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Improved stability and controlled release of CLA with spray-dried microcapsules of OSA-modified starch and xanthan gum

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ABSTRACT

The objective of this investigation was to improve the stability of CLA and to allow for its controlled release by encapsulating it with combinations of octenyl-succinic anhydride (OSA) starch and xanthan gum (XG) in three ratios (OSA/XG: 60/1, 80/1, and 100/1, w/w). The wall material was examined using FTIR and TGA. The microcapsules were characterized by laser particle size analysis (LPS) and SEM. Oxidation of the microcapsules was monitored by headspace method. The results revealed that microcapsules created with an OSA/XG ratio of 60/1 provided superior protection to CLA against oxidation. When CLA-microcapsules were subjected to conditions simulating those in the human gastrointestinal system, 12.1%–50.1% of the CLA was released. CLA encapsulation in spray-dried microcapsules of OSA/XG appears to be an effective technique that provides good protection against oxidation and could be useful in the targeted delivery of functional lipids or other bioactive components to the small intestine.

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1. Introduction

Conjugated linoleic acid (CLA) is a collective term used to describe a mixture of geometric and positional isomers of linoleic acid with conjugated double bonds (Zambell et al., 2000). CLA has been shown to possess helpful biological properties, including anti-diabetic, anti-adipogenic and anti-carcinogenic functions (Bhattacharya, Banu, Rahman, Causey, & Fernandes, 2006). Some authors have suggested that the ingestion of CLA may also enhance the immune response (Cook, Miller, Park, & Pariza, 1993) and reduce body fat accumulation (Blankson et al., 2000; Thom, Wadstein, & Gudmundsen, 2001). However, CLA is highly sensitive to auto-oxidation during manufacturing, storage and consumption due to the inherent structure of its conjugated double bonds, leading to a loss of bioactivity (Yang, Leung, Huang, & Chen, 2000; Yurawecz, Hood, Mossoba, Roach, & Ku, 1995; Zhang & Chen, 1997). Hence, CLA must be packaged to maintain its stability and inhibit gastric digestion while retaining its bioactivity.

The microencapsulation technique is a unique way to package materials as micro- and nanoparticles with functional coatings; while it was originally developed for pharmaceuticals, it has been extended to the food industry (Gibbs, Kermasha, Alli, & Mulligan, 1999). This technique, in particular spray drying (Anal and Singh, 2007; Re, 1998; Reineccius, 2004), has seen increasing application as a tool for the incorporation of bioactive ingredients into food, especially since it protects them from moisture, heat, oxygen, and other adverse conditions (Gosh, 2006); (Tontul & Topuz, 2013). Furthermore, with microencapsulation, the controlled release of flavorings and functional ingredients under specific environmental conditions is possible (Anal & Singh, 2007), making this technique ideal for the controlled, stable release of CLA.

Active ingredients are encapsulated to ensure improved stability in final products and during processing, to decrease evaporation and degradation of volatile actives, to mask unpleasant flavors during consumption, and to prevent the reaction of active ingredients with other components in foods such as oxygen or water. Polysaccharides are widely used for food microencapsulation (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011). However, no single encapsulate material effectively provides the necessary functional properties for specific applications such as anti-oxidation or controlled content release nor does it provide adequate protection for delivery, thereby necessitating the use of modified or hybrid species.

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OSA starch is a type of modified polysaccharide that is widely used because it can lead to the formation of less porous materials (Fontes, Calado, Rossi, & da Rocha-Leao, 2013). Through this modification, the hydrophobicity of OSA is introduced while the hydrophilicity of the starch backbone is retained (Liu et al., 2008; Ruan, Chen, Fu, Xu, & He, 2009). Owing to the amphiphilic nature and consequent biosafety of this starch derivative, it is an ideal wall material for spray drying.

Therefore, OSA starch has been extensively used for encapsulating hydrophobic compounds like vitamin E, bergamot oil, orange oil, and rosemary essential oil (Ascheri, Marquez, & Martucci, 2003; Hategekimana, Masamba, Ma, & Zhong, 2015; Penbunditkul et al., 2012; Rodríguez-Rojo, Varona, Núnez, & Cocero, 2012) given its excellent stabilizing properties in aqueous emulsions. OSA starch can also be a potential carrier for colon-targeted delivery of bioactive food components (Wang et al., 2011). Furthermore, incorporation of polysaccharides such as chitosan (Shen, Augustin, Sanguansri, & Cheng, 2010) and corn syrup (Reineccius, 2001) into OSA starch has been shown to improve oxidative stability of the microcapsules and extend the shelf-life of end products. Mixing OSA starch with other polysaccharides increases the encapsulation yield (Yang, Xiao, & Ding, 2009) and improves dissolution behavior (Deng, Chen, Huang, Fu, & Tang, 2014). Likewise, xanthan gum (XG) is popular because it exhibits high viscosities at relatively low concentrations (Katzbauer, 1998), owing to it being a high-molecular weight extracellular heteropolysaccharide. Some studies have concluded that relatively small quantities of XG can be used to delay *in vitro* drug release and provide zero-order release kinetics (Baichwal & Staniforth, 1991; Lu, Woodward, & Borodkin, 1991). In addition, it has been reported that XG tablets can maintain constant drug plasma levels *in vivo* (Talukdar & Plaiziervercammen, 1993). Therefore, in order to improve stability and control release of CLA, we chose OSA starch and XG combination as wall material of spray-dried microcapsules. To our knowledge, no data have been reported on the preparation and characterization of CLA-loaded OSA/XG microcapsules or their application in controlled bioactive component delivery. Hence, we adopted OSA starch as the main wall material for the digestive delivery of CLA and introduced XG to stabilize and improve the performance of the carrier. We described the development of a new CLA-loaded OSA/XG system, with optimal stability against oxidation, thermal treatments, and dissolution in a simulated stomach. In addition, we demonstrated the stability of CLA in simulated stomach condition and the controlled release of CLA from microcapsules by a mammalian amylase in the small intestine. This new system will help to supplement various staple foods with both CLA and other important bioactive compounds.

