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Gelation of oil-in-water emulsions stabilized by whey protein

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ABSTRACT

The effects of heat treatment and high-pressure homogenization on whey protein (WP) emulsion properties were evaluated. Only emulsion containing non-heated WP was stable, presenting low viscosity, small droplets and higher adsorbed protein content by increasing homogenization pressure. In addition, the influence of high-pressure homogenization and pH on cold-set gel formation by acidification of emulsion (emulsion gel) or emulsion dispersed in heat-treated WP solution (emulsion-filled gel) was studied. Only emulsion-filled gels were self-supported. Stronger gels were observed at pH near WP isoelectric point due to lower acidification rate and electrostatic repulsion that promoted more intense protein aggregation. Varying emulsion homogenization pressure resulted in different gel network strength caused by changing dispersed phase volume fraction and adsorbed protein content. Therefore, depending on process conditions cold-set gel of emulsion can be produced and it shows a great potential to be used in food formulations as texturizing agent and/or as a carrier for heat-labile ingredients.

1. Introduction

Whey proteins (WP) can be used as emulsifiers due to their excellent surface activity, avoiding the coalescence and creaming process of the dispersed phase in oil-in-water emulsions (Dickinson, 2003; McClements, 2005). The heat treatment of these globular proteins can promote unfolding and exposure of reactive sites, resulting in their denaturation and aggregation that allows the use of these natural ingredients of high nutritional value (Millqvist-Fureby et al., 2001) as gelling agent in food products (Dickinson, 2010). The main fraction of WPI, β-lactoglobulin (β-lg), denatures partly if heated by at least 30 min at a temperature around of 60 °C. However β-lg dispersions heated during the same time at temperatures around 95 °C show complete protein unfolding, which is attributed to the extensive exposure of hydrophobic groups and highly reactive nucleophilic sites (−SH and ε-NH^{+3}) (Havea et al., 2001). In this way, the denaturation of WP at different temperatures, but with the same exposure period may lead to different protein denaturation degree (Millqvist-Fureby et al., 2001), resulting in varied surface active properties (Ruffin et al., 2014).

Emulsions can be kinetically stabilized with addition of emulsifying agents (McClements, 2005). A mixture of non-heated and thermal denatured WP as emulsifying agent resulted in improved WP surface activity. Non-heated proteins can move more quickly facilitating the droplet recovering, while denatured proteins contribute to the formation of a thick protective layer on the O/W interface (Kiokias and Bot, 2006). Homogenization process at high pressures also enables the formation of small droplets size (Jafari et al., 2008) as a consequence of intense shear, cavitation and turbulent flow conditions (McClements, 2005). This process promotes a more homogeneous distribution of the surface-active molecules, increasing the emulsion stability. However, the conditions of the homogenization process could also modify the protein structure and its surface activity. It has been reported that high-pressure homogenization can also result in different degrees of protein unfolding, leading to the formation of high molecular weight protein aggregates due to shear and the increase in temperature (Kuhn and Cunha, 2012). Moreover, there is a growing interest in gelation of emulsions due to the possibility of producing food products with different texture properties (Dickinson, 2013; Dickinson and Chen, 1999). In addition, the gelation of WP-stabilized emulsion improves oxidative stability of lipids and promotes a controlled release, resulting in a good potential of emulsion gels as a carrier for active compounds (Ruffin et al., 2014).

The most common way to gel oil-in-water emulsions stabilized

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by whey proteins is by heat treatment (Chen and Dickinson, 1998; Dickinson and Chen, 1999). Another alternative to induce gel formation using this protein emulsifier would be the cold gelation process that involves two steps: (i) pre-heating to promote the protein unfolding and the exposure of reactive groups and (ii) formation of a gel structure through acidification to a pH near protein isoelectric point (pI) by the addition of an acidifier (e.g. glucono-δ-lactone (GDL)) (Mao et al., 2014; Rosa et al., 2006; Sala et al., 2009a, 2009b) or addition of divalent salts (e.g. calcium chloride) (Kuhn et al., 2011; Liang et al., 2010; Pires Vilela et al., 2011; Sok Line et al., 2005) or enzymes (e.g. transglutaminase) (Dickinson and Yamamoto, 1996) to the system. However, the cold-set gelation is more suitable for systems containing heat-sensitive ingredients since the initial step of protein pre-heating involves a less severe heat treatment when compared to the thermal treatment gelation. Such systems can be used as matrices for the release of active ingredients (Liang et al., 2010; Tomczyńska-Mleko and Mleko, 2014). Concerning cold-set gelation of emulsions, the pre-heating that promotes the protein unfolding could be carried out before (Rosa et al., 2006; Sala et al., 2009a, 2009b) or after the emulsification process (Ye and Taylor, 2009). Nevertheless, both possibilities could lead to adverse effects as the reduction of the protein emulsifying properties or the damage of oil properties.

