LWT - Food Science and Technology 51 (2013) 23–29

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Strengthening soy protein hydrogels filled with protein-coated montmorillonite nanoclay by glutaraldehyde crosslinking

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ARTICLE INFO

Article history:
Received 13 July 2012
Received in revised form
9 October 2012
Accepted 19 October 2012

Keywords:
Bio-nanocomposite
Dynamic viscoelasticity
Intercalated montmorillonite
Soy protein
Chemical crosslinking
Glutaraldehyde

ABSTRACT

To strengthen interactions in bio-nanocomposite systems, montmorillonite (MMT) was intercalated by surface-coating with soy protein before being incorporated within soy protein dispersions for cross-linking by glutaraldehyde (GA). Dynamic small strain tests were utilized as a non-destructive method to reveal the improvement of network structure in the bio-nanocomposite system. The storage modulus (G') and loss modulus (G'') gradually increased with increasing GA concentration during isothermal treatment at 23, 60 and 90 °C. The incorporation of intercalated MMT resulted in an order of magnitude increase in the moduli. The moduli were higher at pH 5.5 than those at pH 6.5 and 10.0. At a GA concentration equal to 10 g/100 g mass of soy protein, G' and G'' in the absence of MMT increased with an increase in temperature, while those in the presence of intercalated MMT showed the opposite trend due to restructuring of aggregated MMT. The present results confirmed that the mechanical strength of bio-nanocomposite systems can be significantly improved by first intercalating MMT with protein and subsequently cross-linking with the continuous phase protein molecules. The established chemical cross-linking method and conditions can be beneficial for preparing bio-nanocomposite materials with enhanced mechanical properties.

1. Introduction

Bio-plastics, due to the renewability and bio-degradability, is attractive for substituting petroleum-derived plastics and has been growing by ca. 17–20% annually since 2006 (Anonymous, 2011). Bio-plastic packaging is adopted by companies like Coca-Cola, Frito Lay and Walmart (Marsh & Bugusu, 2007), with poly(lactic acid) being the major biopolymer (Anonymous, 2011). The need of sustainable material supplies calls for other renewable biopolymers such as soy protein that is an abundant plant-sourced biopolymer. Soy proteins have good film-forming properties but the produced films have poor water vapour permeability and less than optimal mechanical properties (Kumar, Sandeep, Alavi, Truong, & Gorga, 2010a, 2010b). Dispersion of nanoparticles in a biopolymer matrix to produce bio-nanocomposites has been proposed as a possible approach to improve film properties (Arora & Padua, 2010; de Azeredo, 2009; Dang, Lu, Yu, Sun, & Yuan, 2010).

Soy protein isolate (SPI)/montmorillonite (MMT) nanocomposite films have been reported for reduced permeability of water vapour and oxygen and increased elastic modulus and tensile strength than controls without MMT (Lee & Kim, 2010). For these nanocomposite systems, achieving intercalation/exfoliation of layered nanoclay is critical for improving film properties. In one study, a melt extrusion technique was applied to achieve intercalation/exfoliation of MMT, and the resultant SPI/MMT cast films were greatly improved for tensile strength, storage modulus, glass transition temperature, water barrier property, and thermal stability (Kumar et al., 2010b). Mechanical properties of some of these SPI/MMT nanocomposite films were comparable to several petro-plastics that are currently used in food packaging applications (Kumar et al., 2010b). In another study, MMT was first intercalated by SPI before being used to prepare SPI-based nanocomposite bio-plastics that showed highly exfoliated MMT, corresponding to significantly improved mechanical strength and thermal stability (Chen & Zhang, 2006). However, the water vapour permeability of SPI-based nanocomposite films, because of the hydrophilic nature of soy protein, still requires substantial improvement to match that of petrochemical-based plastics (Kumar et al., 2010b).

Cross-linkers are commonly used to modify physicochemical properties of biopolymer-based materials. Enzymatic and chemical cross-linking methods have been studied to enhance properties of soy protein hydrogels and films (Caillard, Remondetto, & Subirade, 2009, 2010; de Carvalho & Grosso, 2004; Gan, Latiff, Cheng, & Easa,

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http://dx.doi.org/10.1016/j.lwt.2012.10.012

2009; Jiang, Tang, Wen, Li, & Yang, 2007; Yildirim & Hettiarachchy, 1998). Chemical cross-linkers containing aldehyde groups such as glutaraldehyde (GA), formaldehyde, and glyoxal have been widely utilized to cross-link proteins (Gerrard, Brown, & Fayle, 2003; Gerrard et al., 2005). This category of reactions is called “Maillard-type” crosslinking (Caillard et al., 2009, 2010) because covalent amide bonds are formed between two lysine residues on two separate protein molecules and the aldehyde groups on the cross-linker (Gerrard et al., 2003). Soy proteins cross-linked by GA had lowered solubility, reduced emulsifying properties, and improved ability in stabilizing foams (Gerrard et al., 2003). GA-treated SPI films demonstrated enhanced tensile strength and elongation at break, more significant at a higher GA concentration (Park, Bae, & Rhee, 2000). Crosslinking soy protein concentrate with GA improved the processability of obtained resin, and the subsequently manufactured flax fabric-reinforced composites demonstrated increased tensile properties (Chabba & Netravali, 2005).

