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Viscoelastic properties and fractal analysis of acid-induced SPI gels at different ionic strength

Chong-hao Bi[a,1], Dong Li[a,1], Li-jun Wang[b,*], Benu Adhikari[c]

[a] College of Engineering, China Agricultural University, P.O. Box 50, 17 Qinghua Donglu, Beijing 100083, China
[b] College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China
[c] School of Health Sciences, University of Ballarat, VIC 3353, Australia

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ABSTRACT

The viscoelastic property and scaling behavior of acid (glucono-δ-lactone)-induced soy protein isolate (SPI) gels were investigated at various ionic strengths (0–800 mM) and five protein concentrations ranging between 4% and 8% (w/w). The infinite storage modulus (G'_∞) and the gelation start time (t_g) which indicate the progress of gelation process exhibited strong ionic strength dependence. The storage modulus and critical strain were found to exhibit a power-law relationship with protein concentration. Rheological analysis and confocal laser scanning microscopy (CLSM) analysis were applied to estimate the fractal dimensions (D_f) of the gels and the values were found to vary between 2.319 and 2.729. The comparison of the rheological methods and the CLSM image analysis method showed that the Shih, Shih, Kim, Liu, and Aksay (1990) model was better suited in estimating the D_f value of acid-induced SPI gel system.

1. Introduction

The control of formation of acid-induced soy protein isolate (SPI) gels for industrial applications calls for a better understanding of the relationship between the microstructure of gels and their macroscopic rheological properties (Ould Eleya, Ko, & Gunasekaran, 2004). The fractal analysis is an effective way to link the microstructure with the macroscopic properties of gels. Fractal structure provides an explanation of the micro-structure of macromolecules (Iannaccone & Khokha, 1996) and is usually applied to describe the complexity of branched molecules (Wang, Li, Wang, Wu, & Özkan, 2011). It can also reflect on the type of the organization of the branches (Wang et al., 2011). The fractal dimension, referred to as D_f, is commonly used to describe the complexity prevailing in gel systems. A number of studies have suggested that the aggregation of protein results into structurally disordered protein particles. These particles show a random mass-fractal on a scale that is far larger than the particle itself (Chodankar, Aswal, Hassan, & Wagh, 2010; Kuhn, Cavallieri, & da Cunha, 2010; Nagano & Tokita, 2011).

To investigate the fractal structure of the gel system and to calculate the value of fractal dimension, scientists have developed a number of methods. These methods can be classified into direct method which is based on confocal laser scanning microscopy, small-angle X-ray scattering, dynamic light scattering; and indirect method which is based on rheological and acoustic properties (Hagiwara, Kumagai, & Nakamura, 1996; Hagiwara, Kumagai, Matsunaga, & Nakamura, 1997; Matsumoto, Kawai, & Masuda, 1992; Wu, Xie, Lattuada, & Morbidelli, 2005).

The rheological tests provide an insight on the microstructure of the gel based on the macromechanical properties that are easy to measure, thus, are commonly applied in fractal analysis. Various scaling models have been developed and used in protein and colloidal gels (Bremer, Bijsterbosch, Schrijvers, & van Vliet, 1990; Mellema, van Vliet, & van Opheudsen, 2002; Shih, Shih, Kim, Liu, & Aksay, 1990; Wu & Morbidelli, 2001). Some models correlate the rheological properties of the gels with the size of gel aggregates, the volume fraction of particles and fractal dimension of the aggregates (Shih et al., 1990; Wu & Morbidelli, 2001).

Among these models, the model developed by Shih et al. (1990) allows the estimation of the fractal dimension solely based on the rheological properties of the gel system. This model calculates the fractal dimension values using storage modulus and critical strain of a gel. This model classifies the gel systems into two regimes: strong-link regime (the intermolecular (or interfloc) links are stronger than the intramolecular or intrafloc links) and weak-link regime (the intermolecular links are weaker than the intramolecular links). Based on the characteristics of a gel (whether it forms strong-link regime or weak-link regime), suitable equation can be chosen to represent or predict the experimental

* Corresponding author. Tel.: +86 10 62737351; fax: +86 10 62737351.
E-mail address: wlj@cau.edu.cn (L.-j. Wang).
[1] These authors contributed equally to this work.

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Nomenclature

C	SPI concentration (w/w, %)
D	Euclidean dimension (dimensionless)
D_f	fractal dimension (dimensionless)
G'	storage modulus (Pa)
G'_i	initial G' value (Pa)
G'_∞	estimated value of G' at infinite time (Pa)
k	rate empirical parameter (dimensionless)
m	power law exponent (dimensionless)
n	power law exponent (dimensionless)
N_ε	number of boxes at ε scale (dimensionless)
t	time, s
t_g	time when gelation begins (s)
x	fractal dimension of floc backbone (dimensionless)
α	parameter in Wu model (dimensionless)
β	parameter in Wu model (dimensionless)
γ_c	critical strain (%)
ε	corresponding scale (dimensionless)
φ	particle concentration (%)

data. The Shih et al.'s model has been used in soybean globulin, caseinate, β-lactoglobulin, whey protein isolate and flaxseed gum gels (Hagiwara et al., 1997; Pouzot, Nicolai, Durand, & Benyahia, 2004; Wang et al., 2011). The estimated fractal dimension values in these gels were reported to be between 1.41 and 2.82.

