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Aggregation profile of 11S, 7S and 2S coagulated with GDL

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Abstract

The aggregation process of the proteins coagulated by glucono-δ-lactone (GDL) was monitored by using atomic force microscopy (AFM). Solutions of 11S, 7S and 2S proteins, after heating at 100 °C for 10 min, were mixed with GDL and formed aggregates with different aggregation profiles. When the three protein solutions (11S, 7S and 2S) were mixed with GDL and deposited onto the mica for 1, 2 and 4 min, 11S proteins formed the largest clusters of aggregates, 2S proteins formed smaller clusters of aggregates than 11S but bigger clusters of aggregates than 7S and 7S proteins formed the smallest cluster of aggregates. It was also found, by turbidity measurement, that when GDL was added to the three protein fractions, the level of turbidity was in the order of 11S > 2S > 7S. Both these results showed that, when GDL was added to the three protein fractions, the speed of aggregation was in the order of 11S > 2S > 7S.

Keywords: Soy protein fractions; Turbidity; Aggregation; Gelation; GDL

1. Introduction

Gelation and aggregation are closely related. Gelation takes place when protein aggregates form a network (Lakemond et al., 2003). Doe (1993) reported that granularity gel transparencies were related to aggregate size and Lakemond et al. (2003) reported that aggregation size was related to the thickness of the strands of the gel. Hence, protein aggregation plays an important part in gelation.

Aggregation of proteins can be brought about by heating, or by varying the pH or ionic strength of the protein solution. It has been found that, depending on the conditions, both glycinin (11S) and β-conglycinin (7S) are able to form large aggregates when heated (Mills, Huang, Noel, Gunning, & Morris, 2001). There are various ways to analyze protein aggregates, such as particle size distribution, electron microscopy, dynamic viscoelasticity measurement and spectrophotometric methods (Dybowska & Fujio, 1998). Atomic force microscopy (AFM) is widely used to study the structure of biological macromolecules (McIntire & Brant, 1999). Its high resolution makes it a powerful tool for studying protein aggregation. AFM is able to image the shape of the protein particles and aggregates. It is also able to provide information about the size of particles or even the nature of interactions responsible for gelling (Morris et al., 2001).

Analytical ultracentrifugation of soy proteins in phosphate buffer of pH 7.6, with ionic strength of 0.5, showed that soy proteins contain four major globulins, namely, 15S, 11S, 7S, and 2S protein. These protein fractions are characterized by their sedimentation coefficients. The percentage content each of 15S, 11S, 7S and 2S was found to be 9.1%, 41.9%, 34% and 15%, respectively (Fukushima, 1991). 7S and 11S were shown to have different gel-forming properties (Kohyama & Nishinari, 1993; Tay & Perera, 2004), which could be due to differences in their aggregation processes.

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In our work, the aggregation process of the various soy protein fractions coagulated by GDL was monitored by using atomic force microscopy (AFM). 11S, 7S and 2S proteins, when reacted with GDL, will form gels at different speeds and these differences could be explained by looking at their respective aggregation profiles. It was hoped that a greater understanding of the interactions of the different soy protein fractions would shed more light on the factors that contribute to tofu texture.

2. Materials and methods

2.1. Isolation of 7S and 11S globulin

The 7S and 11S protein fractions were isolated by the modified method of Nagano, Hirotsuka, Mori, Kohyama, and Nishinari (1992). Defatted soybean flour was mixed with 15 volumes of deionised water, and then pH was adjusted to 7.5 with 1 M NaOH. The water-extractable soybean protein was obtained by centrifugation (9000$g \times 30$ min) at 20 °C. Sodium bisulfite (0.98 g/l) was added to the supernatant and the pH was adjusted to 6.4 with 0.1 M HCl, and the mixture was kept in an ice bath overnight. The following procedure was performed at 4 °C: the insoluble 11S fraction was obtained by centrifugation at 6500g for 20 min. The supernatant was adjusted to contain 0.25 M NaCl and to pH 5.0 (with HCl). After 1 h, the insoluble fraction was removed by centrifugation at 9000g for 30 min. The supernatant was diluted 2-fold with ice water, adjusted to pH 4.8 with HCl, and then centrifuged again at 6500g for 20 min. The 7S globulin was obtained as a sediment. Both 11S and 7S fractions were dissolved in deionised water and adjusted to pH 7.5 before dialyzing with deionised water for 24 h at 4 °C, followed by freeze-drying.

