Heat-induced aggregation and sulphydryl/disulphide reaction products of soy protein with different sulphydryl contents

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ABSTRACT

In this study, soy proteins were reduced with 0.1–10 mM dithiothreitol (DTT) to obtain an increasing number of sulphydryl groups (SH) with a similar particle size. Aggregation was promoted by increasing the degree of reduction when heated (100 °C, 30 min), resulting in larger sized aggregates (from 40 to 70 nm) and a higher viscosity of the aggregate dispersion. The disulphide bond (SS) content decreased and the less SS linked polymer, which was composed of acidic (A) polypeptide of glycinin, basic (B) polypeptides of glycinin, and a small amount of α and β subunits of β-conglycinin, was formed with increasing reduction degree, suggesting that SH/SS polymerisation was not the driving force for aggregation. The larger aggregates with increasing degrees of reduction were composed of more B of glycinin and β of β-conglycinin, suggesting that the A and the small amount of α and β in the SS linked polymer have an inhibiting effect on protein aggregates formation.

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1. Introduction

Heat treatment has considerable implications on the behaviour of proteins in food systems. Heat processing results in protein denaturation and aggregation, which is a major determinant for food nutritional and structural properties. Soy proteins are widely used in food applications as an ingredient for this purpose. Soy protein mainly contains β-conglycinin and glycinin. β-conglycinin is a trimeric glycoprotein composed of three subunits (α′, α and β) (Hou & Chang, 2004); while glycinin is a heterogeneous hexamer protein composed of five subunits (AB: A1aB1a, A2B2a, A1B2, A3B4 and A2A3B3). Each subunit is composed of an acidic polypeptide (A) (34–44 kDa) and a basic polypeptide (B) (18–20 kDa), which are linked by an interchain disulphide bond (SS) (Utsumi, Matsumura, & Mori, 1997; Wolf, 1993).

The effect of heat induced aggregation on the nutritional and functional properties of soy protein, such as emulsification, foaming, gelation and in vivo digestibility, has been previously investigated (Campbell, Gu, Dewar, & Euston, 2009; Friedman & Brandon, 2001; Keerati-u-rai & Corredig, 2009). The aggregation mechanism of soy protein is based on the disassociation and association behaviour of the subunits due to their different properties, such as hydrophobicity and isoelectric points (Ren, Tang, Zhang, & Guo, 2009; Utsumi & Kinsella, 1985a). Noncovalent (hydrophobic, hydrogen bond and electrostatic) and covalent (disulphide) interactions were considered to have a combined effort towards an aggregation process in soy protein (Cramp, Kwanyuen, & Daubert, 2008; Utsumi & Kinsella, 1985b). But which interaction as a driving force for soy protein aggregation has not been clearly explained.

It appears that the driving force for aggregation varies from protein to protein (Visschers & De Jongh, 2005). The model of aggregation of β-lactoglobulin can be described by a radical chain polymerisation, in which sulphydryl groups (SH) act as radicals. The relative rates of radical initiation, propagation and termination reactions ultimately determine the average size of the aggregates (Roefs & De Kruijf, 1994). The SH/SS reaction (disulphide interaction) model as a driving force for β-lactoglobulin aggregation has been confirmed and experimentally modified previously (Hoffmann & Van Mil, 1997; Mounsey & O’Kennedy, 2007). However, according to Broersen et al. (2006), the rate of ovalbumin aggregation is not affected by the introduction of SH to the protein molecule, and the formation of the disulphide bond was preceded by noncovalent interactions. Hence, the SH/SS reaction may not be the driving force for the aggregation of ovalbumin. There is little direct evidence as to whether the SH/SS reaction is the driving force for soy protein aggregation.

A common method for studying the SH/SS reaction involves the inclusion of reagents to the protein that modify or prevent the
formation of SS prior to heat processing. These reagents include SS reductants such as 2-mercaptoethanol (ME), and SH blocking reagents, for example N-ethylmaleimide (NEM). However, the presence of such chemicals appears to affect the aggregation properties by interrupting the formation of SS. NEM has been found to severely reduce the hardness of soy protein gel (Shimada & Cheftel, 1988), but NEM did not weaken the soy protein gels reported in some studies (Hua, Cui, Wang, Mine, & Poya, 2005; Utsumi & Kinsella, 1985b). In some studies, low levels of a reductant were shown to result in firmer whey protein gels (Nguyen, Wong, Havea, Kinsella, 1985b). In some studies, low levels of a reductant were involved the SS reduction to increase SH and the removal of excess reductant by gel filtration. In order to improve the use of soy protein as a food ingredient, a better understanding of how these factors relate to the aggregation and functional properties of heat processing protein is required.

