Viability of *Lactobacillus acidophilus* NRRL B-4495 encapsulated with high maize starch, maltodextrin, and gum arabic

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**ABSTRACT**

The effects of different protective agents, high maize starch (HM), maltodextrin (MD), and gum arabic (GA) on the viability of *Lactobacillus acidophilus* after spray drying and during storage at different conditions were investigated. Also, the physicochemical properties of the spray dried powders were evaluated. *Lactobacillus acidophilus* NRRL B-4495 (LA) was suspended in a 20% w/v solution of HM, MD, or GA. The solutions were separately spray dried at 140 °C to obtain LA powders: LAHM, LAMD, and LAGA. The powders were separately placed in aluminum bags and packed under 97 and 10% vacuum. The powders were stored at refrigerated (4 °C) and at room (23 °C) temperatures for 60 days. The moisture content of LA powders ranged from 5.63 to 8.98% with the LAMD powders showing lower moisture content than LAGA and LAHM powders. More than 6 log CFU/g of LA/g powder survived at 4 °C at 60 days of storage. LAGA and LAMD powders packed under 97% vacuum and stored at 4 °C had significantly higher cell viability than other powders. The study demonstrated that viability of LA powders packed under 97% vacuum and stored at refrigerated temperature meets the recommended levels to have therapeutic effects.

### 1. Introduction

Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2001). Some of their benefits on human health include control of irritable bowel syndrome, inflammatory bowel diseases, suppression of endogenous/exogenous pathogens, improving lactose tolerance, and reducing risk factors for colon cancer (Meng, Stanton, Fitzgerald, Daly, & Ross, 2008). These benefits are reached by different protective agents, such as antimicrobial activity, immune, antimutagenic, and antigenotoxic effects, influence on enzyme activity, competitive exclusion and colonization, among others (Nagpal et al., 2012; Papadimitriou et al., 2015). Lactobacilli species, such as *L. acidophilus*, *L. rhamnosus*, *L. paracasei*, and *L. plantarum* are the most common probiotic microorganisms (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). Due to the increasing demand for healthy, nutritious, and functional foods, the food industry is attempting to develop probiotic foods that can retain a high viability during both processing and storage conditions (Siu, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014). However, probiotic viability in food products is negatively affected by several factors, including the presence of antimicrobial compounds, oxygen toxicity, post-acidiﬁcation, and storage temperature (Vasiljevic & Shah, 2008). Poor cell viability during storage has been reported in many probiotic foods as well as low survival after consumption (Evivie, Huo, Igene, & Bian, 2017; Lin, Hwang, Chen, & Tsen, 2006; Martín, Lara-Villoslada, Ruiz, & Morales, 2015).

Encapsulation is one of the approaches to assure probiotic viability. This technology can help protect cell viability and functionality during processing, storage, and delivery through the human gastrointestinal tract (de Vos, Faas, Spasojevic, & Sikkema, 2010). The cells are entrapped within hydrocolloidal agents, resulting in a reduction of cell injury and/or cell loss caused by adverse conditions (Irvanie, Korbekandi, & Mirmohammadi, 2015). Spray drying is defined as a process in which a liquid feed is put in contact with a hot drying medium, leading to the evaporation of the liquid, obtaining a dried product in form of powders, granules, or agglomerates (Solvai, 2011). This microencapsulation technique improves the survival of probiotics in food during processing and storage and also helps to protect the probiotic cells against the harsh conditions in the gastrointestinal tract (Ranadheera, Evans, Adams, & Baines, 2015). Furthermore, this technique allows the production of microencapsulated probiotic bacteria with low production costs and higher productivity (Huang et al., 2017).
However, during this process cells are exposed to high temperature and osmotic stresses due to dehydration, which could result in the loss of viability after spray drying and also during storage (Pérez-Chabela, Lara-Labastida, Rodriguez-Huezo, & Totosaus, 2013).

