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Digestibility and structural parameters of spray-dried casein clusters under simulated gastric conditions

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ABSTRACT

The digestibility of casein clusters prepared from sodium caseinate solution (plain or pH-adjusted (pH = 6.0)) was studied. The prepared solutions were spray-dried at different inlet air temperatures (150 °C and 180 °C), and the properties (i.e. encapsulation efficiency, surface hydrophobicity, and digestibility) of the resultant powders were investigated. The specimens obtained from the pH-adjusted solution had higher encapsulation efficiencies than the specimens obtained from the plain solution. A higher spray-drying temperature resulted in lower encapsulation efficiencies and higher surface hydrophobicities. Simulated gastric digestion tests were carried out to study the digestibility of the obtained casein clusters, which was analyzed in terms of reaction kinetics and structural changes during digestion. The effects of drying temperature and pH on the amount of casein digested were not significant; that is, approximately 30% of casein was digested in 120 min for all specimens. Small-angle and ultra-small-angle X-ray scattering measurements were used to analyze the structure of the obtained clusters and their changes during digestion. The results suggested that all the obtained casein clusters, with an average size of approximately 428 nm, had a rough, fractal-structured surface with many dense primary clusters. These structures changed during digestion; specifically, the cluster size increased both in the overall diameter and on the primary structure scale. The fractal characteristics changed from surface to mass fractals, and simultaneously, the cluster density decreased. The drying temperature affected the cluster size during digestion, and the trends were different in the specimens obtained from the plain and pH-adjusted solutions. These results could be useful in the design of protein-based encapsulation systems with desirable digestibility and bioavailability.

1. Introduction

Encapsulation is an effective technique for stabilizing sensitive nutraceuticals and/or pharmaceuticals, and for endowing a final product with functionalities, such as improved bioavailability and controlled release. The digestibility of encapsulants (wall materials) in the gastrointestinal tract is a key factor that affects the functionality of microcapsules. Numerous studies have reported the release characteristics and digestibility of microcapsules under simulated gastric digestion conditions in vitro. The attributes of the wall materials, such as composition, shape, size, surface charge, and hydrophobicity, have a crucial effect on digestibility (Flores, Singh, Kerr, Phillips, & Kong, 2015; Liu et al., 2015; McClements & Li, 2010; Shapira, Davidson, Avni, Assaraf, & Livney, 2012; Singh & Ye, 2013). Augustin et al. (2014) showed that the in vitro digestibility of microencapsulated oil powders was dependent on the protein composition and processing conditions. Ao and Li (2013) reported the difference in digestive resistance between the positively and negatively charged fractions of alcalase-treated casein hydrolysate. The negatively charged fractions were the most stable, and the antioxidant activity of the encapsulated substances was retained during digestion. Burgain, Gaiani, Cailliez-Grimal, Jeandel, and Scher (2013) and Zhang and Vardhanabhuti (2014) emphasized the effect of structural changes under gastric conditions on digestive resistance.

Casein and caseinate have attracted attention in recent decades for use as encapsulants in food and drug delivery systems owing to their functional properties. Caseins exhibit excellent interfacial properties, thermal stability, and biodegradability, and they are classed as generally recognized as safe (GRAS) (Elzoghby, Abo El-Fotoh, & Elgindy, 2011; Livney, 2010). They offer high nutritional value since they contain approximately 94% proteins and 6% minerals, i.e. calcium, phosphate, magnesium, and citrate, which are known as colloidal calcium phosphate (Fox & Brodkorp, 2008; Fox & McSweeney, 2003). It is well known that casein has a unique structure with a wide size range of approximately 50–500 nm in diameter (Fox & Brodkorp, 2008). Numerous studies have been conducted to investigate the structure of casein and are presented in various models, for example, the sub-units model and the model of internal structure (McMahon & McManus, 1998). According to these models, casein aggregates are formed by the hydrophobic

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interaction of many roughly spherical sub-units that are about 12–15 nm in diameter (Holt, Carver, Ecroyd, & Thorn, 2013; Walstra, 1999). Understanding the structural characteristics of large casein aggregates requires a wide range of analysis covering sizes from nano- to micro-scale. The combination of small-angle X-ray scattering (SAXS) and ultra-small-angle X-ray scattering (USAXS) can be used to evaluate the structures of macromolecules since it provides information about clusters ranging from one to several thousand nanometers (Zhang & Ilavsky, 2010). Pignon et al. (2004) analyzed casein micelles with SAXS and USAXS, and the results showed that casein micelles are bimodally distributed with two size length scales of around 100 nm for the globular micelles and 5.6 nm for the sub-micelles. Marchin, Putaux, Pignon, and Léonil (2007) also used these techniques to evaluate the effects of pH, temperature, and addition of ethylenediaminetetraacetic acid on the structure of casein micelles.

Numerous studies have reported that caseins and their products could be used as natural vehicles for drugs, such as ibuprofen, theophylline, phenytoin, and flutamide (Elzoghby, Helmy, Samy, & Elgindy, 2013; Latha, Rathinam, Mohanan, & Jayakrishnan, 1995; Millar & Corrigan, 1993; Watanabe, Hanawa, Sugihara, & Yamamoto, 1994). Moreover, they were suggested to improve the solubility and bioavailability of hydrophobic bioactives, such as curcumin and vitamin D_2, as well as used to protect probiotic bacteria against gastric digestion (Heidebach, Forst, & Kulozik, 2009; Menéndez-Aguirre et al., 2011; Nag, Han, & Singh, 2011; Pan, Zhong, & Baek, 2013). The authors reported that the encapsulation properties of casein are largely dependent on the nano-microstructures (Jarunglumlert & Nakagawa, 2013; Nakagawa, Jarunglumlert, & Adachi, 2014). Casein was induced to form clusters and incorporated with hydrophobic substances by adjusting the pH of the solution. The degree of aggregation and cluster deformation was closely linked with the encapsulation efficiency and stability of the final products.

