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Evaluation of diacetyl encapsulated alginate–whey protein microspheres release kinetics and mechanism at simulated mouth conditions

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ABSTRACT

In this paper, diacetyl encapsulated alginate–whey protein concentrate (AL–WPC) microcapsule were prepared based on the emulsification/internal gelation method; and diacetyl release was investigated at the simulated mouth condition in different ratios of artificial saliva (0, 1:4 and 1:8) and three various oral shear rates (0, 50 and 100 s-1). The gotten diacetyl release data were fitted to first-order, Korsmeyer–Peppas, Kopcha, and Makoid–Banakar models to evaluate release mechanisms and kinetics. We showed that the shear rate of release media had a significant ($p < 0.05$) effect on the release of diacetyl from AL–WPC microspheres, but the saliva ratio had no any significant effect. The diacetyl release data fit well to the all kinetic expression with R^2 values greater than 0.93. It was found that the release kinetics of diacetyl from AL–WPC microspheres followed a classical Fickian diffusion.

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1. Introduction

Flavor release from food during consumption in the mouth is important in flavor perception and influenced by the food matrix (Madene, Jacquot, Scher, & Desobry, 2006; Roberts & Taylor, 1999). Since food matrix changes biochemically and physically during eating, food flavor microencapsulation results in controlled release at specific situations. On the other hand, the stability and availability of flavors are affected by food processing and storage (Madene et al., 2006). Therefore, it is important to encapsulate food flavor to raise the stability and limit the degradation.

Microencapsulation as a delivery system is a common technology that enables isolating bioactive components. Vesicular delivery system consists of a core with porous or non porous semi permeable shell. During the past decades, considerable attention has been devoted to encapsulation because of the promising potential for almost every area in the human development (Wilcox, Berg, Bernat, Kellerman, & Cochran, 1995). Microencapsulation potentially can be applied in a broad range of fields such as food and beverage technology especially flavor release (Augustin, Sanguansri, Margetts, & Young, 2001; Chen & Subirade, 2006; Gibbs, Kermasha, Alli, & Mulligan, 1999; Heinzen, 2002; Odonnell & McGinity, 1997), drug delivery (Langer, 1998), biotechnology (Chang & Prakash, 2001; Keen, Slater, & Routh, 2012), material science (Zydowicz, Nzimba-Ganyanad, & Zydowicz, 2002), agricultural, and the like.

Flavor release in the mouth depends on microcapsule properties (Naknean & Meenune, 2010), nature and concentration of flavor (Bakker et al., 1996), physical form of food (Taylor, 1996) and mouth characterization (van Ruth & Roozen, 2000), including temperature, pH, shear rate, saliva composition and flow rate. Since, all these parameters are responsible for food flavor perception; simulation of mouth situation should be consisted of all these factors. The composition of human saliva is varied, and usually contains various compounds, including water, protein and peptide, enzyme, mineral salt, acid and mucin (Neyraud, Palicki, Schwartz, Nicklaus, & Feron, 2012). These components can affect the flavor releases depending on the studied compounds (Friel & Taylor, 2001).

Diacetyl (2,3-Butanedione) are well known flavor components of fermented foods, such as alcoholic drinks and dairy products, produced by micro-organisms during fermentation processes (Rodrigues & Barros, 1997).

Different material and various techniques are developed for fabricated diverse microcapsules, but some of them use potentially toxic chemical materials that limit food applications (O'Donnell & McGinity, 1997). Rosenberg and Lee (2004) and Chen and Subirade (2006) developed a safe microcapsule based on alginate–whey protein as a bioactive carrier. Chen and Subirade's (2006) research was limited to the oral administration of riboflavin as a nutrient at simulated gastric and intestinal conditions. This microcapsule was fabricated based on emulsification/internal gelatin method that provides the safe technology for compound encapsulation (Chen & Subirade, 2006). It has been found that whey protein is a suitable compound to be used as encapsulant (Heelan & Corrigan, 1998; Moreau & Rosenberg, 1993; Moreau & Rosenberg, 1999; Rosenberg & Young, 1993; Satpathy & Rosenberg, 2003; Sheu & Rosenberg, 1998). Alginate–whey protein microcapsules as a biocompatible vehicle have a good potential for food flavor delivery.