2.2. Isolation of 2S globulin

The 2S protein fraction was isolated by the modified method of Rao and Rao (1977). Defatted soybean flour was mixed with 10 volumes of deionised water containing 0.1% β-mercaptoethanol, and the mixture was stirred for 30 min. The water-extractable soybean protein was obtained by centrifugation (6000 rpm $\times$ 20 min) at 20 °C. To the supernatant, 0.1 M $MgCl_2$ was added and the suspension was kept at 4 °C for 6 h. The precipitate was removed by centrifugation (10,000 rpm $\times$ 30 min at 4 °C). Then 0.4 M $MgCl_2$ was added to the supernatant and the suspension was kept at 4 °C for 6 h. The precipitate was removed by centrifugation (10,000 rpm $\times$ 30 min at 4 °C). To the clear supernatant, 32% (w/v) of solid $(NH_4)_2SO_4$ was added and the precipitate was obtained by centrifugation (10,000 rpm $\times$ 30 min at 4 °C). The precipitate obtained was dissolved in 1 M NaCl solution and dialyzed against 1 M NaCl for 24 h at 4 °C. Then ethyl alcohol was added to the dialysate in the proportion of 1:1 (v/v) and stirred for 6 h at 4 °C. The resultant precipitation was removed by centrifugation (10,000 rpm $\times$ 30 min at 4 °C). The supernatant, after adjusting to pH 7.5, was dialyzed against deionised water for 24 h at 4 °C and was freeze-dried.

2.3. Atomic force microscopy

The images of the aggregates were obtained by atomic force microscopy (AFM) according to the method of Mills et al. (2001), modified as given below. Protein solutions were heated for 10 min at 100 °C and cooled to room temperature. Then, a freshly prepared GDL solution was added to them such that the resultant mixture contained protein at 4.0% (w/v) and GDL at 0.4% (w/v). A drop of sample solution (30 μl) was deposited onto freshly cleaved mica for 1 and 2 and 4 min. Then argon gas was used to blow-dry the samples. The samples were imaged under ambient conditions with a Multimode™ AFM (Digital Instruments, Veeco Metrology Group; Inc., Santa Barbara, CA) connected to a Nanoscope® IIIa scanning probe microscope controller (Digital Instruments, Veeco Metrology Group Inc., Santa Barbara, CA). All images were acquired in tapping mode using Veeco Nanoprobe™ tips (Veeco Metrology Group Inc., Santa Barbara, CA).

2.4. Turbidity measurement

Turbidity due to aggregation was determined according to the method of Molina and Wagner (1999), modified as given below. Turbidity was measured using a Shimadzu UV–Visible spectrophotometer (Japan). Protein solutions were heated for 10 min at 100 °C. Then, a freshly prepared GDL solution was added to them, such that the resultant mixture contained protein at 4.0% (w/v) and GDL at 0.4% (w/v). The absorbance of 1ml of mixture in a semi micro cuvette was measured at wavelength of 600 nm.

2.5. Statistical analysis

All data points represent the means ± standard deviations. Comparisons of variance of more than two samples were carried out by single factor ANOVA.

3. Results and discussion

3.1. AFM

The aggregation process of 11S with GDL was the fastest. It can be seen clearly from Fig. 1(a) and (b) that

the particles of 11S become a cluster of aggregates in just one minute upon the addition of GDL. When 11S proteins and GDL were deposited onto mica for longer periods of time, the size of aggregates increased as shown in Fig. 1(b)–(d).

The aggregation process of 7S with GDL was the slowest. It can be seen from Fig. 2(a)–(d) that the cluster of aggregates of 7S protein did not change as significantly as the cluster of aggregates of 11S and 2S when GDL was added. This showed that GDL needed more time to react with 7S protein than with 11S and 2S to cause a change in the aggregation profile at room temperature for the same length of time. When 7S proteins and GDL were deposited onto mica for longer periods of time, the number of similar size clusters of aggregates increased.

It can be seen in Fig. 3(a)–(d) that the size of the aggregates increased as the time deposited onto mica increased. For 1, 2 and 4 min, the size of the cluster of aggregates of 2S with GDL was smaller than the cluster of aggregates of 11S with GDL but was larger than the cluster of aggregates of 7S with GDL. Hence the aggregation process of 2S with GDL was slower than 11S with GDL but faster than 7S with GDL.

