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Stirring greatly improves transglutaminase-induced gelation of soy protein-stabilized emulsions

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ABSTRACT

The work reports the influence of stirring (with intermittent or continuous modes) during incubation on the rheological properties, water-holding capacity (WHC) and microstructure of soy protein isolate (SPI)-stabilized emulsion gels, induced by microbial transglutaminase (MTGase), at a constant protein concentration (6 g/100 mL) and three oil fractions (0.2, 0.4 and 0.6). The results indicated that the stirring simultaneously during incubation with the enzyme greatly facilitated the gel network formation and increased stiffness of the formed emulsion gels. The improvement of gel stiffness and WHC for the stirred-type gels was much better with the intermittent than continuous mode. The differences in rheological properties and WHC between different emulsion gels were basically consistent with the differences in their microstructure. Interestingly, the pattern of interaction forces involved in the gel network varied between unstirred and stirred gels, and even between the two stirring modes. The results suggest that the application of shearing could greatly improve the enzyme-induced gelation of protein-stabilized emulsions, and produce stirred-type emulsion gels with better water-holding and rheological properties, within a much shorter periods (relative to enzyme-set gels).

1. Introduction

During the past decades, there has been an increasing interest in the food structuring, especially through formation of soft solids from protein-stabilized emulsions, because of the practical relevance of these structured systems to development of real food formulations. Protein-stabilized oil-in-water emulsions can be converted into soft-solid-like materials by common food processing operations such as heating, acidification, addition of divalent salts, and enzyme action (Dickinson, 2012; Sok Line, Remondetto & Subirade, 2005). Relative to the heat-set emulsion gels that are clearly not suitable as carriers for heat-labile ingredients or nutraceuticals, the gels induced by non-thermal treatments, so called 'cold-set' gels, exhibit more potential to be used for the development of novel functional foods (Yang, Liu, & Tang, 2011). For example, Liang, Sok Line, Remondetto & Subirade (2010) successfully developed a β-lactoglobulin-stabilized emulsion gel induced by Ca^{2+} that acted as the carrier for α-tocopherol (a fat-soluble bioactive compound), and found that the stability of released α-tocopherol was greatly improved during an *in vitro* digestion model. Besides, Lee, Choi, and Moon (2006) found that sodium caseinate- or soy protein isolate (SPI)-stabilized emulsion gels, formed by microbial transglutaminase (MTGase), exhibited much higher storage stability of aroma compounds in the oil phase than the corresponding emulsions.

To date, only the cold gelation, properties and microstructure of the emulsions stabilized by milk proteins (e.g., whey protein and caseins), induced by glucono-δ-lactone (GDL) acidification and $CaCl_2$, have been relatively well investigated (Dickinson, 2012; Rosa, Sala, van Vliet, & van de Velde, 2006; Sok Line et al., 2005; Ye & Taylor, 2009). It is noteworthy that in these emulsion gels, the protein-coated oil droplets not only act as 'active fillers', like that in the heat-set cases (Chen & Dickinson, 1999; Dickinson & Chen, 1999), but also become an integral part of the network, thus greatly reinforcing the gel strength. In contrast, very scarce information is available for the cold-set gelation of protein-stabilized emulsions by transglutaminase (TGase)-induced cross-linking, though the enzymatic technique has been confirmed to effectively induce the gelation of the emulsions stabilized by α_{s1}-casein, soy 11S and 7S globulins, more than 20 years ago (Nio, Motoki, & Takinami, 1986). Dickinson & Yamanoto (1996) investigated the rheological properties of MTGase-induced milk protein (β-lactoglobulin, a major protein in whey proteins, as well as sodium caseinate)-stabilized emulsion gels, and found that the enzyme-set emulsion gels were much less frequency-dependent than the

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equivalent heat-set gels, and the strength of the emulsion gels at much lower protein concentrations was comparable to or even greater than that of the protein gels at much higher protein concentrations (confirming reinforcing effect of oil droplets in the gel network). Kang, Kim, Shin, Woo, and Moon (2003) reported the feasibility to form bovine serum albumin (BSA)-stabilized emulsion gels induced by MTGase, however, in this case, disulfide bond reduction was needed to ensure efficient formation of the emulsion gels. By comparison, sodium caseinate and SPI seem to be much more appropriate protein substrates to form MTGase-set emulsion gels than BSA (Lee et al., 2006), possibly due to the flexible tertiary conformation of these proteins.

In our recent work, we found that the application of MTGase as the coagulant exhibited much higher potential to form SPI-stabilized emulsion gels with higher mechanical strength than that by GDL and $CaCl_2$; it is favorable for the formation of enzyme-set SPI-stabilized emulsion gels, without thermal treatment before and/or after emulsification (Tang, Chen, & Foegeding, 2011). In a more recent work, we further characterized the rheological and water-holding properties and microstructure of these emulsion gels, formed at various oil fractions (ϕ) of 0.2–0.6, and found that their properties and microstructure considerably varied with the applied oil fraction (Yang et al., 2011). The gelling mechanism also varied with the applied ϕ, and at higher ϕ values, it has been considered to be closely related to MTGase-induced aggregation or coagulation of protein-coated oil droplets (Yang et al., 2011).

