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Effects of casein micellar structure on the stability of milk protein-based conjugated linoleic acid microcapsules

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ABSTRACT

The effects of casein micellar structure on the stability of milk protein-based conjugated linoleic acid (CLA) microcapsules were investigated. CLA emulsions were prepared with milk protein concentrates (MPC) or a mixture of whey protein isolates (WPI) and sodium caseinate (SC). Larger droplet sizes and wider size distributions were observed for MPC-CLA emulsions, where the varied sizes and heterogeneous protein particles with different rigidity discontinuously adsorbed on the oil droplets. Aggregated oil droplets with porous walls were found on the inner surface of MPC-CLA spray-dried powders. These microcapsules also had lower encapsulation efficiency and unfavorable oxidative stability. For MPC-CLA, 17% of the CLA remained after 45 days at 35 °C, while ~80% was retained for the WPI + SC-CLA microcapsules. The adverse effect of the casein micellar structure suggested that modification of casein micelles will be needed to improve the efficiency and chemical stability of such microcapsules.

1. Introduction

Milk protein concentrates (MPC) contain ~80% caseins and ~ 20% whey proteins (Uluko, Liu, Lv, & Zhang, 2016). In fresh milk, the caseins are supramolecular aggregates and form micellar structures with colloidal calcium phosphate (Dalgleish & Corredig, 2012). The micellar casein structure is retained in MPC, which is manufactured by spray-drying skim milk after partially removing lactose and minerals through filtration (Luo, Ramchandran, & Vasiljevic, 2015). The size of casein micelles ranges from 0.01 to 0.3 μm. The native micellar structure is lost during the processing of commonly used commercial casein products such as sodium caseinate and calcium caseinate (Choi, Horne, & Lucey, 2011).

Casein micelle-based carriers in solution have been proposed to be used to deliver various bioactive food ingredients (Oehlke et al., 2014). Recently, casein colloidal particles, i.e., the native casein micelles (Ye, Zhu, & Singh, 2013) or the covalently crosslinking casein gel particles (Wang, et al., 2018) have also been reported to be novel food-grade soft particles to stabilize O/W emulsions. On the other hand, the micellar structure, either in MPC or in skim milk powder, adversely affected emulsification when compared with whey proteins and non-aggregated caseinate (Euston & Hirst, 1999), which may limit its applicability for emulsions. Besides the unfavorable emulsification, little research examining the characteristics of MPC as wall material has been carried out.

The overall objective of this study was to examine the possibility of using MPC powder as a microencapsulation wall material using spray-drying and to determine the effects of colloidal casein micellar structure on the chemical stability of the microcapsules obtained. Conjugated linoleic acid (CLA), a group of positional and geometric isomers of linoleic acid with a conjugated double-bond system (Churruca et al., 2009), was selected as a representative bioactive component. CLA is usually supplied commercially as the free acid (Christie, Sébédio, & Juanéda, 2001) and is susceptible to oxidation, which limits its application in the food industry. The oxidation stability of encapsulated CLA coated with MPC was studied. A mixture of whey protein isolates (WPI) and sodium caseinate (SC) with a similar whey protein/casein ratio as MPC but without casein micellar structure were compared. The results will contribute to a further understanding of MPC functionality as encapsulating wall material for nutritional or bioactive molecules.

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2. Materials and methods

2.1. Materials

CLA (c9, t11/t10, c12, 80% pure) was purchased from Penglai Marine Co., Ltd. (Yantai Shandong, China). MPC485 (protein 82%, lactose 5%, fat 1.6% according to the specifications) and sodium caseinate (SC, protein 91.4%, lactose 0.2%, fat 1.1% according to the specifications) were purchased from Fonterra Co., Ltd. (Auckland, New Zealand). WPI (protein 93.5%, lactose 0.4%, fat 0.4% according to the specifications) was purchased from Davisco Foods International, Inc. (Eden Prairie, MN, USA). A CLA standard (c9, t11/t10, c12, 99% pure) was purchased from Nu-Chek-Prep, Inc. (Elysian, MN, USA). All the other chemicals used were at least of analytical grade coming from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Emulsification and spray-drying

Wall material solutions were prepared by dissolving 120 g milk protein powders (MPC, WPI + SC (WPI and sodium caseinate with a ratio of 1:4 w/w)) in 880 g deionized water (NW ultra-pure water system, Heal Force Co., Ltd., Shanghai, China) and stirred overnight at room temperature (20–25 °C) for complete hydration. During the sample preparation, 0.02% (w/w) NaN_3 was added into the wall material solutions to prevent microbial growth as recommended (Silva, et al., 2013).

CLA was added at 1:8 (w/w) owing to the protein powders. After stirring for 2 h, the mixtures were high pressure homogenized using a model JHG-Q54-P110 homogenizer (Pulisehng Co., Ltd., Shanghai, China) at 30 MPa with 3 passes to produce fine emulsions. The emulsions were then spray-dried using a Mini Spray Dryer B-290 (Büchi Laboratoriums AG., Flawil, Switzerland) at an inlet temperature of 160 °C and outlet temperature of 80 °C.