In this context, the aim of this work was to evaluate the influence of WP unfolding caused by high-pressure homogenization and/or heat-treatment on the formation of emulsion gels produced by cold-set gelation induced by acidification with GDL. Firstly, the effect of different denaturation degrees of the WPI on the O/W emulsions stability was evaluated by varying the temperature of heat treatment (70 or 90 °C) and the homogenization pressure (25–60 MPa). Afterwards, the effects of the high-pressure homogenization and the final pH value on the mechanical properties of cold-set gels induced by acidification of the WP emulsions were assessed.

2. Materials and methods

2.1. Materials

Lacprodan® whey protein isolate (WPI) was kindly donated by Arla Foods Ingredients (Denmark). The WPI was characterized by atomic absorption spectroscopy and the following composition of ions was obtained: Na^+ 0.63%, Ca^{2+} 0.05%, and K^+ 0.65%. The protein (N × 6.38) and moisture content (w/w wet basis) of WPI were 92.4% and 5.7%. Soybean oil (Soya, Bunge Alimentos S.A., Brazil) was obtained from the local market. GDL and other reagents of analytical grade were purchased from Sigma Aldrich Co. (USA).

2.2. Emulsion and gels preparation

2.2.1. Stock solutions

WPI stock solutions (10% w/w) were prepared by powder dissolution in deionized water under mild magnetic stirring at room temperature. These solutions were heat-treated at 70, 80 or 90 °C under agitation by 30 min. After heat treatment WPI solutions were immediately cooled using an ice bath. A stock solution containing non-heated protein was also prepared. Sodium azide (0.01% w/w) was added to the stock solutions to prevent microbial growth.

2.2.2. Emulsion

Oil-in-water (O/W) emulsions were prepared at 25 °C by homogenizing the oil in the aqueous phase containing heat treated or non-heated proteins using two sequential homogenization methods. The first step involved the mixture in an Ultra Turrax model T18 homogenizer (IKA, Germany) for 5 min at 14,000 rpm. In the second step, the previously prepared coarse emulsion was subjected to a high-pressure homogenization process using a Panda 2K NS1001L double stage homogenizer (Niro Soavi, Italy) with pressure values ranging between 25 and 60 MPa at the first stage and 5 MPa at the second stage. The WPI and oil contents were fixed at 5% (w/w) and 30% (w/w), respectively. The emulsions were evaluated by creaming stability, mean droplet size, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and rheological measurements.

2.2.3. Cold-set emulsion gels

Before gels preparation, stock emulsions were prepared under the following conditions: 30% (w/w) of oil, 5% (w/w) of non-heated WPI and homogenization pressure of 25 or 45/5 MPa with one pass. Four different kinds of systems were subjected to cold-set gelation. Systems containing only emulsion were called emulsion gels, while those formed by the dispersion of the same emulsion in an aqueous phase with heat-treated WPI were classified as emulsion-filled gels. Moreover, WPI solutions subjected to cold-set gelation in a similar way called hydrogel or hydrogel-filled were used as control in order to evaluate the effect of oil phase on the gelation process.

Emulsion gels were prepared by the addition of GDL directly to the stock emulsion under magnetic stirring for 2 min. Emulsion-filled gels were prepared by the dispersion of stock emulsion into an aqueous phase containing 5% (w/w) heat-treated WPI solution (80 °C/30 min) using magnetic stirring for 2 min, followed by GDL addition under stirring during more 2 min. Systems without oil (hydrogel or hydrogel-filled) were prepared similarly to emulsion and emulsion-filled gels but the stock emulsion was replaced by a solution of non-heated WPI (5% w/w) prepared at the same conditions of stock emulsion.

The mixtures were poured into cylindrical tubes (internal diameter = 20 mm, height = 25 mm) and Petri dishes, sealed and stored at 10 °C for 48 h. The gels composition and GDL content are described in Table 1. GDL content was obtained according to the WPI content of the systems and the desired final pH of the gels after storage. The pH values were measured after the period of storage in order to verify if the theoretical pH values were reached. All gels were evaluated by mechanical properties and scanning electron microscopy (SEM) analysis.

2.3. Emulsions evaluation

2.3.1. Creaming stability

Immediately after preparation, 10 mL of each emulsion was poured into a cylindrical glass tube (internal diameter = 15.5 mm, height = 65 mm), sealed with a plastic cap and stored at 25 °C for a period of one month. The emulsion phase separation was visually observed during 7 days.

2.3.2. Optical microscopy and image analysis

The emulsions were evaluated after 7 days of storage. Emulsions were poured into microscope slides, covered with glass cover slips and observed using an optical microscope Carl Zeiss Model mf-AKS 24 × 36 EXPOMET (Zeiss, Germany). A 40× objective lens was used to visualize the microstructure of emulsions.

2.3.3. Mean droplet size

The emulsions were analysed 7 days after emulsion preparation and each sample was measured in triplicate. The droplet size was measured by laser diffraction measurements. A Mastersizer S (Malvern Instruments Ltd., UK) was used to determine the droplets size distribution. The mean diameter of the oil droplets was evaluated from the volume–surface mean diameter (d_{32}) (Eq. (1)).