All the above-mentioned works have been carried out under static or small-deformation conditions. In usual, a relatively long period of enzyme incubation (e.g., several hours) is needed to warrant the formation of fully structured network for the MTGase-induced emulsion gels, even at high ϕ values (e.g., 0.6; Yang et al., 2011). We should keep in mind that the efficiency of enzyme-induced cross-linking between the proteins (including adsorbed proteins at the interface) can be accelerated if a shearing or stirring treatment was applied during the gelation, since it will greatly increase the chance of individual proteins to meet one another. Thus, the gelation of MTGase-induced protein-stabilized emulsions might be improved by application of shear or stirring during the solidification. Manski, van der Goot, and Boom (2007) investigated the influence of shear during MTGase-induced gelation of sodium caseinate (10–30 g/100 g)-water-fat (15 mL/100 mL) systems, and found that adding fat prior to solidification and mixing (in a mixer) resulted in strong homogenous emulsion gels. This work implies that the application of shear simultaneously during the enzymatic cross-linking can produce the emulsion gels with good quality (e.g., stronger than the systems without fat). On the other hand, it has been confirmed that the application of MTGase-induced cross-linking enhances the viscosity of stirred-type yogurt (an important commercial milk product; Jaros, Heidig, & Rohm, 2007), though stirred yogurt is usually post-processed by meaning of shearing (after set-type yogurt is formed).

The main aim of this study was to highlight the influence of mechanical shearing or stirring during incubation on the rheological and water-holding properties and microstructure of SPI-stabilized emulsion gels, formed at a specific protein concentration (6 g/100 mL) and various ϕ of 0.2–0.6. Two modes of stirring, continuous and intermittent, were chosen for the comparison with the unstirred control. The pattern of interactive forces involved in the gel network was also characterized.

2. Materials and methods

2.1. Materials

SPI was prepared from defatted soybean meal, provided by Shandong Yuwang Industrial and Commercial Co. Ltd. (China), as described in our previous work (Tang et al., 2011). The protein content of this SPI was 92.9 g/100 g (wet basis) as determined by the Dumas method. Rhodamine B (analytical grade) was purchased from Beijing DINGGUO Biological Technology Co. Ltd. (China), and Nile blue from Shanghai BAOAO Biological Technology Co. Ltd. (China). Commercial microbial transglutaminase (MTGase), produced by *Streptomyces hygroscopicus*, was purchased from TAIXIN YIMIN Fine Chemical Industry Co. Ltd. (Jiangsu province, China). The preparation of the stock enzyme solution and determination of its enzyme activity (units (U) mL^{-1}; by a colorimetric procedure using N_{α}-CBZ-GLN-GLY as the substrate and L-glutamic acid γ-monohydroxamate as the standard) were the same as in our previous work (Tang et al., 2011). Soy oil was purchased from the local supermarket (Guangzhou, China). All other chemicals were of analytical grade.

2.2. SPI emulsion preparation

SPI was dispersed in distilled water with 0.04 g/100 mL of sodium azide and stirred at room temperature for 2 h. SPI dispersions were adjusted to pH 7.0 with 0.2 M NaOH and kept overnight at 4 °C to allow the proteins fully hydrated. For the SPI emulsions, the protein concentration in continuous phase and ϕ were 6.0 g/100 g and 0.2–0.6, respectively. The SPI dispersions were mixed with soy oil using a high-speed disperser (Ultra-Turrax T25, IKA-Labortechnik, Staufen, Germany) at 15,000 rpm for 2 min to obtain coarse emulsions. The coarse emulsions were further homogenized at 40 MPa for one pass using a micro-fluidiser (M110EH model, Microfluidics Co., Newton, MA) to produce the original emulsions for the next experiments. All of the obtained original emulsions were stored at 4 °C before use.

2.3. Preparation of emulsion gels

Two sets of stirred-type emulsion gels at ϕ values of 0.2–0.6 were manufactured: one with continuous stirring mode and another with intermittent mode. After pre-incubating in a water bath at 37 °C for 10 min, a total amount (e.g., 50 mL) of each original emulsion in a beaker (with volume of 200 mL) was mixed with MTGase at an enzyme concentration of 10 U per gram (U g^{-1}) of protein in the continuous phase. The resultant mixtures were gently stirred with help of an RW20 Digital stirrer (with propeller 10M/M-D15; IKA® Works Guangzhou) at 220 rpm during incubation periods (up to 30 min), with continuous or intermittent modes. The intermittent mode was carried out as follows: the mixtures were stirred at 220 rpm every 2 min, and then left unstirred for another 3 min. At specific incubation periods of time (e.g., 10, 20 or 30 min), aliquots of enzyme-treated emulsions were taken out, and immediately subject to the following analyses, or refrigerated at 4 °C. Unstirred emulsion gels (control) were prepared according to the same process, but without stirring. All the incubation experiments were conducted at 37 °C, unless stated otherwise.

2.4. Evaluation of emulsion gel characteristics

2.4.1. Steady shear viscosities

Steady shear viscosities of the emulsions or emulsion gels were characterized using an AR550 Rheometer (TA Instruments-Waters LLC Co., New Castle, DE, USA) with parallel plates ($d = 30.0$ mm), at 25 °C. The gap between two plates was set to 1.0 mm. The temperature of samples was monitored through the lower plate. Excess sample out of the plates was trimmed off, and a thin layer of mineral oil applied to exposed free edges of the sample to prevent moisture loss of the samples. After 10 min of equilibrium, the