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Protein oleogels from heat-set whey protein aggregates

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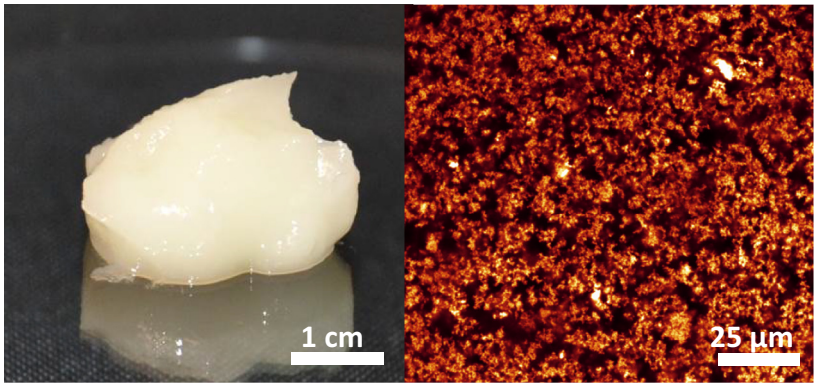
GRAPHICAL ABSTRACT



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ABSTRACT

In this research we use heat-set whey protein aggregates (diameter ~ 200 nm) as novel building blocks for structure formation in liquid oil to form oleogels. To transfer the aggregates to the oil phase, a solvent exchange procedure to sunflower oil was applied using acetone as an intermediate solvent. We found that agglomeration of the aggregates was prevented and the particle size in oil did not change from that in the initial aqueous phase. The small protein aggregates assemble into a space-spanning network, thereby providing solid-like properties to liquid oil. From oscillatory rheology we conclude that the aggregates are highly effective in forming a network. Already at ~3% we found that $G' > G''$ and G' scales with protein concentration as $G' \sim c_p^{5.3}$. Applying a fractal gel network theory to the rheological data we deduce that the gels are in the strong link regime with a fractal dimension of 2.2. The results show that protein aggregates, besides their well-known functionality in aqueous solvents, are capable of forming a network in liquid oil. This provides a novel and promising way to design oleogels with tuneable rheological properties, applicable to e.g. foods, pharmaceuticals and/or cosmetics.

1. Introduction

Gels in which the continuous phase is a nonpolar solvent (so-called organogels, or oleogels in the case of edible oil) is a topic of growing interest. Such organo- or oleogels are studied for various purposes, like drug delivery [1,2], oil spills [3], electronics [4], and foodstuffs [5,6]. Gelators for nonpolar liquids can be classified into low molecular weight organogelators (LMOG's) or polymeric organogelators. LMOG's are the most common and within this class, gelation is achieved by crystallization or by self-assembly into fibrous networks. Common types of LMOG's are fatty

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acids or alcohols [7], waxes [8,9], phytosterols [10,11], oligopeptides [12], and lecithins [13]. The gelation of such systems relies on the self-assembly behavior of the molecules into structures of larger length scales, such as micellar or lamellar phases. The rheological behavior of such organo- or oleogels is influenced by the specific interactions between the building blocks.

In contrast to LMOG's, the number of (bio)polymers used as oleogelator is limited. One of the best studied examples is the cellulose derivative ethylcellulose [14]. Insoluble in water, this biopolymer dissolves in liquid oil at elevated temperatures, where, upon lowering the temperature, the polymer chains interconnect to form a network resulting in an oleogel. By using different surfactants, or by using ethylcellulose of different molecular weight, the interactions between the polymer chains can be altered, and hence the rheological properties of the final oleogel can be tuned [15–17]. Another example of a biopolymer with oil gelling capabilities is chitin. It has been shown that oleogels can be made by using chitin either in a crude form, as nanocrystals [18] or when modified into hydrophobic whiskers [19].