2. Materials and methods

2.1. Materials

OSA-modified waxy corn starch (DS (%): 1.627 ± 0.039, average molecular weight: 302.9×10^4 g/mol) was purchased from Fonovo Food Ingredients Co., Ltd. (Shanghai, China). XG (food grade, moisture: 9.05 g/100 g, average molecular weight: 366×10^4 g/mol) was supplied by Danisco Textural Ingredients (Shanghai, China). CLA (FFA80, a mixture of *cis*-9, *trans*-11 and *cis*-10, *trans*-12 octadecadienoic acids, linoleic acid <1%) was obtained from Auhai Biotech (Qingdao, China). Chemically pure bile salts, *n*-hexane, and light petroleum were purchased from China National Medicines (Shanghai, China). Pancreatin (P7545, from porcine pancreas; analytical grade), with an activity of $8 \times$ USP specifications (EC 232-468-9), was obtained from Sigma–Aldrich (Shanghai, China).

2.2. Preparation of CLA-loaded microcapsules

OSA/XG (60/1, 80/1, and 100/1, w/w) suspensions were prepared for the wall material and stirred overnight at room temperature (25°C). Emulsions were prepared by adding a preweighed quantity of CLA to the wall material suspension. The mass ratios of the wall material to CLA were 4:1. The solid content of the emulsions was maintained at 10% w/w. A blank without XG was prepared as the control. The mixture was pre-homogenized with a rotational speed of 20,000 rpm for 2 min in a high shear mixer (T18 Basic Ultra Turrax, IKA, Staufen, Germany) and homogenized five times with a high-pressure homogenizer (NS1001 L2K, Niro Soavi, Italy) at a pressure of 500 bar. The CLA emulsion was then fed into a nozzle-type spray dryer (SD-1500, Triowin, Shanghai, China) with a peristaltic pump and sprayed into the drying chamber at an inlet air temperature of (160 ± 5)°C and outlet air temperature of (75 ± 5)°C, in which the feed rate and inlet air volume were controlled.

2.3. Determination of the total CLA content of the microcapsules

Total CLA content in the microcapsules was analyzed by the Soxhlet extraction method (Jimenez, Garcia, & Beristain, 2006), in which 0.5 g samples were extracted with *n*-hexane for 8 h in a Soxhlet extraction apparatus. The extracted product was weighed and recorded as the total mass of CLA in the final powder after evaporating *n*-hexane. Total CLA content was determined with the following equation:

$$\text{Total CLA content}(g/100\,g) = \frac{\text{total CLA}}{\text{micro capsule weight}} \times 100$$

2.4. Efficiency of encapsulation

The ratio between the mass of CLA to be encapsulated and its total mass in the final spray-dried powder was defined as encapsulation efficiency. The surface CLA content was determined according to a previously established method (Jimenez, Garcia, & Beristain, 2004). The spray-dried powder sample (1.5 g) was mixed with 20 mL of light petroleum (b.p. 30–60°C) at 30°C for 2 min without destruction of the microcapsules. Surface CLA in the spray-dried powder was extracted by several gentle shakes of the beaker. After filtration through a glass funnel, the retained sample was washed two times with 10 mL of light petroleum. The solvent was evaporated and the residue was dried in an oven at 60°C until a constant weight was obtained. All encapsulation efficiency measurements were performed in triplicate for three sample preparations. Encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation efficiency}(\%) = \left(1 - \frac{\text{surface CLA content}}{\text{total CLA content}}\right) \times 100$$

Researchers have reported that gravimetric method is an effective and accurate way to determine the encapsulated CLA (Choi, Ryu, Kwak, & Ko, 2010; Jimenez et al., 2004, 2006). Hence, we chose gravimetric analysis to quantify encapsulation efficiency.

2.5. Particle size measurement

A laser particle size analyzer (LPS) (Microtrac S3500, Montgomeryville, PA, USA) equipped with a dry dispersion unit was used to measure the median diameter of the microcapsules produced with OSA and the OSA/XG samples. The specific surface area

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