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Material distributions and functional structures in probiotic microcapsules

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ABSTRACT

Smart microstructure design of dosage forms such as microcapsules that protect the microorganism, can improve probiotics survival from gastric pH challenges and prolong their shelf life. In this study, synchrotron radiation X-ray microcomputed tomography (SR-μCT) was applied to quantitatively reveal the material distributions and functional structures of bifidobacterium and lactobacillus microcapsules. The shell layer, middle protective layer, and the microorganisms as particles in the center layer were extracted and visualized. All the microorganisms were encapsulated by the shell completely, which prevents them from being destroyed by external environments. However, the non-uniform thickness of the shell and typical defects in the microcapsules were observed. The quantitative analysis and characterization of internal microstructures provide evidence of the need for further improvement in formulations and processing technologies for the structured system to deliver living microorganisms.

1. Introduction

Probiotics, mainly the bifidobacteria and lactobacilli, are well-known for their unique health care functions (Solanki et al., 2013). Because of potential health promotion benefits, probiotics have been widely incorporated into dairy foods and medicines (Floch, 2014). Their beneficial impacts on a wide range of symptoms have been investigated, for example, relief from diarrhea (Guarino et al., 2015), regulation of intestinal transit (Miller and Ouwehand, 2013), prevention of urogenital infections (Hanson et al., 2016), and treatment of allergic rhinitis (Peng et al., 2015). However, the viability of probiotics is susceptible to dissolved oxygen, lactic and acetic acids, causing reduction in their dose levels and action on the gastrointestinal system (Picot and Lacroix, 2004). Probiotic activity depends on the dose levels and the probiotic viability in products and gut environments. Therefore, it is necessary to increase survival of probiotics when exposed to adverse environmental conditions, or improve the viability of probiotics in products so as to ensure an elongated shelf life and improved effects. Various formulations and processing approaches have been reported to enhance the resistance of sensitive probiotics against adverse conditions (Sarkar, 2010). Encapsulation or microencapsulation is one of the most efficient methods under consideration and investigation (Burgain et al., 2011; Martin et al., 2015), for which the distributions of probiotics and excipients usually form varied core-shell structure systems. The preparation technologies of probiotic microencapsulation including: emulsification, extrusion (Shi et al., 2013), spray drying, freeze drying, coating and agglomeration (Solanki et al., 2013) have been developed.

The distributions of probiotic bacteria in microcapsules prepared using three methods and the real-time viability standard curves for probiotics within microcapsules using confocal laser-scanning microscopy (CLSM) have been reported (Moore et al., 2015; Picot and Lacroix, 2004). Scanning electron microscopy (SEM) was applied to observe the surface and cross sections of probiotics microcapsules to estimate the entrapment of probiotics (Chotiko and Sathivel, 2016; Jimenez-Pranteda et al., 2012). However, for CLSM, not only is a fluorescent marker required but also the focal depth-based three-dimensional (3D) imaging limits the thickness of the object that can be measured. In order to collect the internal 3D microstructures images, the whole microcapsule is usually required to be sectioned destructively. In contrast to conventional light and electron microscopies, X-ray microcomputed tomography (μCT) is able to provide non-invasive visualization of internal and microstructural details at micron resolution (Cooper et al., 2003; Stock, 1999). Synchrotron radiation X-ray is an electromagnetic radiation emitted from radially accelerated charged

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particles and has wide X-ray energy range, high intensity, high brilliance, and is polarized. Using synchrotron radiation X-rays as the light source, synchrotron radiation X-ray microcomputed tomography (SR-μCT) enables non-invasively high-speed imaging and high spatial resolution down to the sub-micron or nanometer scale. Recently, SR-μCT has been successfully applied to reveal the internal 3D microstructures of some solid dosage forms, such as: granules (Noguchi et al., 2013), pellets (Yang et al., 2014), microspheres (Guo et al., 2016), particles (Kajihara et al., 2015) and tablets (Li et al., 2012; Yin et al., 2013).

This current research aimed to investigate the material distributions and functional microstructures of probiotic microcapsules. SR-μCT was initially employed to visualize and non-destructively quantify the internal microstructures associated with probiotic microencapsulation. Distinctive microstructures in probiotic microencapsulations revealed by SR-μCT, suggest that it is possible to enhance the design of microcapsules, to better understand the quality of products and to further improve the manufacturing process.

2. Materials and methods

2.1. Materials

Probiotics oligosaccharide pearl (POP) (Weichangshu™) was obtained from Wanhe Pharmaceutical Co., Ltd. (China). The ingredients are listed as erythritol, edible vegetable oil, cotton seed oligosaccharide, maltodextrin, glycerol, bifidobacterium longum, carrageenan, citric acid, phospholipid, lactobacillus acidophilus, pectin, corn starch, lemon extract, monopotassium phosphate, gardenia yellow, lactobacillus gasseri, β-carotene and dipotassium phosphate. According to the product specification, POP was designed to contain three billion bifidobacteria and one billion lactobacillus, including 0.92 billion lactobacillus acidophilus and 0.08 billion lactobacillus gasseri.