2. Materials and methods

2.1. Materials

Acrylamide, N,N'-methylenebisacrylamide, sodium dodecyl sulfate (SDS), 4,4'-dithiodipiridine and dithiothreitol (DTT) were purchased from Sigma–Aldrich (Shanghai, China) Trading Co., Ltd. All other chemicals were of analytical reagent grade.

2.2. Soy protein preparation

Dehulled and milled soybean flour was treated with n-hexane 5 times to remove the oil. The defatted flour was mixed with hexane and ethanol (1/2, v/v) at 4 °C for 1 h. The slurry was vacuum filtered and the filter cake was mixed with 95% (v/v) ethanol at 4 °C for 1 h. After drying, the meal was dispersed in distilled water (1/10, w/v) and adjusted to pH 7.0 with 2 M NaOH. After stirring for 1 h at 20 °C, the suspension was treated by centrifugation (15,800g, 30 min, 4 °C). The supernatant was adjusted to pH 4.5 with 2 M HCl and centrifuged (6000g, 30 min, 4 °C). The pellet was washed twice with distilled water, re-suspended in 5-fold (w/w) distilled water and neutralised to pH 7.0 with 2 M NaOH. After centrifuging (15,000g, 30 min, 4 °C), the supernatant was freeze-dried and stored at 4 °C. The protein content in the prepared soy protein was 90% (w/w), as determined by the micro-Kjeldahl method.

2.3. Soy protein reduction and heat treatment

Soy protein powder was dispersed in 10 mM sodium phosphate buffer (pH 7.0) at a concentration of 30 g/L and magnetically stirred thoroughly. The soy protein suspensions were centrifuged at 40,000g for 30 min to remove the insoluble particles. The obtained soy protein solutions were incubated with freshly prepared DTT between 0.1 and 10 mM under nitrogen overnight at 25 °C. The excess DTT was removed by placing the samples on a desalting column (Hitrap 5 ml, GE-Healthcare) which was equilibrated and eluted with distilled water. The reduced soy protein was injected into screw caped test tubes and heated in a water bath at 100 °C.

After heating for different time intervals (0.5, 1, 2, 3, 30 min), the samples were taken out and immediately cooled in an ice water bath to ambient temperature.

2.4. Free sulphydryl content determination

The total free sulphydryl (SH) content was determined according to the method from a previous study (Ruan, Chen, Kong, & Hua, 2013). Soy protein solutions were diluted with the SDS-buffer (pH 7.0) to give a final SDS concentration of 2% (w/v). The SH detecting reagent, 4,4'-dithiodipiridine (DPS) was added prior to SDS addition to avoid SH oxidation. The samples were vortexed and detected immediately at 324 nm against the SDS-buffer blank in a UV-2450 UV–VIS spectrophotometer (Shimadzu, Kyoto, Japan) until the absorbance reached the maximum value (this time period was recorded). In the parallel reagent blank, the protein solution was replaced with 0.01 M SDS buffer (pH 7.0), mixed with DPS and incubated in a water bath for the spectrophotometer recorded time period. In the parallel protein blank, the diluted soy protein solution was mixed with water. The SH content was expressed as μmol SH/g protein.

2.5. Cysteine determination using amino acids analysis

Soy proteins were vacuum dried. Performic acid was added and incubated at 0 °C for 20 h. Both the cysteine and cystine could be converted into cysteic acid by performic acid oxidation (Synowiecik & Shahidi, 1991). Cysteic acid was separated from the other amino acids using a Hitachi L-8900 amino acid analyser after digestion in 6 M HCl at 110 °C for 22–24 h. The total cysteine content was determined in the form of cysteic acid. The SS content = (total cysteine residue content – total free SH content)/2.

2.6. Aggregate size measurements

Protein samples were diluted to 1 mg/ml with 10 mM sodium phosphate buffer (pH 7.0) and filtered through a 0.22 μm filter. The aggregates size was determined by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) at 25 °C. The sample was placed in a 1 x 1 cm cuvette. The intensity of the light scattered from the proteins in the dispersions was used to calculate the mean hydrodynamic diameter (Z-average mean), based on the Stokes–Einstein equation and a standard cumulants fit of the autocorrelation function.

