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Improving the stability of wheat protein-stabilized emulsions: Effect of pectin and xanthan gum addition

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ABSTRACT

The potential of two anionic polysaccharides, pectin and xanthan gum, to improve the physical stability of emulsions containing lipid droplets coated by wheat protein (deamidated gliadin) was investigated. Polysaccharide type and solution pH had a major impact on biopolymer interactions in solution: wheat protein interacted with xanthan gum from pH 3.5 to 7, but with pectin from pH 3.5 to 5, which was attributed to different polysaccharide charge densities. Biopolymer interactions in solutions were related to their adsorption behavior in emulsions. Wheat protein-stabilized emulsions were highly unstable to aggregation at pH values around their isoelectric point (pI ≈ 5) and at elevated NaCl (≥100 mM, pH 7) or $CaCl_2$ (≥10 mM, pH 7) levels. Adding xanthan gum improved emulsion stability to high ionic strengths, with no phase separation observed during storage for 4 weeks. Adding pectin improved emulsion stability at acidic pH, but the emulsions were still unstable at elevated ionic strengths. These results are useful for the increased utilization of wheat proteins as functional ingredients in the food industry.

1. Introduction

Many types of food protein can be used as emulsifiers due to their amphiphilic character, polymeric structure, and electrical charge characteristics (Dickinson, 2003; McClements, 2004). The amphiphilic character means that they can adsorb to droplet surfaces during homogenization. The polymeric structure and electrical charge of proteins mean that they can generate strong steric and electrostatic repulsive forces between droplets, thereby inhibiting droplet aggregation. The most commonly utilized protein-based food emulsifiers are caseins and whey proteins derived from bovine milk. However, there is considerable interest in developing protein emulsifiers from other sources for economic, health, and labeling reasons. For example, milk proteins are unsuitable for application in products designed for vegans. Wheat gluten is already widely used as an ingredient in the food industry due to its specific functional attributes and relatively low price (Day, Augustin, Batey, & Wrigley, 2006; Qiu, Sun, Cui, & Zhao, 2013; Qiu, Sun, Zhao, Cui, & Zhao, 2013). However, many proteins from plant sources have limited use as functional ingredients in their native states because of their relatively low water solubilities. Thus, modification of these proteins is required to enhance their solubility and functional attributes. Deamidation by mild acid is a convenient way to increase the overall electrical charge on proteins and thereby improve their water solubility (Hamada, 1994). Deamidated wheat gliadin ("gliadin") has been reported to be a good emulsifier, which has been attributed to its high surface hydrophobicity, molecular flexibility, and interfacial thickness (Day, Xu, Lundin, & Wooster, 2009; Qiu, Sun, Zhao, et al., 2013; Wong et al., 2012). Emulsions formed by this protein were reported to have better thermal stability than those produced using more conventional food-grade proteins, but they still had poor stability near the protein's isoelectric point (pI ≈ 5) and at high salt concentrations (Qiu, Sun, Zhao, et al., 2013).

Protein-coated lipid droplets have poor stability to aggregation at pH values close to the pI and at high ionic strengths when they are primarily stabilized by electrostatic (rather than steric) repulsion (McClements, 2004). Consequently, their aggregation stability can be improved by coating them with polysaccharides that form thick charged interfacial layers that increase the steric and/or electrostatic repulsion between the droplet surfaces (Dickinson, 2008). Most polysaccharides are highly hydrophilic molecules that have little inherent surface activity. Nevertheless, they can be

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made to adsorb to the surfaces of protein-coated oil droplets using electrostatic interactions under conditions where the proteins have opposite charges to the polysaccharides. For this reason, many studies have focused on improving the stability of emulsions by forming interfacial complexes of proteins and polysaccharides (Dickinson, 2003; Gu, Decker, & McClements, 2004; Guzey & McClements, 2006a; Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008). Protein-polysaccharide complexation may occur either before or after homogenization, and may be either covalent or non-covalent.

Covalent protein-polysaccharide complexes are commonly formed by the Maillard reaction, which can then be used to form oil-in-water emulsions containing lipid droplets coated by protein-polysaccharide layers (Dickinson, 2008; Guzey & McClements, 2006b). These emulsions have been shown to have better stability to pH and ionic strength than protein-coated droplets (Wooster, Augustin, 2006). Non-covalent protein-polysaccharide complexes can be formed in aqueous solutions prior to homogenization by electrostatic attraction under conditions where the biopolymers have oppositely charge groups (Dickinson, 2008). Lipid droplets coated by this type of protein-polysaccharide complex have also been shown to have better physical stability than those coated only by proteins (Jourdain, Leser, Schmitt, Michel, Dickinson, 2008; Jourdain, Schmitt, Leser, Murray, Dickinson, 2009). Finally, non-covalent protein-polysaccharide complexes can be formed after homogenization by mixing a charged polysaccharide with an emulsion containing oppositely charged protein-coated lipid droplets (Guzey & McClements, 2006b; McClements, 2010). The resulting protein-polysaccharide coatings have also been shown to increase the physical stability of oil-water emulsions (Gu, Regnier, McClements, 2005; Guzey & McClements, 2006b; Iwanaga, Gray, Decker, Weiss, & McClements, 2008). It should be noted that there may be appreciable differences between the stability characteristics of emulsions containing droplets coated by non-covalent interfacial complexes formed before or after homogenization (Jourdain et al., 2008, 2009).

