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Shear-induced morphological changes in associative and segregative phase-separated biopolymer systems

Chutima Thongkaew [a], Monika Gibis [a], Jörg Hinrichs [b], Jochen Weiss [a, *]

[a] Department of Food Physics and Meat Science, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 25, 70599 Stuttgart, Germany
[b] Department of Dairy Science and Technology, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 21, 70599 Stuttgart, Germany

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ABSTRACT

Shear-induced morphological changes of phase-separated whey protein isolate (WPI; 5%, w/v) droplets dispersed in high methoxyl pectin solutions (2%, w/v) subjected to pH changes (6.1 → 5.2 → 3.2) and addition of salt (200 mM NaCl) were investigated. Systems were sheared (0.1–50 s^{-1}) on an optical rheometer and sizes and morphologies determined by microscopy and light scattering techniques. Apparent viscosities were calculated from shear stress versus strain rate measurements. The microstructures of phase separated WPI – pectin dispersions at pH 6.1 was altered at low to intermediate shear rates (0.1–10 s^{-1}) from initially containing spherical particles to elongated ones. Shearing also led to increases in particle size due to shear-induced coalescence. At high shear rates (>10 s^{-1}) particle size decreased due to droplets being disrupted. Morphologies of dispersions changed substantially when pH was decreased to 5.2 yielding core–shell like WPI droplets surrounded by aggregated pectin – whey protein isolate "shells". Those structures were substantially less sensitive to shear-induced disruption, and high deformational forces were required to break them up. At pH 3.2 small pectin – WPI aggregates were formed that were resistant to superimposed shear stresses. Addition of salt increased shear-induced droplet coalescence at pH 6.1 but had little influence on morphology of structures at pH 5.2 and 3.2. Results were explained in terms of modulations of molecular interactions between the two biopolymers yielding either phase-separated WPI droplets suspended in pectin with ultralow interfacial tensions or electrostatically-networked biopolymer aggregates with high interfacial tensions.

1. Introduction

Binary food biopolymer systems have shown to exhibit a variety of different behavior, including thermodynamic incompatibility, co-solubility, and coacervation depending on pH, temperature, pressure and ionic strength (Doublier, Garnier, Renard, & Sanchez, 2000; de Kruif, Weinbreck, & de Vries, 2004; Salminen & Weiss, 2013; Schmitt & Turgeon, 2011; Seyrek, Dubin, Tribet, & Gamble, 2003). The first phenomena leads to the formation of two separate aqueous phases each rich in one of the respective polymers due to segregative polymer interactions. The latter phenomena leads to the formation biopolymer aggregates due to associative polymer interactions (Chun et al., 2014). Altering environmental conditions induces transitions from one regime to the other. This can be utilized to generate a wide variety of structures that are of interest to food manufacturers. Potential applications that have been reported for binary biopolymer mixtures in the segregative or associative domain include carrier systems for functional compounds, texturing agents, cryoprotectants, fat replacers, and powder or emulsion stabilizers (Chun, Choi, Min, & Weiss, 2013; Karadag et al., 2013; Krzeminski, Prell, Busch-Stockfisch, Weiss, & Hinrichs, 2014; Krzeminski, Prell, Weiss, & Hinrichs, 2014; Salminen & Weiss, 2014; Thongkaew, Gibis, Hinrichs, & Weiss, 2014; Zeeb, Salminen, Fischer, & Weiss, 2014).

Recently we reported that a wide variety of structures can be created by sequentially altering environmental conditions such as pH and ionic strength. There, a binary biopolymer system composed of whey protein isolate (WPI) and pectin that was in its segregative (phase-separated) state was first mildly homogenized. As a result, a water-in-water dispersion composed of WPI droplets

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* Corresponding author. Tel.: +49 711 459 24415; fax: +49 711 459 24446.
E-mail address: j.weiss@uni-hohenheim.de (J. Weiss).

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suspended in a pectin rich aqueous phase was formed. This was then followed by a change in pH and ionic strength, causing the formation of core–shell like structures composed of an inner core rich in WPI and surrounded by a biopolymer aggregate shell composed of WPI and pectin at intermediate pH values. At a low pH, small and dense aggregates were formed that tended to precipitate. The morphologies of these structures could be further modulated by addition of salt (Thongkaew, Hinrichs, Gibis, & Weiss, 2015). Results were attributed to a change in the charge of the biopolymers and/or the extent of their electrical double layer thereby increasing or decreasing attractive or repulsive molecular interactions.

At the time of submission of the article, reviewers raised an interesting question, namely: how stable are the generated structures? More specifically, how do the various structures behave when subjected to superimposed mechanical stresses? In reviewing the literature, one can find a number of studies that have investigated the behavior of phase-separated biopolymer mixtures subjected to mechanical stresses. For example, Van Puyvelde and co-authors wanted to understand the role of block copolymers in modulating the behavior of polymer blends, and therefore investigated changes in rheology and morphology of incompatible polymer blends (Van Puyvelde, Antonov, & Moldenaers, 2002; Van Puyvelde, Velankar, Mewis, Moldenaers, & Leuven, 2002). They obtained important insights into the processes that were involved in droplet break-up. The behavior of phase-separated polymer systems subjected to superimposed fluid flow was also investigated by Al-Mull and Gupta, and authors reported that droplets could be deformed, and disrupted, but were also prone to coalescence (Al-Mulla & Gupta, 2000). Wolf and co-authors also conducted shear experiments on mixtures of gellan and κ-carageenan, and reported formation of anisotropic structures at low shear rates due to ultralow surface tensions (Wolf, Scirocco, Frith, & Norton, 2000). Nevertheless, there are few studies that have investigated the behavior of mixed biopolymer system that have been subjected to a transition from the segregative to the associative interaction regime via pH and ionic strength changes.

