Rheological Study and Fractal Analysis of Flaxseed Gum-Whey Protein Isolate Gels

Yong Wang and Xuqi Lin
COFCO Nutrition and Health Research Institute, Beijing, China
COFCO Tunhe Co.,Ltd., Urumuchi, Xinjiang Uygur Autonomous Region, China
Email: {wang-yong, linxuqi}@cofco.com

Li-Jun Wang and Dong Li
China Agricultural University, Beijing, China
Email: {wlj, dongli}@cau.edu.cn

***Abstract*—The Rheological properties and fractal dimensions of flaxseed gum-whey protein isolate (WPI) gels were analyzed in flaxseed gum concentration range of 1%-2% in this study. The Rheological properties of the mixture were significantly influenced by the flaxseed gum concentration. The gel strength increased with the increase of gum concentration. Two models were used in this study, and the calculated fractal dimension of the flaxseed gum-WPI mixture gels were 2.10-2.44 or 2.12-2.63, based on the model selected and the ionic strength applied. These results were in agreement with the fractal dimensions of other hydrocolloid gels. These results would help to understand the microstructure of the flaxseed gum-WPI gels and the influence of gum concentration on that. However, the difference in results between the two models implied that further study is needed to find suitable model for gum-protein mixtures.**

***Index Terms*—fractal dimension, rheology, scaling model, flaxseed gum, WPI, Gel .**

I. Introduction

Fractal structure is an illustration of the initial structure of macro molecules [1]. The fractal structure was commonly used to describe the branches complexity of the molecules, and also reflect the type of organization of those branches. The fractal dimension, referred as d_f, is introduced to describe this kind of complexity. Fractal analysis is now widely used in the studies on the micro structure of biomacromolecules, such as proteins, carbohydrate polysaccharides, and DNA [2].

Several methods have been used to study the fractal structure and calculate the d_f value. Those methods could basically be classified as direct methods (e. g. confocal scanning laser microscopy, dynamic light scattering, small-angle X-ray scattering), and indirect methods (e.g. Rheological and acoustic methods) [3]-[5]. Rheological methods are commonly used in fractal analysis on gels, as it reflects the gel microstructure based on the macro mechanical properties, which is easily measured. Several Rheological models have been developed to calculate the d_f value of gels.

Cates has developed a model related complex viscosity with the fractal dimension, in the absence of excluded-volume and entanglement effects [6]. This model could be widely applied to the statics and dynamics of a large range of physically interesting fractals, such as gelation clusters, branched polymers, and flexible types of fractal aggregates. Bremer et al. have developed a model to describe gel formation and structure using the concept of fractal geometry [7]. The model was in good agreement with the dependence of the storage modulus of the gels on the volume fraction, thus was successfully used in fractal analysis of acid casein gels.

Shih et al. have developed a model to calculate the fractal dimension based on the critical strain and the corresponding storage modulus [8]. The gels were characterized into strong-link regime and weak-link regime. So that suitable models were chosen before applying the data to the model. This model has been applied to whey protein isolate, caseinate gel, soybean globulin gel, and β-lactoglobulin gel. The calculated d_f of those gels were within 1.5-2.82 [3]. Wu & Morbidelli further developed the model of Shih et al. by introducing new parameters α, β, and x, to describe the link type of the gels [5]. The gels were then assorted into three group based on the α value, the strong-link regime, the weak-link regime, and the transition regime. This model has been successfully used in the analysis of whey protein isolate, sodium caseinate aggregates, soybean protein, β-lactoglobulin gel, and bovine serum albumin gel, the d_f of which were within 1.73-2.87 [9], [10].

Flaxseed gum is natural carbohydrate polysaccharide extracted from flaxseed (*Linum usitatissimum*), which makes up about 8% of the seed weight [11]. Flaxseed gum has shown good performance in viscosity, emulsion property, and gelling properties, thus has the potential to be used in the food industry as thickener, emulsifier, stabilizer, etc. [12]-[14]. The ionic strength is known to influence the gelling properties of flaxseed gum [14]. Thus the flaxseed gum gels microstructure might be altered by the ions. Generally, gels tend to be formed with linear aggregates with a low degree of branching,

Manuscript received May 15, 2013; revised July 13, 2013.

doi: 10.12720/jomb.2.3.201-206

while at a high ionic strength, a random aggregation occurs, leading to the formation of an opaque particulate network [15].