Protein solutions of 11S, 7S and 2S, heated at 100 °C for 10 min and without the addition of GDL, were deposited onto mica for the same periods of time (1, 2 and 4 min) to determine that the changes in the size or number cluster of aggregates, as seen in Figs. 1–3, were due to the coagulating effect of GDL. It was found that all three proteins fractions, when deposited onto mica for 1, 2 and 4 min, formed aggregates that

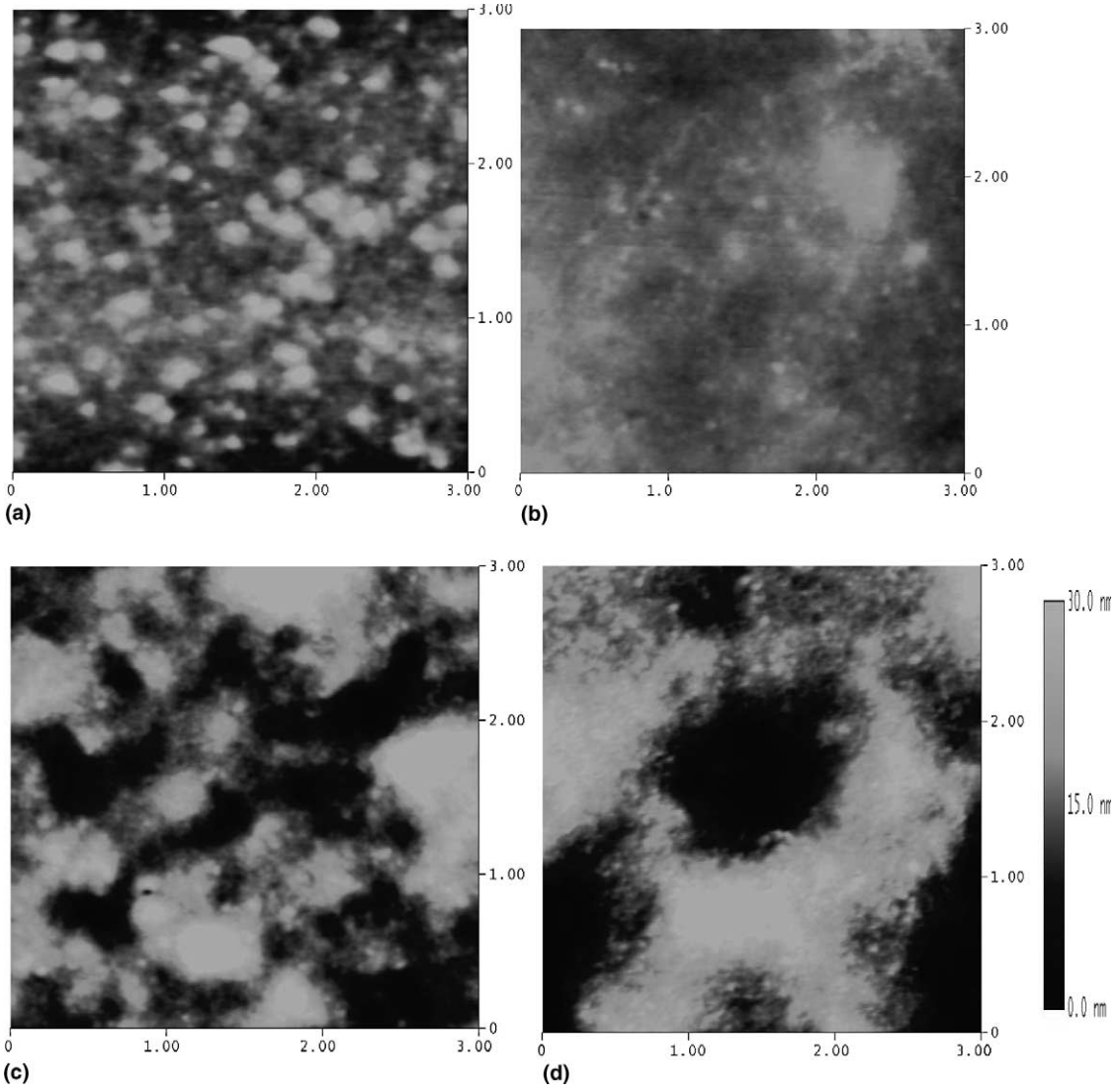

Fig. 1. Images of 11S protein (a) before the addition of GDL, (b)–(d) after addition of 0.4% GDL: (b) 11S and GDL deposited onto mica for 1 min; (c) 11S and GDL deposited onto mica for 2 min; (d) 11S and GDL deposited onto mica for 4 min. Scan size: 3 μm × 3 μm. The colour intensity corresponds to the height of the sample and thus brighter spots denoted a greater aggregate height.