2.7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SDS–PAGE was performed on a vertical slab unit (Beijing Liuyi Instrument Factory, China) according to the method of Laemmli. A 12% resolving gel overlaid with a 3% stacking gel were used. Aliquots of each sample were mixed with 2× sample dissolving buffer (4% SDS, 20% glycerol, 0.125 M Tris–HCl buffer-pH 6.8, 0.02% bro-mophenol blue). The samples were heated at 100 °C for 5 min and centrifuged at 15,000g for 10 min at room temperature. The supernatant was loaded onto SDS–PAGE gels. After running, the gel was stained with Coomassie brilliant blue R250 and scanned using a computing densitometer (Molecular Imager Chemi Doc XR+, Bio-Rad, USA). The intensities of the protein bands were integrated using Image Lab software (Bio-Rad, USA). The content of each component was determined as a percentage of the component in the sample by comparing the individual band intensities with the total intensity of all the bands in a lane.
2.8. Non-reducing and reducing diagonal SDS–PAGE

The protein dissolved in the non-reducing sample buffer was used for the first dimensional electrophoresis. After electrophoresis, the lane of interest was excised from the gel, immersed in distilled water (containing 50 mM DTT) for 10 min at room temperature, and then boiled for 5 min to reduce the SS. The reduced lane was placed on the top of another gel and overlaid with 0.5% low melting agarose (Bio-Rad, USA) to conduct the second dimensional SDS–PAGE. After running, the gel was stained with Coomassie Brilliant Blue R250 and scanned on the computing densitometer as stated above. If the bands had disulphide bonds in the first-dimensional electrophoresis, they would resolve into spots below the diagonal line of the second-dimensional electrophoresis gel. If disulphide bonds were not present in the bands of first-dimensional electrophoresis, the spots in the second-dimensional electrophoresis gel would lie on the diagonal line.

2.9. Shear rate sweeps

The rheological properties of heated soy protein were measured using a controlled-stress rheometer (Physica MCR 301; Anton Paar, Austria), equipped with parallel plate geometry (PP50, 50 mm diameter and 1 mm gap). The temperature was regulated by a circulating bath and controlled with a Peltier system (Anton Paar) and kept constant at 25 °C ± 0.1 °C. The data of all the rheological measurements were analysed with the supporting software Rheoplus/32 v2.81 (Anton Paar).

A shear rate sweep, by increasing the shear rate from 0 to 100/s, was conducted to assess the flow behaviour of the heated soy dispersions. A thin film of mineral oil was applied to the sample surface to minimise sample dehydration. The model used to fit the flow behaviour data was that of Ostwald dewaele, also known as the Power law, and is represented by the following equation:

\[ \eta = \frac{\tau}{\dot{\gamma}} = k\dot{\gamma}^{n-1} \]

where \( \eta \) is the apparent viscosity (Pa s), \( \tau \) is the shear stress (Pa), \( \dot{\gamma} \) is the shear rate (s\(^{-1}\)), \( k \) is the consistency index (Pa s\(^n\)) and \( n \) is the flow behaviour index.

2.10. Atomic force microscopy

AFM images were recorded at room temperature in tapping mode on the Bruker Dimension Icon AFM equipped with a ScanAsyst. 2 µl protein dispersions (diluted to 10 µg/ml with distilled water filtered through a 0.22 µm filter) were placed on a freshly cleaved mica disk and air-dried overnight at ambient temperature. Images were analysed using Digital Nanoscope Analysis software (version 1.40, Bruker).

2.11. Ultracentrifugation experiments

All the unreduced and reduced soy proteins were heated at 100 °C for 30 min and centrifugation at 270,000g for 30 min using a Beckman Optima L-XP ultra centrifuge. The precipitate and supernatant was obtained and the protein contents of supernatant protein were determined. The protein content of the precipitate was calculated by subtracting the supernatant content from the total content. The precipitate and supernatant was analysed by SDS–PAGE as described above. The bands were scanned and quantified according to Section 2.7.

2.12. Statistical analysis

Three separate soy samples were used, and each sample was run in triplicate. Data were subjected to an analysis of variance (ANOVA) using the SAS 9.1 package (SAS 2005). Significant differences among variables were determined by LSD all-pairwise multiple comparisons \((p < 0.05)\). Data were expressed as the mean ± SD \((n = 3)\).