To the best of our knowledge, there is no study on the fractal analysis of the protein and polysaccharide mixture. Thus this paper would focus on the fractal structure of the mixture gel of whey protein isolate and flaxseed gum. The Rheological properties of the mixture gel were studied and modeled to achieve the fractal dimension. This would help to understand the fractal structure of the mixture gel of WPI and flaxseed gum. Moreover, it could also supply information for other studies regarding the mixture of protein and polysaccharide, thus better understand the mechanism of advantage of this kind of mixture.

II. MATERIAL AND METHODS

A. Materials

The flaxseed gum was extracted and dried using the same method as previous study [13].

Flaxseed (100 g) was washed in water for 1 min to remove the surface dust, and then mixed with 900 mL deionized water. The flaxseed and water were then stirred for 5 h at a speed of 300 r/min, in a 60 ^{o}C water bath, according to the method of Cui [16]. The extracted flaxseed gum solution was filtered through 40-mesh screen. After that extracted flaxseed gum solution was precipitated with two volumes of 95% ethanol, collected by centrifugation at 3000 r/min for 10 min using an LG10-2.4 A machine (Beijing Medical Centrifuge Corporation, Beijing, China), according to the method of Cui et al. with some modifications on drying method [17]. The precipitated flaxseed gum was then dried in a hot air oven at 80 ^{o}C for 4 h.

The whey protein isolate was purchased from local market (protein concentration of 90%).

B. Preparation of the Solution

The flaxseed gum was dissolved in deionized water by adding the flaxseed gum powder gradually into the water, to make the 1% flaxseed gum solution. The WPI was then mixed with suitable amount of water and 1% flaxseed gum, to make the final solution has both desired WPI concentration (10%, 12.5%, 15%, 20%, w/v) and flaxseed gum concentration (0%, 0.1%, 0.5%, w/v).

C. Rheological Tests

Rheological properties of the flaxseed gum solutions were measured using AR2000ex rheometer (TA Instruments Ltd., Crawley, UK). An aluminum parallel plate geometry (40 mm diameter, 1 mm gap) was chosen for the gel strength measurements. A thin layer of low viscosity silicone oil was applied on the surface of the samples in order to prevent evaporation. The linear viscoelastic region was determined for each sample through strain sweeps at 1 Hz (data not shown). Viscoelastic properties (storage modulus, G', and loss modulus, G'') of the solutions were determined within the linear viscoelastic region.

The solutions were heated to 90 ^{o}C and held at this temperature for 5 minutes. The solutions were subsequently cooled to 25 ^{o}C and held for 30 minutes for the gel formation. The G' and G'' values were recorded during cooling using an angular frequency of 6.283 rad/s.

Afterward, the gels were subjected to the strain sweep test, in which the strain was varied from 0.001-0.2. The data was collected at 40 points per decade. The average value of G' from 0.001-0.01 strain was calculated as the initial value (G_0). And the point where the G' reached to 95% of G_0 was taken as the critical point of the gel (as shown in Fig. 1). The strain at that point was defined as critical strain (γ_0). The G' and the strain values of critical point were used in the calculation of fractal dimension.