Although examples like that of ethylcellulose and chitin as biocompatible oil gelators do exist, there is a need to identify gelators that are affordable, efficient, and food grade. These requirements are especially important when the desired applications are in foodstuffs, as replacement for high melting crystalline hardstocks, and in drug delivery systems for controlled release of hydrophobic compounds. To cover a wide range of possible applications, it is desirable to control the rheological behavior of such organo- and oleogels in terms of properties as gel strength, yield stress, and plasticity. One interesting alternative is the gelation of liquid oil based on proteins, as they are widely available and food grade. However, since proteins are poorly dispersible in nonpolar solvents, their capability of forming a gel network in solvents such as liquid oil is limited. Nevertheless, attempts have been made to use proteins as oleogelators. To overcome the problem of poor dispersibility, Romoscanu and Mezzenga [20] used an emulsion-templated approach. Here, the protein is adsorbed at the oil-water interface and upon subsequent removal of the water, a gel with a high oil content can be achieved. The continuous phase in these systems is still hydrophilic, and the gel can be easily hydrated and reversed into an oil in water emulsion upon addition of water. Using a similar emulsion or foam-templated approach, Patel and co-workers used a dried foam [21] and a dried oil-in-water emulsion [22], using methylcellulose and a gelatin-xanthan mixture respectively, followed by a shearing step to create oleogels. In these cases, however, the final structure and particle size might be difficult to tune, which will limit specific control over the network structure and subsequent rheological properties of the gel. Moreover, the choice of structuring agents is limited to surface active components that are able to prevent the coalescence of oil droplets and oil separation during drying.

Recently, we have shown that heat-set whey protein hydrogels could be used as macroscopic templates to create protein oleogels by applying a solvent exchange procedure. This procedure relies on replacing the water in the interstitial areas of the heat-set protein matrix by an intermediate solvent (in our case acetone), followed by liquid oil. The gel matrix was shown to be capable of binding a large amount of oil (>91%) and rheological tests showed that the protein oleogels were much stiffer and much more brittle compared to the preceding hydrogels [23]. Although the solvent exchange procedure proved effective, the preparation method gave limited flexibility to alter the rheological properties of the final protein oleogels. In order to do so, control over network formation at smaller length scales is required. Initial building blocks of colloidal size would be more appropriate, as colloidal interactions are essential to control such network formation.

Preparation of protein aggregates of colloidal size is a topic that has already been studied exhaustively. In general, the gelling mechanism of such aggregates into hydrogels is based on protein denaturation (e.g. by applying heat treatment) followed by network formation of the formed aggregates by reducing the electrostatic repulsion [24–26]. However, it is not known whether protein aggregates are able to create networks in oil, and how the network is organized. In the current paper, we extend our previous work by investigating the network formation of protein aggregates of colloidal size in oil and compare this to protein gels obtained in aqueous environments. Understanding the network formation of the colloidal protein aggregates in oil can provide insights to create protein oleogels with a larger diversity of rheological characteristics (such as plastic deformation) and could expand possible applications.

To investigate the network formation and the resulting viscoelastic properties, we create submicron whey protein aggregates as initial building blocks in an aqueous medium, and subsequently transfer the aggregates into liquid oil by applying a similar solvent exchange approach as described previously for macroscopic gels [23]. We show for the first time that submicron protein aggregates can be used directly to form a network in liquid oil, similar to their well-known ability to form a network in water.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI, BiPro) was obtained from Davisco Foods International (Le Sueur, MN, USA). The protein concentration was 93.2% (N × 6.38) and was used as received. Acetone (AR grade) was supplied by Actu-All Chemicals (Oss, the Netherlands). Refined sunflower oil (Vandermoortele NV, Breda, the Netherlands) was bought at a local supermarket and was used without further purification. Demineralized water was used throughout the experiments.

2.2. Methods

2.2.1. Preparation of protein aggregates

To prepare a protein stock solution, WPI powder (4% w/w) was dissolved in demineralized water under continuous stirring at room temperature for 2 h. Afterwards, the stock solution was stored overnight at 4 °C to assure complete protein hydration. The next day, the pH of the stock solution was adjusted to 5.7 using a 1 M HCl solution. The resulting solution was heated in 50 mL plastic tubes with screwcaps at 85 °C for 15 min using a temperature controlled water bath to induce protein denaturation. After cooling in ice water, a weak protein gel was obtained. This weak gel was easily broken into aggregates by hand shaking and vortexing. The resulting protein dispersion was homogenized by using a rotor stator homogenizer (Ultra Turrax, T25, IKA Werke, Germany) at 13.000 rpm for 3 min. The protein aggregates were then collected as a pellet by centrifuging at 3904*g* (Hermle Z383 K, Hermle Labortechnik GmbH, Wehingen, Germany) for 20 min at 20 °C. After collection, the pellet was re-dispersed and centrifuged twice with demineralized water to remove remaining soluble protein material.

2.2.2. Preparation of the protein oleogels

To prepare the protein oleogels, the WPI aggregates were transferred to the oil phase using a solvent exchange procedure. In this procedure, the polarity of the solvent was changed gradually to remove the surrounding water from the WPI aggregates and replace the continuous phase for oil. In short, 15 g of aqueous pel-