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The main aim of this work was to study the release of 2,3-butanedione from alginate–whey protein concentrate (AL–WPC) microcapsules at different ratios of saliva to microcapsule (0, 1:4 and 1:8), three oral shear rates (0, 50 and 100 s^{-1}) simulating mouth conditions. The technique used for diacetyl encapsulated AL–WPC microcapsule fabrication includes the emulsification/internal gelation method presented by Poncelet (2001) for alginate microsphere. The release profile of encapsulated diacetyl was measured by spectrophotometry technique and kinetic models were fitted to the experimental release data. This research may be used to develop novel whey protein microspheres for incorporating flavor compounds into foods. Such microspheres should be degraded by mouth condition, allowing flavor release.

2. Experimental

2.1. Materials

Commercial whey protein concentrate 80% was supplied by Davisco Foods International Inc. Sodium alginate, Deionized water of resistivity 18 2 MΩ · cm (Milli-Q, Millipore), O-phenylenediamine (OPDA) (99.5%), Sodium chloride (99.5) and Calcium chloride (>93%) were purchased from Sigma Aldrich Chemie GmbH (Germany). 2,3-Butanedione (Fluka, analytical standard), Sunflower oil (from the supermarket), Tween 80 (Fluka), Sodium bicarbonate (99%, Fulka) and Hydrochloric acid (Fluka) were used as received. Sodium hydroxide (analytical grade), di-Potassium hydrogen phosphate (99.99%), potato starch, Potassium dihydrogen phosphate (99.99%) and alpha amylase (from Bacillus subtilis, 130U/g) were purchased from Merck.

2.2. Methods

2.2.1. Preparation of AL–WPC microcapsule

The AL–WPC microspheres were prepared using Chen and Subirade (2006) and Hansen, Allan-Wojtas, Jin, and Paulson (2002) methods. For alginate (AL) solution preparation, 2 g of alginate was rehydrated in 100 ml deionized water (2% w/v) using a magnetic stirrer (IKA Werke GmbH&Co. KG, model RH basis), and then, it was held in refrigerator temperature overnight to ensure complete alginate hydration. Whey protein concentrate (WPC) solution was prepared by dispersion of 8 g WPC in 100 ml deionized water (8% w/v) with stirring for 1 h using the magnetic stirrer. The resulted WPC solution was left at room temperature for 2 h, then it was adjusted to pH = 8 and heated for 30 min at 80 °C. WPC solution was cooled and kept at room temperature for 2 h. Then, WPC and AL solutions were mixed (1:2), stirred for 30 min at room temperature and kept at refrigerator temperature overnight.

20 ml of the AL–WPC solution was mixed with 10 mg of diacetyl, then gradually added to 100 ml of 0.05% (v/v) tween 80 in sunflower oil, and blend was mixed for 20 min at 900 rpm using a magnetic stirrer. Afterwards, 32 ml of emulsion containing Ca (including 19.2 g sunflower oil, 0.16 g tween 80 and 12.8 g calcium chloride solution 0.1 M) gently was added to this solution and mixed for 20 min at 100 rpm. later, the emulsion was transferred into an empty cone separation funnel and 40 ml of calcium chloride solution 0.05 M was added to it. After 40 min, the white sediment was separated from the creamed oil. Then, these fabricated microcapsules were washed with the solution of calcium chloride 0.05 M and tween 1%. Later, AL–WPC microspheres solutions were filtered using a Millipore glass vacuum filtration system with 0.65 μm cellulose nitrate membranes filter (ALBET®). The separated microcapsule was weighted and dispersed in deionized water in a ratio of 1 to 9 (w/v).