In order to protect probiotic cells against the harsh conditions they are exposed during the drying process a variety of materials have been evaluated including gum arabic (GA), alginate, gelatin, maltodextrin (MD), pectin, skimmed milk, resistant starch, chitosan, whey protein, among others (De Castro-Cislaghi, Carina Dos Reis, Fritzen-Freire, Lorenz, & Sant’Anna, 2012). GA is a natural composite of proteins and polysaccharides and the most commonly used material in spray drying. Its structure contains a major group with highly branched polysaccharide consisting of a galactose backbone with linked branches of arabinose and rhamnose. The second group is a higher molecular weight arabinogalactan-protein complex (Arslan-Tontul & Erbas, 2017). Maltodextrins are high molecular weight polysaccharides, produced by starch hydrolysis. MD and other carbohydrates may contribute to increase the stability of spray-dried bacteria in terms of water activity, moisture content, pH, solubility, hygroscopicity, nutritional composition, glass transition temperature, color, and fluidity (Sosa et al., 2016). Starches are another important component for microencapsulation that exhibit good film-forming qualities which may further protect encapsulated substances (Nunes et al., 2018). Resistant starch is the small fraction of starch that resists hydrolysis by α-amylase and pullulanase treatment in vitro and is not hydrolyzed to glucose in the small intestine but is fermented in the colon (Raigond, Ezekiel, & Raigond, 2015). Due to these functional characteristics, high maize starch was selected as the encapsulation matrix for the probiotic cells. According to Burgain, Gaiani, Linder, and Scher (2011), resistant starch can be used as an encapsulating agent for targeted delivery of probiotic cells in the human colon. Research regarding the effects of a singular encapsulating material on probiotic survival is common in the literature. A comparison between some of the more widely used encapsulants and their protective effects under different storage conditions is pertinent to developing more effective probiotic delivery systems. The objective of this investigation was to evaluate effects of different encapsulating agents: high maize starch (HM), MD, and GA on the viability of L. acidophilus NRRL B-4495 during storage.

2. Materials and methods

2.1. Microorganism

L. acidophilus NRRL B-4495 (LA) was provided by ARS Culture Collection (Washington, DC). The frozen bacteria were activated twice in deMan Rogosa Sharpe (MRS) broth (Neogen Corporation, Lansing, MI). The strain (75 mL) was inoculated in MRS broth (1500 mL) and incubated at 37 °C for 16 h to reach stationary phase. The LA cell cultures were harvested and washed with sterile distilled water by centrifugation at 10000 × g for 10 min at 4 °C (Model J2-HC, Beckman Coulter, Inc., CA).

2.2. Preparation of probiotics solutions

Three wall material solutions (200 g/L) were separately prepared. HM with approx. 56% resistant starch (High maize® 260, Ingredion Incorporated, NJ), MD with a dextrose equivalent (DE) of 9–13, (Now Foods Company, Bloomingdale, IL), and GA (Frontier Co-op, Norway, IA) were dissolved in distilled water and autoclaved at 121 °C for 15 min. The wall material solutions were then mixed with LA cell cultures (~10^8 CFU/mL) to produce LA solutions.

2.3. Spray drying of probiotic solutions

LA probiotic solutions mixed with the different wall materials were separately fed into a pilot-scale spray dryer (FT80/81 Tall Form Spray Dryer Armfield Inc., Ringwood, UK) under co-current drying conditions at the Food Processing Pilot Plant, Louisiana State University Agricultural Center. The inlet ambient air was electrically heated by a resistance heater to a constant temperature of 140 °C. A feeding pump delivered the probiotic solutions through a two-fluid stainless steel type spray nozzle where they were atomized with an atomizing air pressure of 14.5 psig and sprayed into the main dryer chamber. The three spray dried probiotic powders produced (LAHM, LAMD, and LAGA) were collected from the base of the cyclone vessel. Samples of approximately 1.5 g of the spray dried powders were separately placed in 4” x 6” aluminum bags and packed under 97% and 10% vacuum (Koch UV-550, Kansas City, MO). The packed powders were stored separately at refrigerated (4 °C) and at room (23 °C) temperatures for up to 60 days in order to analyze the cell viability during storage. Temperatures were selected based on our preliminary studies.

2.4. Physicochemical properties of the probiotic powders

The probiotic powders were analyzed for water activity (a_w), moisture content, and color. The water activity was measured using an AquaLab Pawkit (Decagon Devices, Inc., Pullman, WA). The moisture content was determined using a microwave-type moisture analyzer (Model 907875, CEM Corporation, Inc., Matthews, NC). The color of probiotic powders was measured using a chroma meter LabScanXE (HunterLab, VA) equipped with a pulsed xenon lamp and a 13 mm aperture diameter. CIELAB color scales L*, a*, and b* were used to express the results. The L* values measure the degree of lightness to darkness, a* values measure the degree of redness to greenness, and b* values assess the degree of yellowness to blueness.

2.5. Viability of probiotics before and after spray drying and during storage

Probiotic solutions mixed with the different wall materials and powders were determined for cell viability by separately suspending and homogenizing with a vortex mixer 1 g in 9 mL of 0.85 g/100 mL sterile saline solution. Serial dilutions were prepared from the initial suspension in the sterile saline solution. The pour plating method using MRS agar (Neogen Corporation, Lansing, MI) with 0.6 g CaCO3/100 mL (Sigma-Aldrich, St. Louis, MO) was performed in triplicate. The plates were incubated at 37 °C, enumerated after 48 h, and results expressed as colony forming units per gram sample (CFU/g).