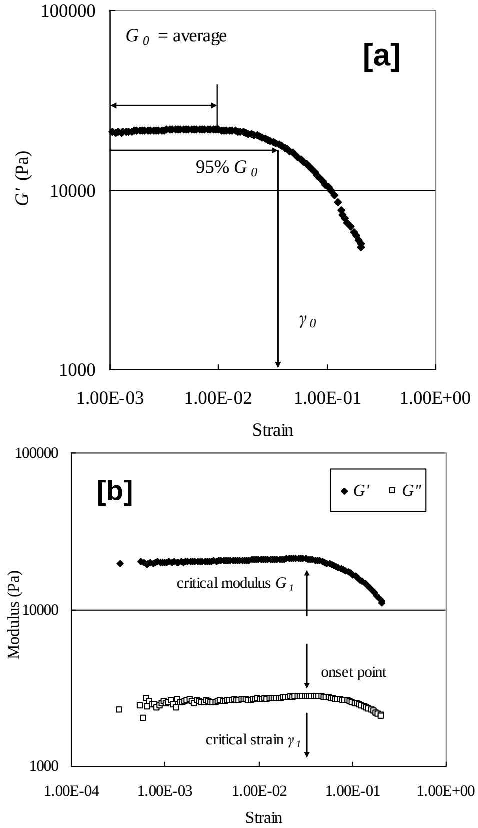


Figure 1. Illustration of the location of onset point, critical strain, and critical modulus.

D. Theory

A scaling model has been developed by Shih et al. to relate the storage modulus G' and the critical strain γ_0 to the volume fraction ϕ for a colloidal gel [8]. Based on the strength of the inter- and intra-floc links, two regimes were defined: strong-link regime (inter-floc links are stronger than intra-floc links) and weak-link regime (inter-floc links are weaker than intra-floc links).

In the strong-link regime:

$$G' \propto \phi^{(d+x)/(d-d_f)} \quad (1)$$

$$\gamma_0 \propto \phi^{-(1+x)/(d-d_f)} \quad (2)$$

In the weak-link regime:

$$G' \propto \phi^{1/(d-d_f)} \quad (3)$$

$$\gamma_0 \propto \phi^{1/(d-d_f)} \quad (4)$$

where d is the Euclidean dimension, d_f is the fractal dimension of the flocs and x is the fractal dimension of the floc backbone ($1 \leq x \leq d_f$).

Wu & Morbidelli have developed the model above to a new model which related G' and γ_0 of the gel to volume fraction of primary particles [5]. A constant α (where $0 \leq \alpha \leq 1$) was introduced to account for the elastic contributions of both inter- and intra-floc links. It allows identifying the gelation regimes prevailing in the system, and serves as indicator of the relative importance of the two links type.

$$G' \propto \phi^{\beta/(d-d_f)} \quad (5)$$

$$\gamma_0 \propto \phi^{(d-\beta-1)/(d-d_f)} \quad (6)$$

$$\beta = (d-2) + (2+x)(1-\alpha) \quad (7)$$

where x is the fractal dimension of the floc backbone ($1 \leq x \leq d_f$) as in the Shih et al. model.

E. Determination of Fractal Dimension

Two methods were used to determine the fractal dimension in this study.

The volume fraction of particles (ϕ) in the gels was assumed to be proportional to the gum concentration (C). Fractal dimension values of flaxseed gum gels were calculated using the slope values of log G' versus log C and of log critical strain γ_0 versus log C, according to the models listed above (Eqs. (3), (4), (5) and (6)).

III. Results and Discussions

A. Gelation Process of Flaxseed Gum

The effects of concentration on the gelation process of WPI were shown in Fig. 2. The gelation increases rapidly and was mostly finished in the first 200 s. After that the storage modulus increases slowly with the time. Also, the storage modulus increases with the increasing of concentration. The same trends were also observed when flaxseed gum concentration was added with 0.1% to 0.5% (data not shown). Similar gelation trend and concentration effect were also found in gellan gum and xanthan gum.

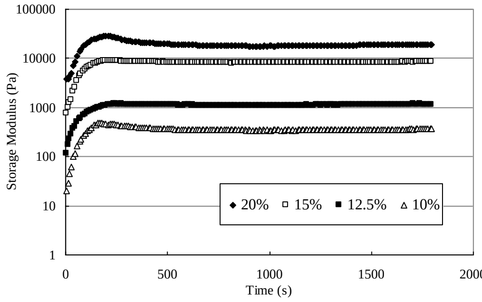


Figure 2. Gelling process of the whey protein isolate at different concentration.

B. Strain Dependent of G'

Changes in storage modulus of WPI with strain was presented in Fig. 3, when no flaxseed gum was added. All the samples show similar trends. Firstly the storage modului are almost constant and then suddenly decrease as strain increase. The sudden decrease in G' indicated breaking of bonds within the gel network and a transition from a linear to a non-linear behavior. The flaxseed gum gels with WPI have similar patterns with the gels shown in Fig. 3 (results not shown).