3. Results and discussion

3.1. Soy protein reduced with 0.1–10 mM DTT without heat treatment

3.1.1. The total free sulphydryl (SH) content

Fig. 1a shows that the total free SH content of the unreduced soy protein was 7.5 ± 0.26 µmol/g protein. The total free SH content slightly increased by 0.1–0.5 mM DTT reduction. When the DTT concentration was ≥2.5 mM, the total free SH content greatly increased. When DTT was 10 mM, the total free SH content was 75.9 ± 0.5 µmol/g protein. The total free SH content increased with the increasing DTT concentration. Wolf (1993) also found that the SH content of glycinin increased with increasing DTT concentration in the absence of denaturants.

3.1.2. Particle size

The hydrodynamic diameter of unreduced soya protein determined by dynamic light scattering (DLS) was 23.0 ± 1.6 nm. The average particle size of unreduced soy protein was considerably

![Fig. 1. Effect of increasing the degree of reduction (0–10 mM DTT) on the total free sulphydryl (SH) content and article size (a), and the cleaved disulphide bond (SS) (b) of soy proteins.](image-url)
lower than that of the native soy protein isolate (SPI) reported by Wang et al. (2012), but is consistent with the glycinin diameter (pH 7.0, I = 0.05) analysed by Pizones, Henestrosa, Martinez, Patino, and Pilosof (2012). No significant change in particle size was observed in the reduced protein (shown in Fig. 1a). It has been reported that hydrophobic interactions are very important in the formation of the glycinin hexamer and β-conglycinin trimer (Guo et al., 2012; Utsumi & Kinsella, 1985a). Thus, the SS reduction by DTT appeared not to affect the subunits association of either glycinin or β-conglycinin in the aqueous environment (Pizones et al., 2012).

3.1.3. The cleaved disulphide bond (SS) by DTT
Nonreducing SDS–PAGE was used to determine which disulphide bonds (SS) were cleaved by DTT. Fig. 1b shows the profiles of soy protein with different degrees of reduction (0–10 mM DTT). The unreduced soy protein was dissolved into β-conglycinin subunits (α, α’, β), AB (glycinin subunit), A2B2 (SS linked portion of A2A2B2B2, one type of glycinin subunit) and several other bands. Diagonal electrophoresis showed that Band 2 was the SS linked dimer of α and/or α’, and Band 3 was the SS linked dimer of AB (result not shown), which had previously been observed (Petruccelli & Anon, 1995a; Wolf, 1993). Band 5 was the Kunitz trypsin inhibitor (KTI), which was confirmed by isoelectric focusing in our lab (see Supporting material). With increasing DTT, the intensities of Band 2, Band 3 and AB became weaker. The α, α’, A (on Band 4 position) and B (on Band 5 position) appeared and gradually became denser. When the DTT concentration was >2.5 mM, Band 2 and 3 disappeared completely. These results showed that the SS between A and B and the SS between α and α’ was gradually disrupted with reduction, which resulted in the SH increase in soy protein.

3.2. Soy protein reduced by 0–10 mM DTT with heat treatment
3.2.1. Effect of increasing degrees of reduction on heat-induced aggregates size
All protein solutions (22.5 mg/ml, pH 7.0) were heated at 100 °C for various time intervals from 0 to 30 min. The particle size of heated soy protein determined by dynamic light scattering (DLS) is shown in Fig. 2a. At the early heating stage (0–3 min), the particle size (average hydrodynamic diameter) increased rapidly for all unreduced and reduced proteins, which suggested that aggregates were formed quickly. When the heating time was prolonged to 30 min, the aggregates size increased slowly. Interestingly, the aggregates size significantly increased with increasing degrees of reduction. When heated for 30 min, the size of the aggregates formed with unreduced soy protein was 40 ± 2 nm, the aggregates size was increased with increasing degrees of reduction and the size was 70 ± 2 nm for 10 mM DTT reduced soy protein. AFM was used to obtain information concerning the morphologies of the aggregates. Fig. 2b also showed that the aggregates of the unreduced protein were small, while the aggregates of 10 mM DTT reduced protein appeared to be larger.

