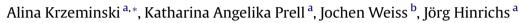
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Environmental response of pectin-stabilized whey protein aggregates



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A R T I C L E I N F O

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ABSTRACT

Electrostatically-stabilized complexes are highly sensitive to changes in their local environment. The current study investigated the formation and stability of particles by heating (90 °C, 5 min) whey protein isolate (WPI) and pectin together as affected by the degree of esterification (DE), whey protein (WP):Pectin ratio (8:1, 5:1, and 2:1), and pH (6–4). At pH 6.0, primary complex formation between WP and pectin was shown, whereas this effect was more pronounced for low-methoxyl pectin (LMP) than for high-methoxyl pectin (HMP) based systems. At pH 4.0, maximum opposite net charges on both biopolymers and maximum biopolymer interactions were observed. Heat-treated WP-Pectin mixtures tended to form more compact and stable structures than unheated ones, associated with the lower pH sensitivity of protein. LMP-stabilized WP systems were characterized by many small aggregates structures, which is associated with interpolymeric complexation. Since LMP-stabilized WP aggregates meet the size characteristics of milk fat globules, they might have potential to replace parts of fat in fermented milk products.

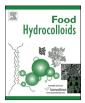
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1. Introduction

In the past decade, a lot of work has been done to understand associative interactions and their resulting associated structures in ternary polymer solutions (de Kruif, Weinbreck, & de Vries, 2004; Krzeminski et al., 2006; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003; Zintchenko, Rother, & Dautzenberg, 2003) due to their potential application in a variety of fields e.g. drug release, microencapsulation (Wang, Gao, & Dubin, 1996), and fat substitution (Mattison, Dubin, & Brittain, 1998). For example, complexes impart several interesting abilities such as hydration (solubility, viscosity), structuration (aggregation, gelation), and surface properties (foaming, emulsifying) (Schmitt & Turgeon, 2011). Complexes with improved functional properties can be formed between proteins and polysaccharides via electrostatic interactions, hydrogen bonds, hydrophobic interactions, and covalent interactions (Schmitt et al., 1998). Among those interactions, electrostatically-stabilized complexes are highly sensitive to dissociation due to changes in their local environment. Thus, an ongoing challenge for food scientists and food producers is to generate complexes that maintain their expected functionalities during food processing. Processed foods usually are multi-component systems consisting of e.g. different biopolymers (proteins and polysaccharides), which in turn comprise dispersed particles (droplets, granules, bubbles). Product features in terms of microstructure, rheology, texture, and overall stability largely depend on the state of aggregation of the dispersed particles affecting structural characteristics of complexes such as size and conformation, nature of the interactions involved (protein-protein, proteinpolysaccharide, polysaccharide-polysaccharide) and the number of interacting groups (Tolstoguzov, 1997). In addition, it is important to determine the influence of other food components such as lipids, sugars, and simple salts in order to control the resulting associative interactions (Dickinson & Euston, 1991; Samant, Singhal, Kulkarni, & Rege, 1993). Previous studies, performed on whey protein (WP)pectin mixtures, have shown that heating preserves the functionality of electrostatically-stabilized complexes and improves their stability (Gentès, St-Gelais, & Turgeon, 2010; Jones, Decker, & McClements, 2010a). This article focuses on the formation of particles by heating whey protein isolate (WPI) and pectin together and on the investigation of the aggregation behavior of those mixed dispersions as a function of environmental conditions. Because of their 'natural' image and their various nutritional and functional abilities, WP and pectin have a widespread acceptance by producers







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and consumers as potentially safe food ingredients (Dickinson, 1998). Whey has a high economic value as it is a co-product from the cheese-making process, accounting for approximately 80-90% of the total volume of milk. It comprises the globular WP, which mainly include β -lactoglobulin (56%) and α -lactalbumin (21%). On heating at 85–95 °C for 1–2 min, WP aggregates to comparable sizes to milk fat globules (1–10 um) while possessing a compact and stable structure and thus high serum binding capacity (Spiegel, 1999). In addition, the combination of heat-induced WP aggregation with an intensive shearing load results in the formation of WP aggregates with particle sizes that usually range between 0.5 and 10 µm (Spiegel & Huss, 2002). The incorporation of those microparticulated WP into fat-reduced milk products improves their textural attributes such as creaminess (Janhøj, Petersen, Frøst, & Ipsen, 2006; Koxholt, McIntosh, & Eisenmann, 1999; Schenkel, Samudrala, & Hinrichs, 2013; Torres, Janhøj, Mikkelsen, & Ipsen, 2011). In contrast, native WP and very small particles are less effectively incorporated into a fermented milk matrix. Pectin was chosen since it is often used in fermented milk products such as yogurt, cheese, and curd cheese due to its remarkable stability at low pH values (pH 3.5–3). For instance, pectin is used to coat casein micelles in acid dairy drinks and thus preventing casein from aggregation by electrostatic and steric stabilization (Parker, Boulenguer, & Kravtchenko, 1994). Many studies have dealt with WP-Pectin complexes and examined the complexation behavior near neutral pH (pH 6.4–6.0) (Beaulieu, Corredig, Turgeon, Wicker, & Doublier, 2005; Zhang, Zhang, Lin, & Vardhanabhuti, 2012), the complex formation between WPI and amidated pectin (Gentès et al., 2010), emulsion stabilizing effects (Neirynck et al., 2007), the formation of nanoparticles with narrow size distributions (diameter ~200-300 nm) (Jones et al., 2010a) or small assemblies (Girard, Sanchez, Laneuville, Turgeon, & Gauthier, 2004) for microencapsulation issues. Based on those investigations and size characteristics of microparticulated WP, we focused our objectives on examining the size and morphology of pectin-stabilized WP aggregates upon heating at 90 °C for 5 min (high WP denaturation rate) and the response of those heat-induced aggregates to changes in degree of pectin esterification (unstandardized pectin with 38, 53, and 70% DE), WP:Pectin ratio (8:1, 5:1, and 2:1), and pH (6-4). In particular, laser diffraction spectroscopy was used to determine the aggregate size characteristics of WP-Pectin mixtures, which can be used as indicative parameters for assessing the ability of WP-Pectin complexes to replace parts of fat in fermented milk products.