Although bio-nanocomposites have been studied extensively, there is currently no report of integrating intercalation of MMT and crosslinking of biopolymer matrix and that of nanofillers. In this work, MMT was first coated with soy protein by adopting an intercalation method (Chen & Zhang, 2006), and the intercalated MMT was dispersed in soy protein dispersions for crosslinking. In our preliminary tests, formaldehyde and glyoxal were observed to be less effective than GA in improving elasticity of soy protein hydrogels, consistent with the literature when these cross-linkers were applied to cross-link proteins (Gerrard et al., 2003, 2005). The objective of this study was to improve the mechanical strength of composite hydrogels with constant soy protein and MMT concentrations by studying crosslinking variables of GA concentration, pH, and temperature. Small-amplitude oscillatory shear tests were adopted as a non-destructive method to study the dynamics of structural development during crosslinking.

2. Materials and methods

2.1. Materials

Na^+-montmorillonite (Cloisite® Na^+ Nanoclay) was a product of Southern Clay Products, Inc. (Gonzales, TX). Defatted soy flour was obtained from MP Biomedicals, LLC (Solon, OH). GA was purchased from Sigma–Aldrich Corp. (St. Louis, MO) and used as received. Mineral oil and other chemicals were analytical grade products from Fisher Scientific (Fair Lawn, NJ) or Sigma–Aldrich Corp. (St. Louis, MO).

2.2. Extraction of soy protein

Soy protein used in the present study was extracted from defatted soy flour using a modified protocol for preparation of SPI (L’Hocine, Boye, & Arcand, 2006). Soy flour was further reduced for particle size by using a coffee grinder (Hamilton Beach Proctor-Silex, Inc., Southern Pines, NC). The flour was then suspended at a solid:liquid mass ratio of 1:10 in 2 L deionized water, adjusted to pH 9.0 using 4 mol/L NaOH, vigorously stirred at 48 °C for 3 h, and centrifuged at 3778 × *g* for 30 min (Sorvall RC 5B Plus, Newtown, CT). The supernatant was transferred and adjusted to pH 4.5 using 6 mol/L HCl to precipitate protein. After centrifugation, the precipitate was collected, suspended in deionized water, adjusted to pH 9.0, and re-precipitated at pH 4.5, which was repeated twice to improve protein purity. The final protein precipitate was suspended in deionized water, adjusted to pH 6.5 using 4 mol/L NaOH, and spray-dried using a B-290 Mini Spray Dryer (BÜCHI Labortechnik AG, Postfach, Switzerland) at a feed rate of 6.67 mL/min, a compressed air line with a pressure of 600 kPa and an air flow rate of 35 m^3/h, and inlet and outlet temperatures of 150 and 55–65 °C, respectively. The spray-dried soy protein powder (SPP) was collected and stored at −20 °C until use. The SPP as prepared had a protein content of 83.85 g/100 g, based on nitrogen analysis, and a solids-content of 92.11 g/100 g, corresponding to a dry-basis protein content of 91.03 g/100 g (Jin, 2012).

2.3. Intercalation of MMT with soy protein

MMT was intercalated with soy protein using a solution intercalation protocol (Chen & Zhang, 2006). Dispersions of 0.5 g/100 mL SPP and 2 g/100 mL MMT were separately prepared in 10 mmol/L NaH_2PO_4 buffer, adjusted to pH 9.0 using 1 mol/L NaOH, heated at 60 °C, and stirred at 300 rpm for 30 min using a magnetic stirring hot plate. The MMT dispersion was slowly added to an equal volume of the SPP dispersion, while being vigorously agitated at 1000 rpm and maintained at 60 °C. The resultant slurry was agitated for 3 h at 60 °C.

2.4. Rheological analyses

For samples containing MMT, the above slurry after intercalation and cooling to room temperature (21 °C) was added with 8 g/100 mL SPP and hydrated overnight. Samples without MMT were prepared by directly hydrating the same amount of SPP in 10 mmol/L NaH_2PO_4 buffer overnight. The slurries were adjusted to pH 5.5, 6.5 or 10.0 using 1 mol/L HCl or NaOH and added with GA at an amount equivalent to 2.0, 4.0, or 10.0 g/100 g mass of SPP immediately before the rheological test.

Oscillatory small strain analyses were performed using an AR2000 rheometer (TA Instruments, New Castle, DE). A sample was placed in a concentric cylinder geometry consisting of a bob with an outer diameter of 28 mm and a cup with an inner diameter of 30 mm. After removing excess sample, the top of sample was covered with a thin layer of mineral oil to minimize moisture evaporation. Developments of the storage modulus (G') and loss modulus (G'') were acquired during isothermal incubation at 23, 60, or 90 °C for 5 h using a strain of 0.01 and a frequency of 1 Hz. A strain sweep test at 23 °C was performed after cross-linking, at a strain range of 0.001–1 and a frequency of 1 Hz. All experiments were performed in duplicate and the averages were reported.

3. Results and discussion

3.1. Representative rheograms

Figs. 1–3 show developments of G' and G'' during 5-h incubation at 23, 60, and 90 °C for samples added with the lowest level of GA – 2.0 g/100 g mass of SPP. For dispersions without MMT, both G' and G'' increased slowly during incubation at 23 °C, indicating the progress of crosslinking, but all samples remained flowable, corresponding to G' and G'' both smaller than 1 Pa. The G' of samples at pH 6.5 and 10.0 was lower than G'', while there was a cross-over of G' and G'' for the sample at pH 5.5. In contrast, G' was already greater than G'' at the beginning of data collection for the corresponding samples with MMT. At 60 and 90 °C (Figs. 2 and 3), the pH 5.5 samples without MMT demonstrated significantly higher G' than that at 23 °C, those at pH 6.5 remained non-gelling, while the pH 10.0 sample formed a weak gel after incubation at 90 °C. Figs. 1–3 show that rheological properties of samples are a strong function of temperature, pH, and presence of MMT. The impacts of these variables, in addition to GA concentration, are presented in the following sections by comparing the data at the end point of incubation.