3.2.2. Effect of increasing degrees of reduction on aggregates dispersion viscosity
The apparent viscosities versus shear rates of soy protein aggregates dispersions (100 °C, 30 min) were determined and are shown in Fig. 2c. The values of the flow behaviour index (n) and the consistency index (k) determined by plotting shear rate (γ) versus apparent viscosity (η) are presented in Fig. 2c. The experimental data fitted well to the Power law, with R² values ranging from 0.98 to 0.99. Low n values (n < 1) were recorded for all dispersions, suggesting they exhibited pseudoplastic behaviour. The consistency index, k, increased from 0.20 ± 0.02 to 0.40 ± 0.01, indicating that the viscosity of the dispersions increased with the increasing degree of reduction (Mudgal, Daubert, Clare, & Foegeding, 2011).

3.2.3. Effect of increasing degrees of reduction on SH and SS content of heated soy proteins
The total free SH and SS contents of the heated soy proteins (100 °C, 30 min) were determined and shown in Fig. 3a. The SS content was obtained by subtracting the total free SH content from the total cysteine residue content. The total free SH content of heated soy proteins increased from 0.75 ± 0.1 to 50.2 ± 0.3 μmol/g protein with increasing degrees of reduction. As expected, the SS content of heated soy proteins were decreased from 56.6 ± 0.56 to 31.9 ± 0.5 μmol/g protein with the increasing degrees of reduction.

3.2.4. Effect of increasing degrees of reduction on subunits and polypeptides distributions in aggregates
The aggregates dispersions (100 °C, 30 min) were treated by ultracentrifugation (270,000g) to get a supernatant and precipitate. The protein concentration in the supernatant decreased from 14 ± 0.7 to 9 ± 0.6 (mg/ml) with increasing degrees of reduction, clearly revealing that the aggregate formation was promoted by increasing degrees of reduction. However, the SS contents of the heated soy proteins (100 °C, 30 min) decreased with increasing degrees of reduction (Fig. 3a), revealing that SS was not necessary for formation of the protein aggregates (Broersen et al., 2006).

The compositions of the precipitate and supernatant were determined by reducing SDS–PAGE. Before ultracentrifugation, the compositions of all the samples were identical and the amount of A was equal to that of B (~30%) (Fig. 3b). A was more than B in the supernatant and was less than B in the precipitate; in contrast (Fig. 3c and d). In the precipitate, the content of α’ + α + A increased from 53% to 60% and β + B decreased from 33.7% to 25.8% with increasing degrees of reduction. In the precipitate, the content of α’ + A + A increased from 25% to 12% and B + B increased from 51% to 60% with increasing degrees of reduction (Fig. 3e). These results suggested that the larger sized aggregates with increasing degrees of reduction were composed of more B and β, rather than A, α’ and α. Ren et al. (2009) reported that B should be located inside the protein aggregates, whereas the A, α’ and α are located outside. The β is preferentially associated with B (Ono, Choi, Ikeda, & Odagiri, 1991; Utsumi, Damodaran, & Kinsella, 1984). Thus, in this study, it was deduced that B and β formed the core of the protein aggregate, while A, α’ and α formed on the surface of the protein aggregate. The results above show that the heated unreduced soy protein had a smaller average particle size with less β + B and more α’ + A compared to the heated reduced soy protein. It is known that the surface-area-to-volume ratio is an important parameter used to characterise particles and a small ratio means a large size and large ratio means small size, which is in agreement with the above findings.

3.2.5. Effect of increasing degrees of reduction on SS linked products
After heating at 100 °C for various time intervals from 0 to 30 min, the samples were applied to non-reducing SDS–PAGE (Fig. 4) to determine the SS linked products in the aggregation process. It was found that the glycinin subunits, AB and A2B2, gradually become lighter with heating time, these results are similar with the results that are reported by Petruccelli and Anon (1995b). For the 0–0.5 mM DTT reduced protein, AB and A2B2 disappeared completely after 3 min; for 2.5–5 mM DTT reduced protein, they disappeared completely after 2 min; for 10 mM DTT reduced protein, they disappeared completely within 1 min. Band 1 and Band 4 gradually became denser with heating time. Band 5 slightly increased after 1 min of heating, and then decreased to
the original level. The other bands showed no obvious changes when heated. Thus, Band 1 and Band 4 were the main SH/SS reaction products when heated. In order to clarify their compositions, diagonal SDS–PAGE was conducted.