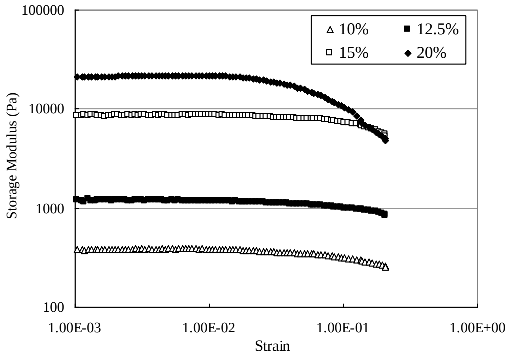


Figure 3. Storage modulus-strain profile of WPI at zero gum concentration and different concentration.

C. Effects of Gum Concentration on G'

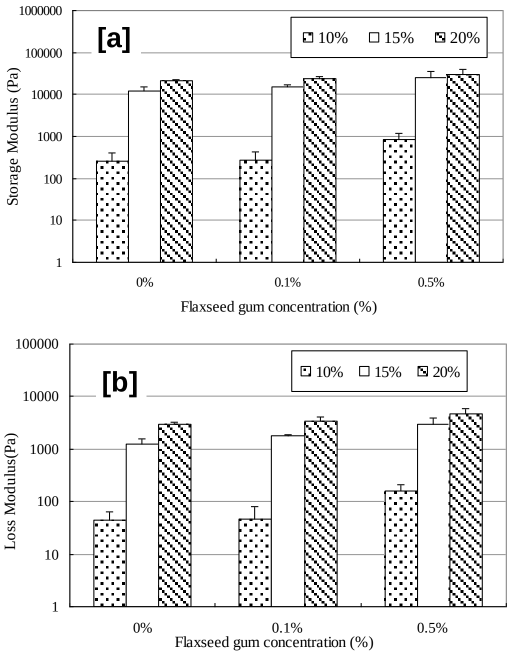


Figure 4. Effects of gum concentration on the storage modulus and loss modulus of WPI.

Fig. 4. The G' values increase with the gum concentration increase. It reflects that the increase of gel strength of WPI could be achieved by adding suitable amount of gum. Aggregation becomes increasingly random and gel rigidity decreases [15]. Similar trends of ionic strength of $CaCl_2$ were also reported in previous study, where the G' values first increased to the maximum around 0.3% (w/w) then decreased [14]. The storage modulus values of 0.8% gellan gum gel and 0.8%

κ-carrageenan gum gel were also dramatically increased by adding 0.1%-0.5% NaCl [18].

D. Scaling Behavior of Flaxseed Gum Gels

The scaling behaviors of strain of flaxseed gum-WPI gels as a function of gum concentration are shown in Fig. 5. For all the gum concentration in this study, the critical strain γ_0 of flaxseed gum-WPI gels shows a power-law relationship, i.e. $\gamma_0 \sim C^m$, where m is the power-law exponent. The m values for all the gels are negative, as shown in Table I. The m values were strongly influenced by the gum concentration. The highest value was obtained when gum concentration was 0% mM (m=-0.91), while the lowest value was at 0.5% gum concentration (m=-3.66).