Wu and Morbidelli (2001) modified Shih et al.'s model by introducing three parameters α, β and x to define the type of the link in the gel system. Based on the α value, the gels can be classified into three regimes: the strong-link, the weak-link and the transition regimes. Wu and Morbidelli's model was also applied to soybean globulin, bovine serum albumin and flaxseed gum gels (Caillard, Remondetto, & Subirade, 2010; Hagiwara, Kumagai, & Nakamura, 1998; Wang et al., 2011). The estimated fractal dimension values in these gels were reported to be between 1.42 and 2.87.

Acid gelation of SPI is important in food processing industry, such as lactone tofu, and can affect the texture and acceptability of foods. Functional properties of foods such as visco-elasticity and water holding capacity may also get altered during the acid gelation process (Hu, Stanley, Goff, Davidson, & LeMaguer, 1992; Paraskevopoulou & Kiosseoglou, 1997). Soy protein isolate is commonly used as an ingredient in food formulations in order to impart better mechanical properties. The process of making SPI gels involves two steps: heating of soy protein isolate at neutral pH followed by heating of the denatured protein solution at acidic pH after acidification with glucono-δ-lactone (GDL) (Campbell, Gu, Dewar, & Euston, 2009). The ionic strength is known to influence the gel forming characteristics of soy protein isolate (Ikeda, Foegeding, & Hagiwara, 1999; Izumi, Kikuta, Sakai, & Takezawa 1996). Therefore, the microstructure of SPI gels is expected to undergo some changes when the salts are added and the ionic strength (i.e. the salt concentration) is allowed to vary. Commonly, a random aggregation of protein in gels leads to the formation of non-transparent particulate network, especially when subjected to a high ionic strength (Ikeda, Foegeding, & Hagiwara, 1999).

To the best of our knowledge, no research has been undertaken on the fractal structure of acid-induced SPI gels. In this study, the scaling behavior and the fractal dimension of acid-induced SPI gels were investigated using both rheological and the confocal laser scanning microscopy (CLSM) methods. The fractal dimension values obtained from rheological (and associated models) and CLSM image analysis methods were compared. In addition, the effect of ionic strength on the gelling process and the viscoelastic properties of the acid-induced SPI gels were also investigated.

2. Materials and methods

2.1. Materials

Commercial Soy protein isolate (MSZ-90B) (containing 35% of 7S, 52% of 11S) was obtained from Messenger Biotechnology Co. Ltd. (Beijing, China) as a gift. GDL (glucono-δ-lactone) was obtained from Beijing Wohai Technology Ltd. Rhodamine B (BS) was purchased from Beijing Yinghai fine chemical industry. Sodium chloride (AR) was purchased from Beijing Chemical works. All materials are used as supplied.

2.2. Methods

2.2.1. Sample preparation

For rheological tests, the SPI was dissolved in deionized water (pH = 6.7) using a magnetic stirrer (500 r/min) at 25 °C for 20 min in order to prepare 5–8% (w/w) SPI stock solutions. Sodium chloride was then added into the SPI solution to attain ionic strength values between 200 mM and 1000 mM. The stock solutions were then heated at 90 °C for 20 min to fully denature the protein. After cooling to 20 °C, these samples were stored overnight in a refrigerator maintained at 4 °C. For CLSM tests, the stock solutions were also prepared following the same procedure.

2.2.2. Rheological tests

Rheological properties of these acid-induced SPI gels were measured using AR2000ex rheometer (TA Instruments Ltd., Crawley, UK). Aluminum parallel plate (40 mm diameter, 1 mm gap) was chosen to follow the gelling process and measure the gel strength. The temperature was controlled using a peltier unit attached with a water circulation system. GDL (2 g) was added as a powder to 100 ml of stock solution just before the rheological tests. The samples were transferred between parallel plates and the gel was allowed to form in situ at 60 °C. The storage modulus (G') and loss modulus (G'') values were recorded every 30 s during the gel formation process using a constant frequency of 1 Hz and a strain of 1%. A thin layer of silicone oil was applied on the surface of the samples in order to prevent the loss of water due to evaporation. The linear viscoelastic region (LVR) was determined for each sample using strain sweep tests at 1 Hz (data not shown). The viscoelastic properties (G' and G'') of the gels were determined within the LVR.

The end of the gel formation process was considered to be reached when the increase in two consecutive G' values was less than 1%. Once the gelling process was completed, the fully formed gels were subjected to strain sweep measurement, in which the strain was varied from 0.001 to 0.1. The data were recorded at a rate of 20 points per decade. The initial value (G'_i) was calculated as an average value of G'_i at the strain values between 0.001 and 0.01. A critical point was defined as the point at which the G' value reduced to 95% of G'_i (Fig. 1, inset) (Wang et al., 2011). The strain at the critical point was determined and defined as the critical strain (γ_c). The fractal dimensions of the gels were calculated using the G'_i and γ_c.

2.2.3. Confocal laser scanning microscope tests

In all cases, a LEICA TCS SP5 II confocal laser scanning microscope (CLSM) was used to investigate the structural features of acid-induced SPI gels and to determine their fractal parameters. The CLSM was equipped with an inverted microscope (model Leica DMI6000) and He–Ne visible light lasers (Leica Microsystems (CMS) GmbH., Mannheim, Germany). The objective lens used was a 40 × 0.85 HC PL APO lens. Before gelation, the SPI gels were stained with an aqueous solution of Rhodamine B (1 ml of a 0.01% (w/w) Rhodamine B + 10 ml of sample) and then allowed to gel inside a sealed concave slide in a 60 °C water bath for 30 min. The