**Fig. 2.** Effect of increasing the degree of reduction (0–10 mM DTT) on soy protein aggregates size and flow behaviour. (a) Aggregates size of soy proteins with different reduction degrees heated at 100 °C for various time intervals. (b) Topographical AFM images of unreduced (i) and 10 mM DTT reduced soy protein (ii) heated at 100 °C for 30 min. (c) Apparent viscosities versus shear rates of soy proteins with different reduction degrees heated at 100 °C for 30 min. \( k \) is the consistency index (Pa s\(^n\)), and \( n \) is the flow behaviour index.

**Fig. 5.** Shows the diagonal SDS–PAGE profile of soy protein with different reduction degrees heated for 30 min. In the case of unreduced soy protein (Fig. 4a), Band 1 was resolved into A, B, and a small amount of \( \alpha' \) and \( \alpha \) below the diagonal line, revealing
that Band 1 was SS linked polymers composed of A, B, and a small amount of \( \alpha \) and \( \alpha \) (Ren et al., 2009). Its composition gradually changed with increasing degrees of reduction. The content of B in the polymer appeared to be slightly higher than that of A. Less \( \alpha \) and \( \alpha \) was present in the polymer and disappeared completely when DTT was \( >5 \) mM. Band 4 was resolved into two spots, one (A) was on the diagonal line and the other (B) was below the diagonal line. This showed that Band 4 contained two components: an
SS linked dimer of B and monomer of A (and/or A4 α). The other minor SS linked products were also confirmed: Band 2 was SS linked dimer of α' and/or α; Band 3 was the SS linked dimer of α'/α and A; the wide zone, below α and above β, contained SS linked dimer of A and trimer of B. β did not participate in the SH/SS reaction because β (Utsumi et al., 1997) had no SH. Thus, it was found that the polymer and dimer of B + monomer of A was the main SH/SS reaction products.

The production of the polymer and dimer of B + monomer of A by 30 min heating was determined (Fig. 6). The total
production of the polymer and dimer of B+ monomer of A
decreased from 45% to 20% and the production of polymer
was decreased from 40% to 8% with increasing degrees of
reduction, which was positively correlated with the decrease
of SS content of heated soy protein (\(R^2 = 0.9\)). This suggested
that the formation of intermolecular SS contributing to
aggregation was not necessary, and the non-covalent molecular
interactions were important for protein aggregation. With
increasing DDT, the polymer, which was composed of A, B,
and a small amount of \(\alpha'\) and \(\alpha\), was gradually decreased,
revealing that monomer of B and dimer of B were gradually
increased. It can be considered that this should be the mecha-
nism for the size increase of protein aggregates. As stated
above, it is reasonable to consider that A and small amounts
of \(\alpha'\) and \(\alpha\) of the polymer would have some inhibiting effect
on protein aggregates core formation (Guo et al., 2012)

Fig. 5. Non-reducing and reducing diagonal SDS–PAGE profile of (a) unreduced soy protein; (b) 0.1 mM DTT reduced soy protein; (c) 0.5 mM DTT reduced soy protein; (d) 2.5 mM DTT reduced soy protein; (e) 5 mM DTT reduced soy protein; and (f) 10 mM DTT reduced soy protein heated at 100 °C for 30 min.
In this study, soy proteins were reduced by 0.1–10 mM DTT to obtain samples with an increasing SH content (7.5–75.9 mol/g protein). Through heating, larger amounts and sizes of protein aggregates were formed, and aggregates dispersions with higher viscosity were obtained with increasing degrees of reduction. However, the SS content decreased and the less SS linked polymer, which was composed of A of glycinin, B of glycinin, and a small amount of \( \alpha \) and \( \alpha' \) of \( \beta \)-conglycinin, was formed with increasing degrees of reduction, revealing that intermolecular SS are not necessary for protein aggregation and the polymer has a somewhat inhibiting effect on the protein aggregate formation. This study was meaningful in supplying a strategy to control aggregation in soy protein processing.

4. Conclusion

Fig. 6. The production of polymer and dimer of B + monomer of A by SH/SS reaction after heating soy proteins with increasing degrees of reduction at 100 °C for 30 min. The contents of polymer and dimer of B + monomer of A were determined by densitometric scanning of Band 1 and Band 4 in non-reducing SDS–PAGE. B: basic polypeptide of glycinin; A: acidic polypeptide of glycinin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.01.083.

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