2.2.2. Characterization of AL–WPC microcapsules

A Leo 1450VP SEM microscope was used to observe diacetyl encapsulated microcapsule. For this purpose, a drop of AL–WPC microcapsule aqueous phase was air-dried on stainless steel SEM sample holder overnight, then dried microsphere was sputter coated using platinum (40 mA, 60 s, 1 × 10^{-3} mbar, in an argon environment), and t observed using SEM at 5.0 kV. To observe the shape and structure of AL–WPC microspheres, an Olympus BX41 transmitted light microscope coupled to Olympus DP12 color Digital Camera and capture software (Microsuite^{TM} five) were used. Also using image J software (version 1.46r), optical images were analyzed to estimate the average size of AL–WPC microcapsules. Sizing and size distribution analyses of AL–WPC microspheres were also conducted by dynamic light scattering (DLS) using a Shimadzu-Sald 2101 instrument. About 1 ml of microcapsule aqueous phase was dispersed in deionized water, and then the clear solution was placed in a sample dispersion cell. The mean hydrodynamic diameter of AL–WPC microsphere was estimated from three measurements. Zeta potential of AL–WPC microcapsules were examined by CAD-Zetacompact instrument using the clear solution of microsphere. Dilution of AL–WPC microcapsules dispersion was conducted to avoid multiple scattering effects, and then the clear microcapsule solution was injected into the instrument chamber. All zeta potential measurements were performed in three separated injections.

2.2.3. Artificial saliva preparation and characterization

The artificial saliva was prepared using Roth and Calmes (1981) instruction. The produced saliva consisted of 20 mM sodium bicarbonate ($NaHCO_3$), 15 mM sodium chloride, 2.75 mM di-potassium hydrogen phosphate (K_2HPO_4), 12.2 mM potassium dihydrogen phosphate (KH_2PO_4) and 200 U/ml of α-amylase. The final pH was adjusted to 7.0. For investigation of artificial saliva properties alpha amylase activity was determined using Moor method (van Ruth & Roozen, 2000). For this purpose, at first, potato starch was dispersed in distilled water (1% w/v) and boiled. Then, the gelatinized starch solution was diluted with distilled water in a ratio of 1:to 9, and warmed to 37 °C. Later, 1 ml of artificial saliva was added to 100 ml diluted starch solution, and 1 ml sample was withdrawn at 10 s intervals and mixed with 0.5 ml iodine solution 0.5% (v/v). This work was repeated until no blue tinge was produced (the achromic point). Alpha amylase activity (D) was measured by the time to rich the achromic point (n, in min) and saliva initial volume (v, in ml) using Eq. (1).

$$D = \frac{10}{v \times \frac{5}{n}} \tag{1}$$

2.2.4. In vitro diacetyl release studies

Diacetyl release was determined by placing AL–WPC microcapsules in a simulated mouth condition including three shear rates (0, 50 and 100 s^{-1}), three saliva to microcapsule ratios (0, 1:4 and 1:8), neutral pH and 37 °C. Shear force and temperature were applied by a magnetic stirrer with hot plate, and pH adjustment was accomplished using sodium hydroxide (0.1 N) and hydrochloric acid (0.1 N). Shear rate was calculated from revolutions per minute using Steffe equation (Steffe, 1992):

$$\dot{y} = 2\mathrm{N}\left[\frac{\left(\frac{\mathrm{D}}{\mathrm{d}}\right)^{\frac{(2-n)}{n}}}{\left(\frac{\mathrm{D}}{\mathrm{d}}\right)^{n} - 1}\right]\left(\frac{\mathrm{d}}{\mathrm{h}}\right)^{\frac{n}{2}}. \tag{2}$$

Where N is agitation speed (s^{-1}), D is a diameter of the beaker (mm), d is the diameter of stirrer bar (mm), h is stirrer bar thickness (mm) and n is AL–WPC solution's flow index behavior. 30 ml of AL–WPC microsphere solution was incubated at various shear force and saliva ratio. 1 ml of sample was collected via syringe at specific time intervals and filtered through 0.22 μm Biofil syringe filter. Then, the sample was mixed with 0.05 ml of 1% OPDA in 4 M hydrochloric acid and blend left for 20 min in a dark place .2 ml of 4 M hydrochloric acid was added to the solution, and absorbance of the mixture was measured

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