Previous studies have shown that the ability of deamidated wheat proteins to stabilize oil-in-water emulsions can be improved by forming covalent (Maillard) complexes with polysaccharides (dextran) (Wong, Day, & Augustin, 2011, Wong, Day, McNaughton, Augustin, 2009). In the present study, we focus on improving the emulsion stabilizing properties of deamidated wheat proteins by formation of non-covalent protein-polysaccharide complexes with anionic polysaccharides. Xanthan gum (XG) is a stiff high molecular weight anionic polysaccharide produced by the microorganism *Xanthomonas campestris* (Moschakis, Murray, & Dickinson, 2005). It is a non-adsorbing polysaccharide with high viscosity and strong shear-thinning character. Addition of XG to oil-in-water emulsions increases the viscosity of the continuous phase, which may improve their stability to gravitational separation. On the other hand, XG addition at relatively low concentrations may accelerate creaming of emulsions due to depletion or bridging flocculation (Moschakis et al., 2005). Pectin from citrus fruits is mainly composed of a backbone of galacturonic acid with protruding hairy regions containing rhamnose, galactose, arabinose, xylose and glucose (Nakamura, Yoshida, Maeda, & Corredig, 2006). Pectin addition may also either increase or decrease emulsion stability depending on the amount added and solution conditions (such as pH and ionic strength).

The purpose of the present study was to investigate some of the major factors that impact the preparation of stable oil-water emulsions coated by gliadin-anionic polysaccharide interfacial layers. Non-covalent electrostatic protein-polysaccharide complexes were formed after homogenization, and then the stability of these emulsions to environmental stresses (pH and ionic strength) was investigated. This work is novel since it may lead to an increase in the utilization of wheat proteins as emulsifiers in foods and other commercial products.

2. Materials and methods

2.1. Materials

Wheat gluten with 71.5% crude protein was purchased from Lianhua Co. Ltd. (Zhoukou, China). Xanthan gum (XG) (Prehydrated Ticaxan Xanthan powder) was provided by TIC Gums (Maryland, USA), which had a reported average molecular weight of 6000 kDa. Pectin extracted from citrus fruit (DE > 50%) was provided by CPKelco (Houston, TX) which was reported to have an average molecular weight of 200 kDa. Sodium phosphate buffer (5 mM, pH 7.0) was prepared using milli-Q water. Fluorescent dyes, fluorescein isothiocyanate (FITC) and Nile Red, were obtained from the Sigma Chemical Co. (St. Louis, MO). Corn oil was purchased from a local supermarket. All other reagents were of analytical grade.

2.2. Preparation of protein material

Deamidated wheat gliadin was prepared according to Qiu et al. (Qiu, Sun, Zhao, et al., 2013). Citric acid (0.2 M) was added to wheat gluten solution (8 wt%) at 70 °C with constant stirring in a sealed glass container for 16 h. The dispersion was then neutralized, dialyzed, and freeze-dried. The gliadin-rich fraction was extracted from deamidated wheat gluten by stirring with 70% ethanol for 2 h. The ethanol in the supernatant was removed by rotary evaporation (50 °C). The final freeze-dried deamidated gliadin fraction contained 79% of protein (N × 5.7) and 6.4% moisture. The average molecular weight of deamidated gliadin determined by SDS-PAGE was between 31 and 74 kDa.

2.3. Solution preparation

An emulsifier solution containing gliadin was prepared by dispersing powdered deamidated wheat gliadin into 5 mM phosphate buffer (pH 7) and stirring for at least 2 h to ensure complete hydration. The solution was centrifuged (4000 rpm, 15 min) and the final supernatant was collected. Solutions of 0.6 wt% xanthan gum and 0.4 wt% pectin were prepared by dispersing powdered biopolymers into phosphate buffer and stirring for at least 4 h to ensure complete hydration. Mixed solutions containing gliadin (0.25 wt%) and either xanthan gum or pectin (0–0.3 wt%) at different pH values were prepared by mixing the biopolymer solution to the required concentration and stirring for at least 1 h to ensure complete dispersion.

2.4. Emulsion preparation

Stock emulsions were prepared by pre-homogenizing corn oil (10 wt%) with the emulsifier solutions (90 wt%) using a high-speed mixer (M133/1281-0, Biospec Products Inc., ESGC, Switzerland) for 2 min. The coarse emulsions formed were then further homogenized using a microfluidizer (M110Y, Microfluidics, Newton, MA) for 3 passes at a pressure of 10,000 psi (69 MPa). After homogenization, 0.01% (w/w) of sodium azide was added as a microbial preservative.

2.4.1. Post-homogenization complex formation

Different proportions of stock emulsion were mixed with sodium phosphate buffer or polysaccharide solution to form primary emulsions (2.5 wt% corn oil, 0.25 wt% gliadin, pH 7) or secondary emulsions (2.5 wt% corn oil, 0.25 wt% gliadin, 0–0.3 wt% pectin or