The present study was therefore undertaken to answer some of the questions that arose from our previous investigations. As the basis of this study, we postulate that low shear rates may quickly lead to deformation and disruption of phase separated WPI droplets in the segregative interaction regime, but that higher shear rates may be needed to deform structures in the associative regime since strong electrostatic attraction forces are at work. To this purpose, we manufactured a WPI-pectin dispersion, and subjected it to pH and ionic strength changes by adding HCl or NaCl, and then superimposed shear at shear rates ranging from 0.1 to 50 s^{-1} on a rotational rheometer. We then used microscopy and light scattering techniques to record changes in size and morphology of the systems.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) with 97.8% protein, 2.0% ash, 0.2% fat, and 4.6% moisture content was obtained from Davisco (BiPRO, Davisco Foods International, Inc., Le Sueur, MN, USA). Apple pectin was kindly donated by Herbstreith and Fox (Neuenbürg, Germany). According to manufacturer, the degree of esterification (DE) of the apple pectin was 71%. Analytical grade sodium chloride, hydrochloric acid and sodium hydroxide were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Distilled, deionized water was used throughout the study.

2.2. WPI and pectin solution preparation

Solutions were prepared following a previously described method (Thongkaew et al., 2014; Thongkaew et al., 2015). Briefly, powders of WPI (5% w/v) and apple pectin (2% w/v) were dissolved in 10 mM sodium phosphate buffer (pH 7.0), and allowed to dissolve at ambient temperature under stirring for >2 h to ensure complete hydration. The protein solution was heated to 65 °C, held there for 40 min, then cooled down to room temperature, and finally filtered (filter type 595½, Whatman GmbH, Dassel, Germany) to remove remaining undissolved WPI. The heat treatment of WPI was done to promote the later required phase separation with pectin (Bryant et al., 2000; Mounsey, O'Kennedy, Fenelon, & Brodkorb, 2008).

2.3. Preparation of mixed WPI – pectin systems

Mixed systems were prepared using a modification of a previously described method (Thongkaew et al., 2014; Thongkaew et al., 2015). Briefly, WPI and pectin stock solutions were mixed together at a ratio of 1:1 using a rotor-stator system (AR 19 Anchor-Type Impeller size 60 × 40 × 5 mm, length 350 mm, ⌀ shaft 8 mm, Type RZR, Heidolph Elektro KG, Kelheim, Germany) at 50 rpm for 3 min. After combining the stock solutions, the mixed system had a protein concentration of 2.5% and a pectin concentration of 1.0% (w/v) and a pH of 6.1. The mixture was then allowed to stand for 1 h during which time a phase separation occurred. The now two phases were then mildly homogenized at 10 rpm (Type RZR, Heidolph Elektro KG, Kelheim, Germany) to form a so called water-in-water dispersion consisting of protein droplets suspended in pectin (see also results). The generated dispersion was then subjected to pH and ionic strength changes by adding either 1 N or 0.1 N hydrochloric acid, or 200 mM NaCl, respectively. All samples were equilibrated for >1 h before mechanical stress tests were conducted.

2.4. Superimposition of mechanical stresses

Systems were subjected to mechanical stresses using a Modular Compact Rheometer MCR300 (Anton Paar Physica, Graz, Austria) equipped with a transparent counter rotating parallel plate shearing device (Anton Paar, Graz, Austria) and an optical microscope system. The system consisted of two parallel quartz plates with a diameter of 43 mm and a gap setting of 0.5 mm. The rheometer was controlled by the US 200 software version 2.0 (Physica Messtechnik GmbH, Stuttgart, Germany). For rheological tests, samples (1 mL) were placed between the plates and allowed to equilibrate at 20 °C for 5 min. Samples were then sheared for up to 600 s at a constant shear rate (0.1, 0.5, 1, 5, 10, 20, 30, 40, 50 s^{-1}). For each shear rate experiment, a new and undisturbed sample was used. Shear stress versus time was recorded, and apparent viscosities as a function of time calculated from these values. The microstructure of samples before and after 600 s of shearing was characterized with an optical microscope (M Plan Apo series M 84022407, Mitutoyo, Japan).

2.5. Particle size

Determination of particle mean sizes and distributions were carried out using both image analysis and light scattering (Thongkaew et al., 2014; Thongkaew et al., 2015). For light scattering, samples were first diluted to 1:300 using appropriate buffer solutions having the same pH and salt concentration and then analyzed in a static light scattering instrument (LS230 Small Volume Module Plus, Becker Coulter Inc., Miami, FL). Diameter