This work aims to investigate the nano- and microstructures of the pH induced casein clusters spray-dried at different drying temperatures. This is to clarify how the drying temperature affects the cluster structures that would relate to their encapsulation efficiency and digestibility. A simulated digestion test was carried out to analyze digestion kinetics and structural changes during digestion by using small angle X-ray scattering technique.

2. Materials and methods

2.1. Materials

Sodium caseinate (SC) from bovine milk, β-carotene (type I, synthetic, ≥93%), and pepsin from porcine gastric mucosa (≥2500 units/mg protein) were purchased from Sigma-Aldrich (St Louis, MO, USA). Acetic acid, hexane, hydrochloric acid, acetone, sodium acetate, sodium tetrahydroborate, dimethyl sulfoxide (DMSO), 1-anilinonaphthalene-8-sulfonic acid (ANS), and trichloroacetic acid (TCA) were purchased from Wako Pure Chemical Industries, Osaka, Japan. All chemicals used were of analytical grade.

2.2. Particle preparation

The casein solution was prepared by adding 25 g of SC to 400 mL of distilled water. Rehydration was accomplished by stirring overnight at ambient temperature. β-Carotene in acetone was added to the casein solution at a 1:400 weight ratio (β-carotene:SC) with stirring in a thermostatic bath at 37 °C for 2 h. To prepare the aggregated casein clusters (A60), this mixture was then adjusted to pH 6.0 with 1% (v/v) acetic acid solution using a pH meter (SK-620PH, Sato Keiryoki Mfg Co., Tokyo, Japan). The final volume of the mixture was adjusted to 500 mL with distilled water. The mixture prepared without pH adjustment (A70) was used as control. The prepared solution was dried using a rotary atomization-type spray-dryer (LB-8, Ohkawara Kakohki Co., Yokohama, Japan). The operational conditions (i.e. inlet air temperature, outlet air temperature, feed flow rate, and atomizer rotational speed) were adjusted according to the inlet air temperature and are described in Table 1. The obtained powders were stored in a desiccator for further analysis.

2.3. Encapsulation efficiency measurement

Dried casein clusters (0.1 g) were dispersed in 10 mL of hexane and stirred for 5 min and 24 h to estimate the free and total amounts of β-carotene loaded in the specimens, respectively. The mixture was then filtered through a 0.45-μm membrane filter, and the amount of β-carotene in the filtrate was determined by measuring the absorbance at 450 nm with a UV–visible spectrophotometer (U-5100, Hitachi High-Technologies Corporation, Tokyo, Japan). The encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation efficiency } [\%] = \left[\frac{\text{Amount of total loaded β-carotene} - \text{Amount of free β-carotene}}{\text{Amount of β-carotene added in the original solution}}\right] \times 100. \quad (1)$$

2.4. Surface hydrophobicity measurement

An ANS hydrophobic fluorescence probe was used to determine the surface hydrophobicity (SH) of the dried casein clusters, following the procedure described by Hayakawa and Nakai (1985) with slight modifications. A series of specimen solutions with concentrations ranging from 0.0005% to 0.01% (w/v) were prepared by diluting the prepared sample (before spray-drying) or dissolving dried casein clusters in 0.05 mol/L phosphate buffer at pH 7.0. ANS solution (25 μL of 8 mmol/L ANS in 0.05 mol/L phosphate buffer) was added to 5 mL of the specimen solution. The fluorescence intensity (FI) of the mixture was measured using a spectrofluorophotometer (RF-1500, Shimadzu Corporation, Kyoto, Japan) with excitation and emission wavelengths of 390 nm and 470 nm, respectively. The relative fluorescence intensity (RFI) of each specimen concentration was determined by subtracting the FI of the control specimen solution (25 μL of 0.05 mol/L phosphate buffer was added instead of ANS). The SH index was obtained from the initial slope of the RFI versus the specimen concentration (% (w/v)).

2.5. Simulated gastric digestion

The simulated digestion method described by Mandalari et al. (2009) was used with modifications to estimate the digestibility of the dried casein clusters. Dried casein clusters (0.1 g) were dissolved in 10 mL of the simulated gastric fluid (SGF; pH 2.0 HCl solution). The mixture was stirred for 2 h to ensure complete dissolution. Pepsin in SGF (5 mg/mL) and protein solution were incubated at 37 °C in a thermostatic bath for 10 min prior to the reaction starting. Pepsin solution (1 mL) was added to the mixture to give a pepsin:dried casein cluster ratio of 1:20 (w:w). To study the digestion behavior, 0.6-mL aliquots of the digested solution were collected at predetermined intervals, and the reaction was stopped by adding a mixture of 0.05 mol/L phosphate buffer and 0.1 mol/L NaOH to adjust the pH to 6.0. This digested solution was used immediately for further analyses.

2.6. Estimation of digestion kinetics

The amounts of amino acids produced by digestion were determined using the ninhydrin reaction according to the method described by Takahashi (1978) with modifications. Ninhydrin solution was prepared by dissolving 29.5 mg of $NaBH_4$ in 300 mL of 0.15 mol/L ninhydrin in DMSO. The digested casein solution (0.5 mL) was mixed with 0.5 mL of 5% (w/v) TCA to precipitate large peptides and undigested proteins;