2.3. Characterization of wall material solutions and emulsions

2.3.1. Composition of wall material solutions

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with reducing conditions was carried out using a Bio-Rad protein II xi cell (Bio-Rad Laboratories, Inc., Hercules, CA, USA). After preparing the gel (12% acrylamide), 15 μL of wall material solution and the marker (0–250 kD) were injected into sample channels and separated at 350 V. The gel was photographed after staining with Coomassie Brilliant Blue R-250 (0.1% w/w) for 4 h and destained using the aqueous solution of acetic acid and methanol.

2.3.2. Size distribution of wall material solutions and milk protein-based CLA emulsions

Before the size distribution measurement, all the wall material solutions were treated at 30 MPa for 3 times and then diluted to 0.5 mg/mL using deionized water. The hydration radius and size distributions of various milk protein solutions were characterized using dynamic light scattering (DLS) using a NanoBrook Omni (Brookhaven Instruments Co., Austin, TX, USA) at 25 °C with a scattering angle of 173°. The detection range is within 1 μm.

The size distribution of milk protein based-CLA emulsions were measured using a Microtrac S3500 laser particle size analyzer (Microtrac Inc., Largo, FL, USA) with the detection range between 1 and 100 μm. These emulsions were also observed using an Eclipse Ti-U Inverted Microscope (Nikon Co., Ltd., Shanghai, China).

2.3.3. Transmission electron microscopy (TEM) of wall material solutions and MPC-based CLA emulsion

TEM images of MPC and WPI + SC solutions and MPC-CLA emulsions were observed using a HITACHI 765 TEM (Hitachi, Ltd., Tokyo, Japan) using the method of McMahon, Du, McManus and Larsen (2009). The wall material solutions without CLA or MPC-CLA emulsions after homogenization were mixed with warm melted (50 °C) low-temperature gelling agarose (3%) (Sigma-Aldrich, Shanghai, China) at a ratio of 1:1 and then cooled for 15 min in an ice bath. The samples were cut into small strips (~1 × 1 × 1 mm) and preserved in 0.1 M sodium phosphate buffer solution (pH 6.8) containing 2.5% (w/w) glutaraldehyde and stored overnight at 4 °C. Samples were post-fixed in 1% (w/w) osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA, USA) in phosphate buffer for 3 h at 4 °C. Dehydration was done by transferring samples into increasing concentration of ethanol (50, 70, 90 and 100%) for 15 min in each solution. After dehydration, the samples were transferred into propylene oxide solutions with various proportions of 33, 50, 67, and 100% in sequential steps. The infiltrated samples were incubated at 60 °C for 72 h. Ultrathin sections were cut using an Ultracut E ultramicrotome (Leica Inc., Gilroy, CA, USA) and were post-stained with uranyl acetate and lead citrate. The samples were observed at an accelerating voltage of 80 kV with magnification from 5000 to 50000x.

2.4. Characterization of microcapsules

2.4.1. CLA retention, microencapsulation efficiency and a_w of microcapsules

The total extractable CLA of microcapsules was determined using the method of Kim, Chen and Pearce (2009). One g of the microcapsule was added to 5 mL of warm water (50 °C) and was Vortexed (Vortex-Genie2, Scientific Industries, Inc., New York, USA) for 15 min. The resulting solution was extracted with 90 mL of hexane/isopropanol (3:1 v/v) twice and centrifuged at 2000*g* for 15 min. The clear organic phase was collected and evaporated (RV-10 digital rotary evaporator, IKA Works Guangzhou Co., Ltd., Guangzhou, China) at 45 °C until the extracted fat residue achieved constant weight.

Extractable surface CLA was determined by gently shaking 5 g of microcapsules in 80 mL petroleum ether for 10 min. The organic solvent was filtered through filter paper (double ring type with pore < 5 μm, GE healthcare Co., Ltd., Beijing, China) and evaporated until the extracted fat residue achieved constant weight. Extractable surface CLA was determined gravimetrically (Jimenez, Garcia, & Beristain, 2004). Total CLA retention and microencapsulation efficiency (ME) were calculated as:

$$\text{Retention}\,(\%) = (\text{Total extractable CLA} / \text{Total CLA in the initial formulation}) \times 100$$

$$\text{ME}\,(\%) = [(\text{Total extractable CLA} - \text{Extractable surface CLA}) / \text{Total extractable CLA}] \times 100$$

a_w of the microcapsules was measured using a LabSwift-a_w analyzer (Novasina AG., Lachen, Switzerland) at a temperature of 25 °C. Duplicate experiments were done for all of these measurements.

2.4.2. Confocal laser scanning microscopy (CLSM)

The encapsulation and distribution of CLA in the milk protein-based microcapsules were analyzed using a Zeiss LSM 710 confocal microscope (Carl Zeiss AG., Oberkochen, Germany). Both CLA and protein were stained using the method of Kosasih et al. (2016). Nile red and rhodamine B were used at a concentration of 0.1 g/L to label fat and protein, respectively, at a ratio of 1:100 (dye:powder, w/w). The nile red and rhodamine B were excited at wavelengths of 488 and 561 nm, respectively. The resultant images were photographed at 400× magnification.

2.4.3. Scanning electron microscopy (SEM)

The microcapsules were sprinkled onto two-sided adhesive tape, then scraped with a razor blade to see the inner structure and sputter-coated with gold using a MSP-1S sputter coater (Shinkku, Ltd., Tokyo,