2. Materials and methods

2.1. Materials

Native WPI (DSE 5627), obtained from sweet whey, was purchased from Fonterra Co-operative Group Ltd. (Auckland, New Zealand). As stated by the manufacturer, the protein fractions were as follows: 66.9% β -lactoglobulin, 17.4% α -lactalbumin, 4.4% glycomacropeptide, 2.2% bovine serum albumin and 1.4% immunoglobulin G (IgG). Protein content of the samples was calculated from the nitrogen content as determined by a nitrogen analyzer (LecoFP-258, LecoInstruments GmbH, Moenchenladbach, Germany), following the Dumas method (International Dairy Federation (IDF) 185:2002). A conversion factor of 6.38 was used. The total protein content determined according to the Dumas method was 92.14 \pm 0.08%, which was used for further calculations.

Three unstandardized citrus pectins of classic type having a different DE were kindly provided by Herbstreith & Fox KG (Neuenbuerg, Germany) and used without further purification. According to the DE, pectin is classified in high-methoxyl (HMP, >50% DE) and low-methoxyl (LMP, <50% DE) forms. As stated by the

manufacturer the DE and the apparent molecular weight (MW, determined by capillary viscometry) of the used citrus pectins was: 38% DE, 55 kDa MW (CU-L057/11), 53% DE, 65 kDa MW (CU-L058/11), and 70% DE, 85 kDa MW (CU L061/11), respectively.

2.2. Preparation of WP-Pectin mixtures

WP and pectin aqueous stock solutions were prepared in percent by weight (wt%) by dispersing known amounts of biopolymer powder in bidistilled water under gentle magnetic agitation (350 rpm) at room temperature for at least 3 h and then left overnight at 10 °C to ensure complete hydration of biopolymers.

WP-Pectin mixtures were prepared by adding a pectin solution to a WP solution having a constant protein concentration of $c_{\text{Protein}} = 0.5$ wt%. The concentration of the pectin solution was defined according to the desired WP:Pectin ratio of 8:1 (high ratio), 5:1 (intermediate ratio), and 2:1 (low ratio). Half of the WP-Pectin mixtures were heated in a water bath to 90 °C for 5 min (to ensure high WP denaturation) while being unstirred. Corresponding pure WP and pure pectin solutions were prepared as control samples.

The acidification step of WP-Pectin mixtures was performed after thermal treatment (post-heating acidification) as this favours protein—polysaccharide interactions over protein—protein aggregation (Tolstoguzov, 1997).

Solutions were acidified by adding lactic acid from pH 6.0 to pH 4.0, and 0.1 M NaOH was used if necessary to correct the pH to the desired pH value. Sodium azide was added to the solutions as a preservative, the final concentration was 0.02 wt%. All analytical grade reagents were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany).

2.3. Electrophoretic mobility and ζ-Potential determination

The Zetasizer Nano Series particle size analyzer (Nano-ZS ZEN 3600, Dispersion Technology Software DTS v5.1, Malvern Instruments Ltd., Worcestershire, UK) was used to measure the electrophoretic mobility and calculate the ζ -Potential of the samples (Henry, 1931) by applying the Smoluchowski constant $f(\kappa a)$ of 1.5 (Delgado, González-Caballero, Hunter, Koopal, & Lyklema, 2007; Ryan et al., 2012; Sağlam, Venema, de Vries, Shi, & van der Linden, 2013). Thereby, the κa term relates to the radius (a) of the particles to the thickness of their electrical double layer. A refractive index of 1.42 was used for calculation. The temperature of the electrophoresis cell was maintained at 20 °C. The ζ -Potential was determined in triplicate.

2.4. Particle size determination

The particle size distribution of samples was determined using a Horiba Laser Scattering Particle Size Distribution Analyzer (LA-950 V2, Software LA-950, Horiba Ltd., Kyoto, Japan). The calculation is based on the Mie theory allowing a particle detection within a range of 0.01–3000 µm. The broad detection range of this instrument allowed the particle size determination of precipitates (acidified WP solutions) and aggregates (WP-Pectin mixtures). Prior to analysis, an appropriate refractive index of the WP-Pectin particles was determined by means of the Method Expert option within the LA-950 Software. Here, the parameters χ^2 and R compare the measured raw data in each channel (i.e. detector) to the amount of light scattering predicted for the reported particle size distribution. A lower value for either χ^2 or *R* indicates a better fit of the raw data to the calculated particle size distribution. Thus, a refractive index of 1.42 was determined and used for all measurements of the samples. Particle size was measured in triplicate.

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