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S/O/W emulsions prepared with sugar beet pectin to enhance the viability of probiotic *Lactobacillus salivarius* NRRL B-30514

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ABSTRACT

Encapsulation is a common approach to improve the viability of probiotic bacteria against environmental stresses. However, conventional biopolymer beads have a millimeter dimension and can cause texture defects. The objective of this work was to study solid/oil/water (S/O/W) emulsions as a delivery system by suspending spray-dried probiotic *Lactobacillus salivarius* NRRL B-30514 in soybean oil followed by emulsification in sugar beet pectin solution. The encapsulation efficiency was up to 87%, and the droplets were smaller than 17 μm. Encapsulation of *L. salivarius* in S/O/W emulsions improved the viability under different conditions, including 2-week storage at 4 °C, pasteurization at 63 °C for 30 min, and the *in vitro* gastric and intestinal digestions containing proteases and bile salt. Cross-linking sugar beet pectin on emulsion droplets by divalent calcium ions further improved the viability of *L. salivarius* against various environmental stresses, enabling the survival of a significant portion of viable *L. salivarius* following treatment with simulated gastrointestinal digestions. Findings from the present work suggest the possibility of the studied S/O/W emulsions to deliver probiotic bacteria in foods to improve their viability during processing, storage, and digestion.

1. Introduction

Probiotics are viable microorganisms and substances that have positive physiological effects on intestinal microflora populations of the host (Guerra, Bernárdez, Méndez, Cachaldora, & Pastrana Castro, 2007). In recent years, probiotics also have drawn considerable attention as natural antibiotics in poultry products (Guerra et al., 2007; Woraharn, Chaiyasut, Sirithunyalug, & Sirithunyalug, 2010). Common probiotics include *Lactobacillus* such as *Lactobacillus salivarius*, *L. acidophilus*, and *Lactobacillus casei*, *Bifidobacterium*, and yeasts such as *Saccharomycces cerevisiae* (AFRC, 1989). Lactic acid bacteria are major microorganisms in the gastrointestinal tract of humans and animals, and bacteriocins and organic acids produced by these bacteria have positive effects against infections (Twomey, Ross, Ryan, Meaney, & Hill, 2002). Studies also concluded that probiotics as feed supplements can improve the growth rate, general health, and ability to resist diseases of poultry (Ahmad, 2006; Guerra et al., 2007).

However, there are several challenges in the application of probiotics as supplements, including the viability during processing and prolonged storage, and the survival in acidic conditions and digestive fluids especially the bile (Lin, Yu, Jang, & Tsen, 2007). Encapsulation is a commonly-studied technique to immobilize probiotics to enhance their survival rate in adverse environments. Entrapment of probiotics in calcium alginate beads is a widely used encapsulation approach. Encapsulation of nine *Bifidobacteria* strains in calcium alginate microspheres improved their survival ability in milk during refrigerated storage (Hansen, Allan-Wojtas, Jin, & Paulson, 2002). *L. acidophilus* CSCC 2400 was encapsulated in calcium alginate beads to improve the survival ability in simulated gastric conditions (Chandramouli, Kailasapathy, Peiris, & Jones, 2004). *L. casei* NCDC-298 encapsulated in beads produced with different alginate concentrations had a better ability to survive low pH, high bile salt conditions, and during heating (Mandal, Puniya, & Singh, 2006). Pectin, chitosan and carrageenan are other biopolymers studied to encapsulate probiotics (Audet, Paquin, & Lacroix, 1991; Krasaekoopt, Bhandari, & Deeth, 2006; Sandoval-Castilla, Lobato-Calleros, García-Galindo, Alvarez-Ramírez, & Vernon-Carter, 2010).

Pectin is a heteropolysaccharide in the cell wall of higher plants (Thakur, Singh, Handa, & Rao, 1997). Commercial pectin ingredients

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are mainly extracted from citrus peels, sugar beet pulp, and apple pomace (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). Pectin is widely used as a food additive for gelling and stabilizing functions. High-ester pectin (degree of esterification, DE, >50%) from citrus and apple pomace can form gels at pH below 3.5 due to hydrogen bonding and hydrophobic interactions (Walkinshaw & Arnott, 1981), while low-ester pectin (DE < 50%) needs divalent cations such as calcium ions to form gels (Grant, Morris, Rees, Smith, & Thom, 1973). Sugar beet pectin (SBP) has poor gelling ability but has good emulsifying properties (Leroux et al., 2003). The emulsification property of SBP is attributed to the protein segment glycated to polysaccharides and the acetyl group (Williams et al., 2005).

Various methods have been studied to encapsulate probiotic bacteria, such as spray drying, emulsion, and extrusion (Sohail, Turner, Coombes, Bostrom, & Bhandari, 2011). The extrusion method is the oldest and most common one. This is typically done by extruding a suspension with probiotic bacteria in a hydrocolloid solution through a syringe needle into a gelling solution to obtain beads that have a diameter ranging from 2 to 5 mm (Krasaekoopt, Bhandari, & Deeth, 2003). The dimension of beads is a drawback because calcium alginate beads bigger than 1 mm can cause sandy texture (Hansen et al., 2002). The big dimension of beads also can limit the release rate of encapsulated bacteria (Krasaekoopt et al., 2003). Smaller beads with a dimension from around 25 μm to 2 mm can be produced by the emulsion template method (Krasaekoopt et al., 2003). This can be prepared by emulsifying a biopolymer (e.g., carrageenan, gelatin and locust bean gum) suspension with bacteria in oil to prepare water-in-oil (W/O) emulsions. The gelling agents are then mixed to form beads from water droplets. Although the emulsion template method is more advantageous than the extrusion method (Manojlović, Nedović, Kailasapathy, & Zuidam, 2010), these beads can also potentially cause the sandy texture because it can be detected when particulates bigger than about 10 μm are present in dairy products (Walstra, Walstra, Wouters, & Geurts, 2005).