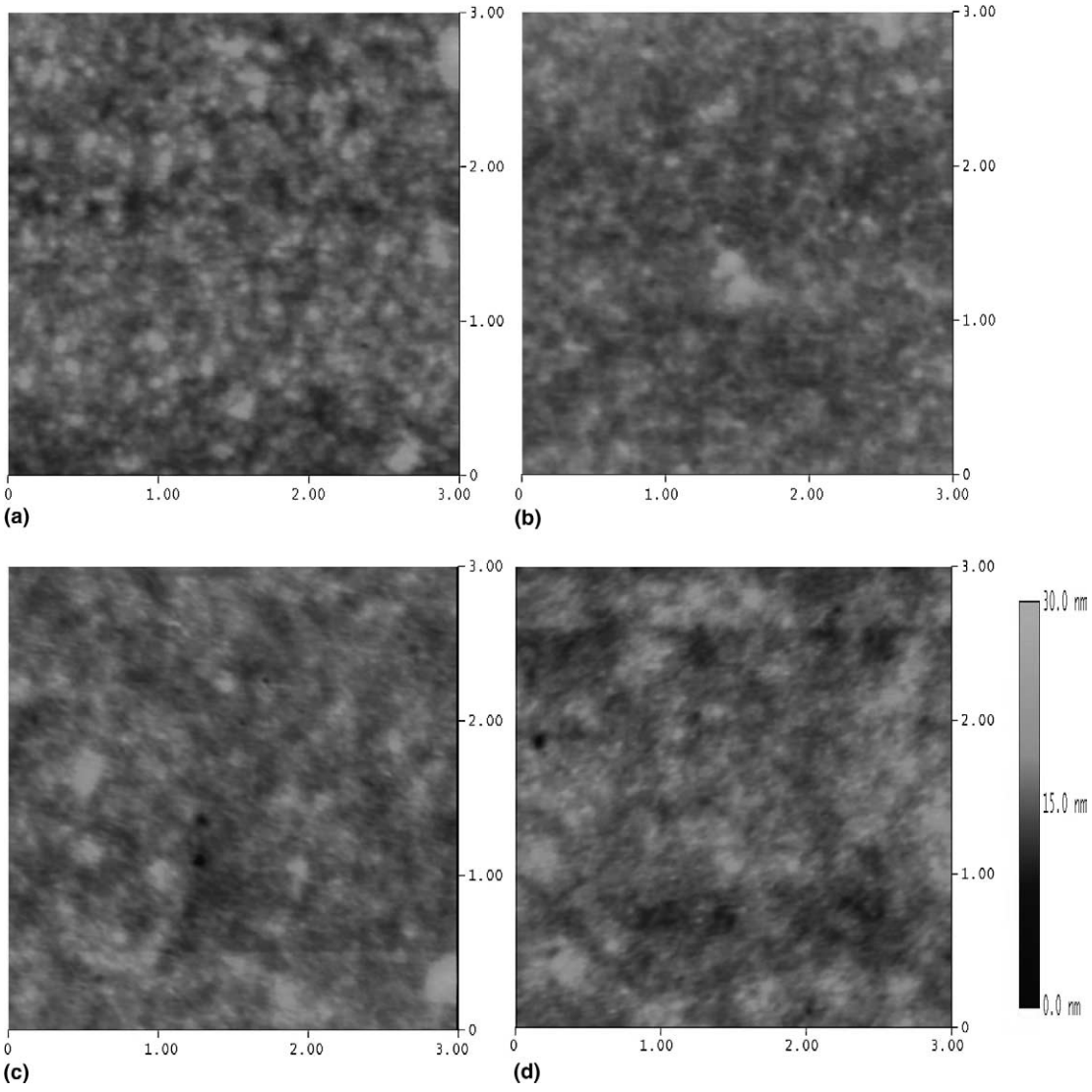

Fig. 2. Images of 7S protein (a) before the addition of GDL, (b)–(d) after addition of 0.4% GDL: (b) 7S and GDL deposited onto mica for 1 min; (c) 7S and GDL deposited onto mica for 2 min; (d) 11S and GDL deposited onto mica for 4 min. Scan size: 3 μm × 3 μm. The colour intensity corresponds to the height of the sample and thus brighter spots denoted a greater aggregate height.

had similar aggregation sizes at a confidence level of 95% (Table 1). This shows that the differences in the aggregate sizes in Figs. 1–3 were due to the effect of GDL.