2.6. Scanning electron microscopy

The morphology of probiotic powders was observed using a scanning electron microscope (SEM, JSM-6610LV, JEOL Ltd. Japan). Samples were mounted on aluminum SEM stubs and then coated with platinum in an Edwards S150 sputter coater (Edwards High Vacuum International, Wilmington, MA) for 4 min prior to observation at both 1000 × and 3000 × magnification.

2.7. Statistical analysis

The data were analyzed using SAS (Statistical Analysis System) software version 9.4 (SAS Institute Inc., Cary, NC). Experiments were performed in triplicate and the data were reported as means ± standard deviation. Tukey’s test at an alpha of 0.05 was carried out to determine significant differences among the treatments.

3. Results and discussion

3.1. Physicochemical properties of the probiotic powders

Water activity (a_w) of LA powders was not affected by type of wall material. As shown in Table 1, a_w values of LA powders ranged from 0.26 to 0.35 and were not significantly different. The a_w indicates free
Table 1
Water activity and moisture content of probiotic powders.

<table>
<thead>
<tr>
<th>Wall Material</th>
<th>Water activity</th>
<th>Moisture content (wet basis g/100g)</th>
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<tbody>
<tr>
<td>HM</td>
<td>0.35 ± 0.01A</td>
<td>8.98 ± 0.50A</td>
</tr>
<tr>
<td>MD</td>
<td>0.26 ± 0.00A</td>
<td>5.63 ± 0.02A</td>
</tr>
<tr>
<td>GA</td>
<td>0.31 ± 0.05A</td>
<td>8.94 ± 1.62A</td>
</tr>
</tbody>
</table>

Values are means ± SD of triplicate determinations. *Means ± SD with the same letter in a column are not significantly different (P > 0.05). LA = L. acidophilus NRRL B-4495, HM = high maize starch, MD = maltodextrin, and GA = gum arabic.

3.2. Scanning electron microscopy of spray-dried probiotic powders

The spray-dried LA powders were observed by SEM (Fig. 1). Spray-dried powders had a surface without mechanical fissures and the presence of concavities. These concavities were the result of the rapid evaporation of the atomized liquid drops during spray drying (Fritzen-Freire et al., 2012). LAMD powders consisted of particles with a wrinkled surface or with concavities. Rodríguez-Huezo et al. (2007) reported that the use of moderate inlet drying temperatures in spray-dried powders (140 °C) could produce concavities, making the particles stronger against mechanical fracture and solute diffusion. LAGA powders showed an overall spherical shape with both smoothness and shrinkage on their surface, which was also found in other studies using GA as a wall material for the production of spray-dried probiotic powders (Guergoletto, Busanello, & Garcia, 2017; Kingwatee et al., 2015).

3.3. Effect of wall materials on the viability of spray-dried L. acidophilus NRRL B-4495 powders

The viability during storage of LA powders with different wall materials is illustrated in Fig. 2. Initial cell counts in LAHM, LAMD, and LAGA probiotic solutions before spray drying were 9.06, 9.05, and 9.24 log CFU/g, respectively. The number of viable cells of LA powders decreased by less than 1.25 log CFU/g after spray drying. After spray drying, the number of viable cells in LAGA, LAHM, and LAMD powders was 8.10, 7.90, and 7.82 log CFU/g, respectively. These results showed that GA was the best protective agent of cells during the spray drying process. Regarding the storage temperatures, the results showed that the cell viability of LA powders was more stable at refrigerated temperature (4 °C) than those stored at ambient temperature (23 °C). These results are in agreement with other studies that evaluate the effect of temperature on the viability of L. acidophilus during storage (Nunes et al., 2018; Ranadheera et al., 2015; Soukoulis et al., 2014). According to Chávez and Ledeboer (2007) and Santivarangkna, Kulozik and Foerst (2007), the survival of probiotic bacteria is inversely related to the temperature during storage conditions. Furthermore, De Castro-Cislaghi et al. (2012) state that the encapsulating agent also has a direct effect on the stability of the microencapsulated cells. Regarding the vacuum conditions, LA showed a higher survival at 97% vacuum than at 10% vacuum when they were kept at 4 °C and at 23 °C. This suggests that lower levels of oxygen improved cell viability during storage (Chávez & Ledeboer, 2007). According to Champagne, Gardner, and Roy (2005), oxygen affects probiotic cells due to the intracellular production of hydrogen peroxide. In addition, Tripathi and Giri (2014) revealed that the production of free radicals from the oxidation of cellular fats can be toxic to probiotic cells.

