^{-1} in the supplemented media containing 20 and 30% TS. In the latter, the strain developed better than in MRS broth (9 log cfu mL^{-1}). The medium containing 40% TS, however, inhibited *Lb. casei* growth. Interestingly, regarding casein peptone, the dependency of *Lb. casei* final population on supplementation decreased while TS increased. As to SD, bacteria had a 40% viability count in the 30% TS medium, a 60-fold increase when compared with the 5% TS, indicating that sugar and protein contents represent an important protection for this process. What is more, *Lb. casei* with 20–40% TS had a maximum log reduction of 0.89 during storage (4 °C for 120 days), when the population was 8.84 log cfu g^{-1}. These were the best results among all the other media tested. In conclusion, authors have shown that the total solid contents of the medium not only enhance biomass production, but also tend to offer the best protection to drying processes and storage. This may be related to osmotic stress tolerance: since bacterial growth occurred in a concentrated medium, the high osmolality played a role in selecting the most resistant bacteria, which will obviously show higher survival than non-resistant bacteria. Higher solids also help to create thicker capsules in the spray-drier, which will eventually offer better protection, increasing viability in drying and storage.

Other strategies to create better capsules for SD can be studied; for example, the addition of extra carbon or nitrogen sources to the fermentation medium. Under this perspective, the ability of whey supplemented with yeast extract for supporting the growth of *Lactobacillus fermentum* K73 in a 1-L bioreactor was evaluated, and an extra addition (after 10 h of cultivation) of carbon sources such as maltodextrin, Arabic gum, and whey itself were also tested. In this case, Aragón-Rojas, Quintanilla-Carvajal, and Hernández-Sánchez (2018) found that the mixture of maltodextrin and whey offered the highest ($p \leq 0.05$) enhancement in survival under gastric pH conditions, with reduction of 0.12 log cfu mL^{-1}, and also provided support in the presence of bile salts, allowing *Lb. fermentum* to grow 0.15 log cfu mL^{-1}. After selecting the carrier material (whey + maltodextrin), the samples were spray-dried under different conditions. Aragón-Rojas et al. (2018) observed that there was cell decrease when microorganisms were exposed to simulated GIT conditions regardless of drying condition; results have shown that the atomising pressure was the most influential value to determine survival in the GIT, followed by outlet temperature. Subsequently, optimal drying was analysed with atomising pressure of 0.117 MPa and outlet temperature of 90.8 °C; when using the selected carrier materials, viability loss was of 2 log cfu g^{-1} after SD, 0.61 log cfu g^{-1} after exposure to in vitro conditions of gastric pH, and 0.2 log cfu g^{-1} after the addition of bile salts. With exactly the same parameters, whey alone (the culture medium used for the growth of *Lb. fermentum*) showed the highest viability after drying (0.96 log cfu g^{-1} reduction) and after simulated GIT conditions (0.58 log cfu g^{-1} reduction in gastric pH and 0.67 log cfu g^{-1} reduction with bile salts). In conclusion, extra carbon or nitrogen

sources may not always lead to better survival or higher cell viability under stress conditions. Actually, fermented whey alone showed the best protective effect of all the tested media, which corroborates the findings above and in section 5.2.2 of the present paper.

Scaling up is another important aspect in the production of dried bacterial cultures. Huang et al. (2017a) used CW (30%, w/v) as growth medium for the same strain aforementioned, using a static fermentation in a bioreactor (37 °C for 48 h). After reaching the stationary phase, the bioreactor was slightly agitated and the liquid resulting from the fermentation was directly submitted to three spray-dryers at different scales: laboratory, semi-industrial, and industrial scales. All experiments were conducted under the same inlet and outlet temperatures (140 °C and 60 °C). A survival of 60% was observed in the first two spray-dryers, with no significant difference ($p \leq 0.05$) between them. At the industrial scale, *Lb. casei* increased its viability, which shows that industrial-scale projects are absolutely attainable for producing LAB cultures.

7. Challenges in using CW and/or SCW as culture media and encapsulating agents of LAB

The implementation of both CW and SCW for the applications in the industry proposed in this paper is challenging. One of the challenges that must be highlighted is medium standardisation, since bacterial growth is very sensitive to variation in composition. Most of the reports cited in this article used whey powder for the growth and encapsulation of LAB. Of course, drying whey into a powder is an adequate solution, but it will significantly increase the cost of the whole operation. On the other hand, both CW and SCW in natura have been reported (Bernárdez et al., 2008; Lavari et al., 2014; Secchi et al., 2012; Soriano-Perez et al., 2011) for the multiplication of LAB, as they represent not only cost reduction but also a convenient process, since liquid media could be readily used for biomass production, only requiring previous heat treatment. This two-step process is exactly what this review is proposing.

Heat treatment is an important issue, especially concerning CW, due to the presence of proteins. In the studies cited in this article, treatments varied from complete sterilisation in autoclave (121 °C for 15 min) to pasteurisation in water bath (65 °C for 30 min). Pelegrine and Gasparetto (2005) and Wijayanti, Bansal, and Deeth (2014) observed that whey proteins such as β-lactoglobulin are completely denatured in a few minutes at high temperatures (>70 °C), while α-lactalbumin is more resistant to heat, not showing irreversible aggregation up to 90 °C. The effects of protein denaturation on the growth of LAB are yet to be studied. So far, considering the reports discussed in this paper, heat treatment of CW apparently does not affect biomass production, since values shown in Table 1 are very similar among different reports. Regarding SD, aggregation and gelation of proteins is highly desired, since these configurations are prone to forming good capsules (Bernard, Regnault, Gendreau, Charbonneau, & Relkin, 2011). Therefore, heat treatment of whey would not represent an obstacle for the encapsulation of LAB, although testing this process is highly recommended.

8. Conclusions

The development of LAB cultures starts with the selection of adequate strains for the product desired. Either as probiotic or as starter, LAB are prone to process standardisation, contributing to increased safety and quality of food products. The second step would be to implement a biomass production method. This typically requires the use of bioreactors, which automatically leads to the need for choosing a suitable culture medium. Dairy whey, especially CW, is a suitable material for this process, since it contains the majority of milk nutrients that supports well the growth of LAB. As seen, the supplementation with other compounds such as yeast extract is not a guarantee that higher biomass yield will be attained, since the effects of supplements vary among genera and/or strains. Therefore, testing the ability of specific strains to grow in CW and SCW, with or without supplements, is highly recommended. After growth, bacteria must still be introduced in the industry in an easy-to-use configuration. SD is reported to enable the production of powders with high cell viability, especially probiotic cultures. Thus, CW plays an important role in producing stable capsules that protect microorganisms from the drying process itself, from storage time, and from gastrointestinal tract conditions. Similarly, FD is a drying technique that allows the production of starter and probiotic LAB cultures, in viable concentrations for industrial applications. To the best of our knowledge, CW alone has not been reported as a cryoprotectant for the FD of LAB. Hence, it is possible to conclude that the versatility of dairy industry by-products still needs to be better explored, especially regarding SCW, as reports on its applications are very poor.

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