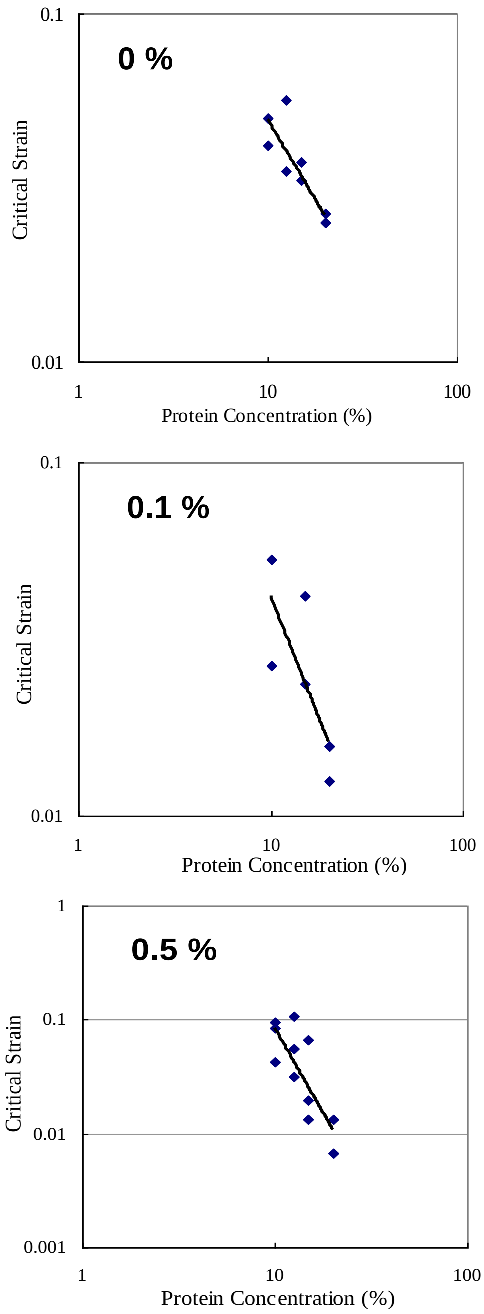


Figure. 5. Effects of gum concentration on the scaling behavior of critical strain of flaxseed gum-WPI gels.

TABLE I. EFFECT OF GUM CONCENTRATION ON THE FRACTAL DIMENSION OF FLAXSEED GUM-WPI GELS CALCULATED BY FRACTAL MODEL.

Gum conc.	Power Law exponents		Model of Wu and Morbidelli (2001)			
	n	*m*	d_f	*β*	*α* at *x*=1	*α* at *x*=1.3
0	6.33	-0.91	2.63	2.34	0.55	0.59
0.10%	6.56	-1.37	2.61	2.53	0.49	0.54
0.50%	5.22	-3.66	2.12	4.59	-0.20	-0.09

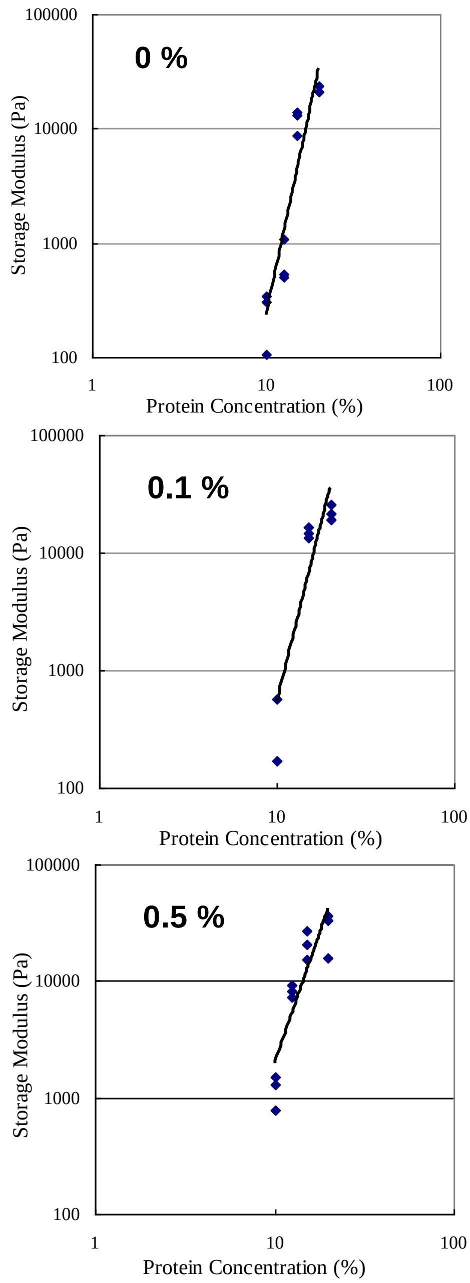


Figure. 6. Effects of gum concentration on the scaling behavior of storage modulus of flaxseed gum-WPI gels.