The images of protein solutions of 11S (Fig. 1(a)), 7S (Fig. 2(a)) and 2S (Fig. 3(a)) without the addition of GDL, all showed large aggregates instead of the uniform particles or linear fibrous aggregates found by Mills et al. (2001) using 7S protein solution of 0.005% (w/v). These large aggregates could be due to the effect of heating the 4% (w/v) protein solution at 100 °C for 10 min. Similar 'macro aggregates' have been observed for heating 1% (w/v) solutions of 7S at 100 °C for 10 min (Mills et al., 2001). Mills et al. (2001) suggested that the 'macro aggregates' may be conglomerates of the smaller fibrous structures observed at lower protein concentrations.

3.2. Turbidity measurement

Molina and Wagner (1999) have found that turbidity ($A_{600\ nm}$) can be used to estimate the degree of protein aggregation. When GDL was added to the three proteins fractions, it was found from the turbidity graph ($A_{600\ nm}$ against time) that, as time increased, the $A_{600\ nm}$ increased and thus the level of turbidity increased, which in turn meant that the degree of aggregation also increased; thus the turbidity graph can be used to determine the speed of aggregation. From Fig. 4, the level of turbidity increased with time as more aggregates were formed and reached a maximum when a homogenous gel was formed. The order of turbidity was in the order of 11S > 2S > 7S. These results suggested that the speeds of aggregation of the proteins were in the same order of 11S > 2S > 7S.