POP is a seamless microencapsulation delivery system for probiotics. Fig. 1 shows the appearance, structural diagram and manufacturing method of the probiotics microcapsules in POP. Apart from oligosaccharide, sweetener and flavor mixed grains (Fig. 1a, white), POP contains two types of microcapsules: one for bifidobacterium microcapsules (Fig. 1a, yellow) and the other for lactobacillus microcapsules (Fig. 1a, light yellow). According to the product specification, the microcapsules have three layers: i) an acid resistant external shell layer, ii) a protective substance oil layer and iii) a core substance layer (Fig. 1b). The three-layered structure protects the embedded probiotics from inactivation by acidic gastric juice and allows them to be active in the intestines (Taki et al., 2005). When observed with the naked eye, the probiotic microcapsules are seen to have a regular spherical shape and smooth surfaces. They are manufactured by a “Dropping Method” (Fig. 1c), for which the shell composition and contents composition are injected from a concentric multiplex nozzle. The core substance is totally wrapped by the microcapsule shell. The drops of ejected liquid develop into a spherical form due to surface tension effects after contacting the curing liquid (Kikuchi and Kamaguchi, 1994).

2.2. Weight of microcapsules

In light of color difference, the yellow bifidobacterium microcapsules and the light yellow lactobacillus microcapsules were picked out from the mixture. Total weights of about 76 yellow bifidobacterium microcapsules and about 24 light yellow lactobacillus microcapsules in one dose were then determined respectively using an electronic analytical balance (CPA225D, Sartorius, Germany). From these measurements, carried out in triplicate, the mean weight of single microcapsule was calculated.

2.3. Particle size and roundness

Based upon their color, yellow bifidobacterium microcapsules and light yellow lactobacillus microcapsules were manually separated and photographed using an Eclipse TS-100F inverted phase contrast microscope (Nikon, Japan). Particle size and roundness of the capsules were calculated using a statistical method from the data for 50 capsules using image analysis (Image-Pro Analyzer 3D software, version 7.0, Media Cybernetics, Inc. USA). The roundness value of circular objects is “1.0”, while the roundness value is larger than “1.0” for non-spherical shapes (Wu et al., 2016a). Then the particles size and roundness distributions were calculated.

2.4. Scanning electron microscopy

In order to observe the internal structures of microcapsules at high resolution, a field-emission scanning electron microscope (SEM) (JSM-6360, JEOL/EO, Version 1.1), the microcapsules were manually dissected into two halves using a sharp blade. These cross-sections cut through probiotic microcapsules were mounted on carbon tabs attached to specimen stubs without any metal coating. The examination was carried out in high vacuum mode using a beam acceleration voltage of 10.0 kV. SEM images were recorded at magnifications of 40×, 100× and 500×.

2.5. Sample fixture and SR-μCT image acquisition

SR-μCT measurements were carried out on beamline BM13W1 at the Shanghai Synchrotron Radiation Facility (SSRF, China). X-rays were

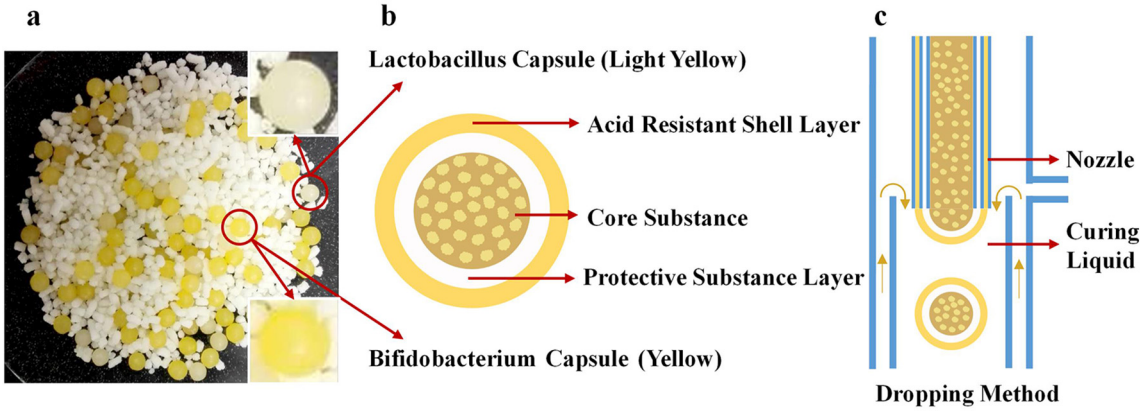

Fig. 1. Structural diagram and manufacturing method of probiotic microcapsules. POP Composition: two kinds of probiotic microcapsules physical mixed with white excipients (a). Diagrammatic representation of the three-layered structure of probiotic microcapsules (b). “Dropping Method” used for the fabrication of three-layered probiotic microcapsules (c).