The effects of gum concentration on the scaling behavior of G' of flaxseed gum-WPI gels are shown in Fig. 6. The G' values of all the samples show power-law behavior with the increase of concentration, which can be fitted to the form: $G' \sim C^n$, where n is the power-law exponent. Similar with the m value in critical strain scaling, the n values were also strongly sensitive to the ionic strength.

E. Fractal Analysis

Wu & Morbidelli have developed the above model by introducing new parameter α, to indicate the regime that the gel belongs to (strong-link, weak-link, or transition gels) [5]. The fractal dimension of flaxseed gum gels were also evaluated using Eqs. (5) - (7) and results were shown in Table I. The d_f values were among the range of 2.12-2.63 using this method, which was lower than the fractal dimension values calculated from model of Shih et al. [8]. But the d_f values from two different models show similar trends with the increase of ionic strength. Although a good coincidence was found in the strong-link and weak-link gels using these two models, the difference in fractal dimension values calculated by these two models were also found in whey protein isolate gels and β-lactoglobulin gels, which were all transition gels.

IV. CONCLUSION

The scaling behavior of Rheological properties and fractal analysis of flaxseed gum-WPI gels were studied. Both storage modulus and critical strain showed scaling behavior, i.e. the power-law relationship, with the gum concentration. Two different models were used to estimate the fractal dimension of the gels. The fractal dimension values were 2.12-2.63, based on the model selected and the ionic strength applied. These values were in good agreement with the fractal dimensions of other hydrocolloid gels. And the fractal dimension values were found to be strongly dependent on the ionic strength. The gels were classified in transition regime in using model of Wu & Morbidelli. This study provides useful information on the fractal dimensions of flaxseed gum-WPI gels, which also help understand the microstructure of the gels.

ACKNOWLEDGMENT

This work was supported by Beijing Municipal Science and Technology Commission Project (D12110003112002), China Postdoctoral Science Foundation Project (2012M520462), National Natural Science Foundation of China (31000813), Chinese Universities Scientific Fund (2012QJ009), High Technology Research and Development Program of China (2011AA100802), and Commonwealth Guild Agricultural Scientific Research Program of China (201003077).

REFERENCES

[1] P. M. Iannaccone and M. K. Khokha, *Fractal Geometry in Biological Systems: An Analytical Approach*, CRC Press, Inc., Boca Raton, Florida, USA, pp. 15-30, 1996.

[2] Z. Y. Su, T. Wu, and S. Y. Wang, "Local scaling and multifractal spectrum analyses of DNA sequences - genbank data analysis," *Chaos, Solitons and Fractals*, vol. 40, no. 4, pp. 1750-1765, 2009.

[3] T. Hagiwara, H. Kumagai, and K. Nakamura, "Fractal analysis of aggregates in heat-induced BSA gels," *Food Hydrocolloids*, vol. 12, no. 1, pp. 29-36, 1998.

[4] T. Matsumoto, M. Kawai, and T. Masuda, "Viscoelastic and SAXS investigation of fractal structure near the gel point in alginate aqueous systems," *Macromolecules*, vol. 25, no. 20, pp. 5430-5433, 1992.

[5] H. Wu, J. Xie, M. Lattuada, M. Morbidelli, "Scattering structure factor of colloidal gels characterized by static light scattering, small-angle light scattering, and small-angle neutron scattering measurements," *Langmuir*, vol. 21, no. 8, pp. 3291-3295, 2005.

[6] M. E. Cates, "Statics and dynamics of polymeric fractals," *Physical Review Letters*, vol. 53, no. 9, pp. 926-929, 1984.

[7] L. G. B. Bremer, T. Vliet, and P. Walstra, "Theoretical and experimental study of the fractal nature of the structure of casein gels," *Journal of the Chemical Society, Faraday Transactions*, vol. 85, pp. 3359-3372, 1989.

[8] W. H. Shih, W. Y. Shih, S. I. Kim, J. Liu, and I. A. Aksay, "Scaling behavior of the elastic properties of colloidal gels," *Physical Review A*, vol. 42, no. 8, pp. 4772-4779, 1990.