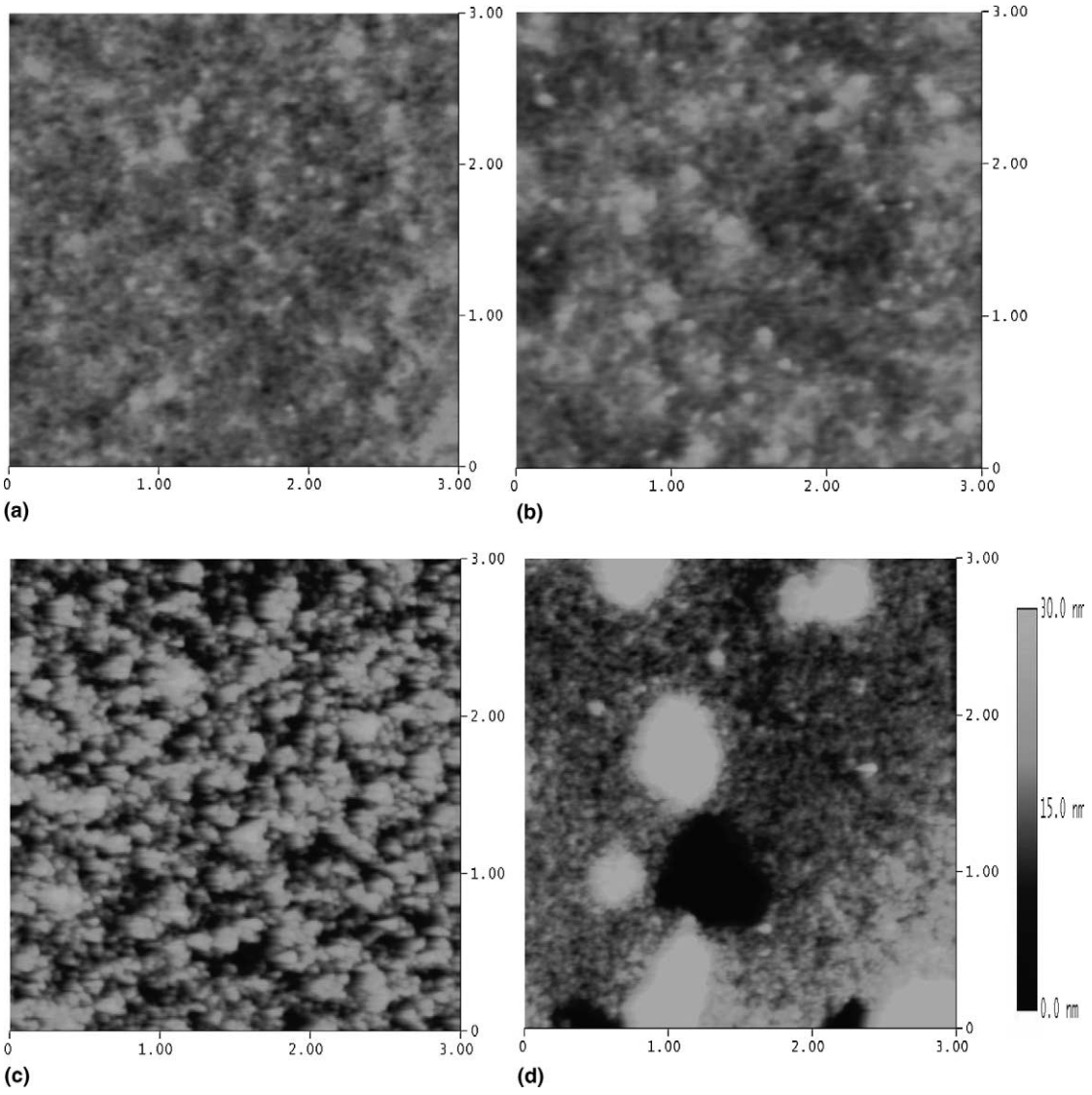

Fig. 3. Images of 2S protein (a) before the addition of GDL, (b)–(d) after addition of 0.4% GDL: (b) 2S and GDL deposited onto mica for 1 min; (c) 2S and GDL deposited onto mica for 2 min; (d) 11S and GDL deposited onto mica for 4 min. Scan size: 3 μm × 3 μm. The colour intensity corresponds to the height of the sample and thus brighter spots denoted a greater aggregate height.

Table 1
Average size of protein particles formed when 4% protein solution heated at 100 °C for 10 min was deposited onto mica for 1, 2 and 4 min

	Average size of protein deposited onto mica for 1 min (μm)	Average size of protein deposited onto mica for 2 min (μm)	Average size of protein deposited onto mica for 4 min (μm)
11S protein	0.22 ± 0.02[a]	0.23 ± 0.03[a]	0.21 ± 0.03[a]
7S protein	0.09 ± 0.02[b]	0.09 ± 0.02[b]	0.08 ± 0.03[b]
2S protein	0.13 ± 0.03[c]	0.12 ± 0.04[c]	0.14 ± 0.04[c]

[a] Denotes that average size of 11S was not significantly different at $P < 0.05$.
[b] Denotes that average size of 7S was not significant different at $P < 0.05$.
[c] Denotes that average size of 2S was not significant different at $P < 0.05$. Single factor ANOVA was carried out.

4. Conclusion

Aggregation profile of soy protein could be used to explain the difference in the gelation of protein with GDL. The aggregation profiles of 11S, 7S and 2S proteins with GDL at room temperature (Figs. 1–4) showed that 11S proteins formed large aggregates fastest, followed by 2S and then 7S. Faster aggregations could lead

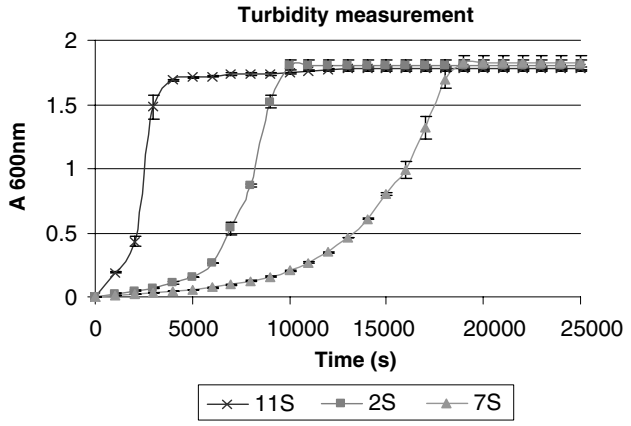

Fig. 4. Turbidity measurement of 11S, 7S and 2S protein solutions.

to faster gelation which was reported by Kohyama, Murata, Tani, Sano, and Doi (1995) who used a remoter to determine the gelation speed of 7S and 11S. They reported that 11S underwent faster gelation with GDL. Thus, from our results, 2S should form faster gels with GDL through 7S with GDL but slower gels than 11S with GDL. The presence of 2S will affect the coagulation of soy protein with GDL. 2S, despite having the least percentage of soy protein (as compared to 7S and 11S) will play a significant role during the gelation process.

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