Table 2
Color values of probiotic powders.

<table>
<thead>
<tr>
<th></th>
<th>HM</th>
<th>MD</th>
<th>GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>91.02 ± 0.96A</td>
<td>93.67 ± 1.09A</td>
<td>87.03 ± 0.32A</td>
</tr>
<tr>
<td>a*</td>
<td>-0.04 ± 0.01A</td>
<td>-0.39 ± 0.01A</td>
<td>0.11 ± 0.01A</td>
</tr>
<tr>
<td>b*</td>
<td>5.55 ± 0.29A</td>
<td>2.47 ± 0.63A</td>
<td>3.16 ± 0.71A</td>
</tr>
</tbody>
</table>

L*, a*, and b* are the degree of lightness to darkness, redness to greenness, and yellowness to blueness, respectively. Values are means ± SD of triplicate determinations. *Means ± SD with the same letter in a row are not significantly different (P > 0.05). LA = L. acidophilus NRRL B-4495, HM = high maize starch, MD = maltodextrin, and GA = gum arabic.

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L. acidophilus survived at 4 °C at 60 days of storage and that the concentration of the wall materials used was able to protect the encapsulated cells. This is in agreement with the results reported by other studies that evaluated L. acidophilus viability using different encapsulating agents during refrigerated storage. Their results showed that encapsulation by spray drying and storage at refrigerated temperatures were able to improve the number of viable cells (Behboudi-Jobbehdar, Soukoulis, Yonekura, & Fisk, 2013; Maciel, Chaves, Grosso, & Gigante, 2014; Pispan, Hewitt, & Stapley, 2013; Riveros, Ferrer, & Borquez, 2009). Zhao, Sun, Torley, Wang, and Niu (2008) claim that a minimum concentration of L. acidophilus equivalent to 10^6 CFU/mL or gram of product is needed to have therapeutic benefits in the human body. Functional foods such as yogurt containing above 6 log CFU/g or mL of viable probiotic L. acidophilus at the time of consumption help to promote health benefits to the consumers (Machado et al., 2017).

3.3.2. Viability of spray-dried L. acidophilus NRRL B-4495 microcapsules stored at room (23 °C) temperature

L. acidophilus survived at 4 °C at 60 days of storage and that the concentration of the wall materials used was able to protect the encapsulated cells. This is in agreement with the results reported by other studies that evaluated L. acidophilus viability using different encapsulating agents during refrigerated storage. Their results showed that encapsulation by spray drying and storage at refrigerated temperatures were able to improve the number of viable cells (Behboudi-Jobbehdar, Soukoulis, Yonekura, & Fisk, 2013; Maciel, Chaves, Grosso, & Gigante, 2014; Pispan, Hewitt, & Stapley, 2013; Riveros, Ferrer, & Borquez, 2009). Zhao, Sun, Torley, Wang, and Niu (2008) claim that a minimum concentration of L. acidophilus equivalent to 10^6 CFU/mL or gram of product is needed to have therapeutic benefits in the human body. Functional foods such as yogurt containing above 6 log CFU/g or mL of viable probiotic L. acidophilus at the time of consumption help to promote health benefits to the consumers (Machado et al., 2017).
viability is critically affected by the operating spray drying conditions. Also, Fu and Chen (2011) classify L. acidophilus as a heat sensitive bacteria. According to Soukoulis et al. (2014), some lactobacilli strains have greater thermotolerance throughout spray drying and this characteristic is strictly strain specific. It is likely that the effects of spray drying on cell damage could subsequently affect the viability of L. acidophilus during the storage conditions.

4. Conclusion

The present study investigated the effect of three wall materials on the viability of L. acidophilus NRRL B-4495 after spray drying and during storage at different conditions and the physicochemical properties of the spray dried powders. The LAMD powders had lower moisture content and water activity than the LAGA and LAHM powders. L. acidophilus powders with GA and MD packed under 97% vacuum and
stored at 4 °C had significantly higher cell viability than the other powder samples. The data obtained showed that more than 6 log CFU/g of *L. acidophilus* survived at 4 °C at 60 days of storage which meets the recommended levels to have therapeutic effects in the human host. The technologies used to produce and store encapsulated LA are commonly used in food applications, and are scalable to an industrial level. The obtained results may be used to optimize combined processing and storage conditions for survival of *L. acidophilus* NRRL B-4495 in more complex food systems.

References