The objective of the present work was to study solid/oil/water (S/O/W) emulsions as a novel approach to encapsulate *L. salivarius* NRRL B-30514 using spray-dried cells as the solid core, soybean oil as the oil phase, and SBP as a polymeric surfactant. This is the first time that SBP was applied to encapsulate probiotics in S/O/W emulsions with particles significantly smaller than millimeter-sized beads. *L. salivarius* is isolated from the digestive tract of mammals and has been used as a potential probiotic (Neville & O'Toole, 2010) with functions such as preventing the colonization of *Salmonella enteritidis* in chickens (Pascual, Hugas, Badiola, Monfort, & Garriga, 1999) and the infection by pathogenic *Listeria monocytogenes* (Corr et al., 2007). The *L. salivarius* NRRL B-30514 in the present study is very effective in reducing *Campylobacter jejuni* in poultry (Stern et al., 2006). In addition to physical properties of emulsions, we studied the viability of encapsulated *L. salivarius* during storage, after thermal treatment, and after simulated gastrointestinal digestion.

2. Materials and methods

2.1. Materials

SBP was a GENU® Explorer YA-400 product from CP Kelco (Atlanta, GA, USA). Soybean oil was purchased from MP Biomedicals, LLC. (Santa Ana, CA, USA). Pectinase from *Asperigillus niger* was purchased from MP Biomedicals, LLC. (Santa Ana, CA, USA). Other chemicals were from either Sigma–Aldrich Corp. (St. Louis, MO, USA) or Thermo Fisher Scientific, Inc. (Pittsburgh, PA, USA).

2.2. Emulsifying property of SBP

2.2.1. Preparation of O/W emulsions

SBP was hydrated in distilled water on a stir plate overnight, and the obtained SBP solution had a pH of 3.9. After centrifugation at 5372 *g* for 20 min using an RC-5B Plus centrifuge (Sorvall, Inc. Norwalk, CT, USA) to remove impurities, the clarified SBP solution was used to prepare O/W emulsions by homogenization at 12,000 rpm for 4 min (IKA® 25 digital ULTRA TURRAX®, IKA® Works, Inc., Wilmington, NC, USA). Sodium azide (0.02% w/v) was added as an antimicrobial agent to prevent microbial spoilage. SBP concentrations in the aqueous phase were studied for 1.0%, 2.0%, 3.0% and 4.0% w/v, and the volume ratios of soybean oil and SBP solution (O:W) were 1:4, 1:6, and 1:8. Emulsions were stored at 4 °C in a refrigerator if not analyzed immediately.

2.2.2. Analysis of droplet dimension

The dimension of emulsion droplets was measured using an LS 13,320 laser diffraction particle size analyzer (Beckman, Brea, CA, USA). Area-volume ($d3,2$) and volume fraction-length ($d4,3$) mean diameters were calculated using the following Eq. (1) and Eq. (2), respectively.

$$d_{3,2} = \frac{\sum_{i=1} n_i d_i^3}{\sum_{i=1} n_i d_i^2} \tag{1}$$

$$d_{4,3} = \frac{\sum_{i=1} n_i d_i^4}{\sum_{i=1} n_i d_i^3} \tag{2}$$

where d_i is the diameter of the ith group of droplets and n_i is the corresponding number of droplets.

2.2.3. Evaluation of surface adsorption of SBP

SBP adsorbed on the surface of emulsion droplets was measured after centrifuging emulsions at 15,000 *g* for 30 min using a MiniSpin Plus centrifuge (Eppendorf, Inc. Hauppauge, NY, USA). The serum phase was collected for determination of SBP concentration using a colorimetric method (Ye, Flanagan, & Singh, 2006). Briefly, 1 mL of the serum was mixed with 1 mL of a 5% w/w phenol solution and 5 mL of sulfuric acid (98%, w/w) and incubated for 10 min at room temperature (21 °C). After mixing by a vortex thoroughly, the mixture was incubated for additional 30 min at room temperature before measuring absorbance at 485 nm with a UV/Vis spectrophotometer (model Evolution 201, Thermo Fisher Scientific, Waltham, MA, USA). Solution series with different SBP concentrations were used to construct a standard curve. Surface load (Γ_s, mg/m^2) was then determined to evaluate the emulsifying property of SBP with the following Eq. (3) (Wu, Lin, & Zhong, 2014).

$$\Gamma_s = \frac{M_s \; d_{3,2}}{6V_{\text{oil}}} \tag{3}$$

where M_s is the mass of SBP adsorbed on oil droplets, and V_{oil} is the volume of soybean oil.

2.2.4. Confocal laser scanning microscopy (CLSM)

The structure of O/W emulsions was studied by CLSM. The microscope (model Leica TCS SP2, Leica Microsystems, Heidelberg GmbH, Germany) was equipped with three excitation sources, an Ar ion laser with 488 nm excitation wavelength and two HeNe lasers with excitation wavelengths of 543 and 633 nm. Before preparing O/W emulsions, SBP and soybean oil were labeled by