[9] A. Maltais, G. E. Remondetto, and M. Subirade, "Mechanisms involved in the formation and structure of soya protein cold-set gels: A molecular and supramolecular investigation," *Food Hydrocolloids*, vol. 22, no. 4, pp. 550-559, 2008.

[10] R. Caillard, G. E. Remondetto, and M. Subirade, "Rheological investigation of soy protein hydrogels induced by Maillard-type reaction," *Food Hydrocolloids*, vol. 24, no. 1, pp. 81-87, 2010.

[11] G. Mazza and C. G. Biliaderis, "Functional properties of flaxseed mucilage," *Journal of Food Science*, vol. 54, pp. 1302-1305, 1989.

[12] Y. Wang, L. J. Wang, D. Li, N. Özkan, *et al.*, "Effect of flaxseed gum addition on rheological properties of native maize starch," *Journal of Food Engineering*, vol. 89, pp. 87-92, 2008.

[13] Y. Wang, L. J. Wang, D. Li, J. Xue, *et al.*, "Effects of drying methods on rheological properties of flaxseed gum," *Carbohydrate Polymers*, vol. 78, no. 2, pp. 213-219, 2009.

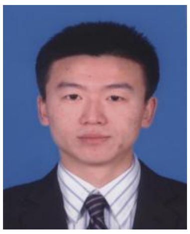
[14] H. H. Chen, S. Xu, and Z. Wang, "Gelation properties of flaxseed gum," *Journal of Food Engineering*, vol. 77, pp. 295-303, 2006.

[15] S. Ikeda, E. A. Foegeding, and T. Hagiwara, "Rheological study on the fractal nature of the protein gel structure," *Langmuir*, vol. 15, no. 25, pp. 8584-8589, 1999.

[16] S. W. Cui, *Polysaccharide Gums from Agricultural Products: Processing, Structures & Functionality*, Technomic Pub. Co., Lancaster, Penn., USA, 2001, pp. 59-66.

[17] W. Cui, G. Mazza, and C. G. Biliaderis, "Chemical structure, molecular size distribution and rheological properties of flaxseed gum," *Journal of Agricultural and Food Chemistry*, vol. 42, pp. 1891-1895, 1994.

[18] H. Moritaka, M. Takahashi, and K. Kubota, "Effects of cooling rate and sodium chloride on polysaccharide gelation," *Food Science and Technology Research*, vol. 13, no. 4, pp. 345-350, 2007.



Yong Wang, Beijing, born 1983.7, got PhD on agricultural engineering from China Agricultural University, Beijing, China, at 2011. He has been conducting research on food engineering as joint PhD student in University of California, Davis, USA.

He now works as research specialist for COFCO Nutrition & Health Research Institute, Beijing, China. Some publications are listed as below: Yong Wang, et al., (2011). Rheological study and fractal analysis of flaxseed gum gels. Carbohydrate Polymers, Volume 86, Issue 2, Pages 594-599. Yong Wang, et al., (2010). Effects of drying methods on the functional properties of flaxseed gum powders. Carbohydrate Polymers 81 (1), pp. 128-133.

He has conducted researches on extraction, drying, rheology, and fractal study of agricultural products. The current research focuses on dietary fiber production and application, and the processing technology of tomato sauce.

Dr. Wang is a member of IFT.

Xuqi Lin, Beijing, born 1963.9, got PhD on Chemical Engineering for the University of Auckland, Auckland, New Zealand.
He now works as senior expert for COFCO Nutrition & Health Research Institute, Beijing, China, also serves as R&D director for COFCO Tunhe, Urumqi, China. He has worked years on chemical engineering and food engineering. He has worked as visiting scholar in University of California, Davis, USA, as senior lecturer in Monash University, Melbourne, Australia.
Dr. Lin is a member of IFT.

Li-Jun Wang, PhD on Food Science & Engineering, is now associate professor in College of Food Science & Nutrition Engineering, China Agricultural University, Beijing, China. Her research interests focus on soybean processing, novel food processing, and fractal analysis of food material.

Dong Li, PhD on Agricultural Engineering, is now professor in College of Engineering, China Agricultural University, Beijing, China. His research interests focus on food properties, novel food processing, agricultural product processing, and biomass utilization.