

# Comparative Study About Some Physical Properties, *In vitro* Digestibility and Immunoreactivity of Soybean Protein Isolate for Infant Formula

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**Abstract** The objective of this study was to determine molecular weight subunit distributions of soy protein isolate (SPI) by SDS-PAGE and gel size exclusion chromatography, and further to investigate the differences of thixotropy, viscosity, *in vitro* digestibility and immunoreactivity of SPI for infant formula produced in Chinese domestic companies and world famous SPI manufacturers such as Dupont and Fuji. The molecular subunit distributions were analyzed by SDS-PAGE and gel size exclusion chromatography, indicating that Solae and Fuji were hydrolyzed by proteolytic enzymes. The thixotropy of Fuji and Solae significantly reduced around by 95 %, compared with those of Mantianxue and Dupont Zhengzhou. The allergen contents of Fuji and Solae strikingly decreased by 60 and 84 % respectively, in contrast to that of Mantianxue. The *in vitro* protein digestibility of Solae at the end of pepsin and trypsin digestion markedly increased by 11.7 and 11.3 %, respectively, in comparison to those of Mantianxue. Suitable enzymatic hydrolyzed SPIs showed lower thixotropy, viscosity, immunoreactivity and higher *in vitro* protein digestibility than those from the other SPIs. The lower thixotropy indicates low difficulty or shear stress in swallowing for infants. The lower immunoreactivity will improve the safety of SPI for cow milk allergic babies.

**Keywords** Soy protein isolate for infant formula · Thixotropy · Immunoreactivity · *In vitro* digestibility of protein

## Introduction

Soy protein-based formulas have been available for almost 100 years [1]. The use of soy-based formulas is recommended for only those infants who cannot have milk-based products because of healthy, cultural or religious reasons, such as cow milk allergy (CMA), lactose intolerance and a vegan lifestyle [2, 3]. With respect to its application in CMA, the use of soy-based formulas is only contraindicated for non-immunoglobulin E (IgE)-mediated CMA [4]. This contraindication stems from the high rate of coincident soy allergies in milk allergic children (range 17 to 47 %) [5].

Despite very limited indications for its use, soy protein-based formulas in the United States may account for nearly 25 % of the formula market [1] and the utilization rates of soy-based formula were up to 20 % among infants in Canada [2]. The chief reasons for selecting the soy-based formulas are their reduced cost when compared with the hydrolyzed formulas and their superior palatability [4]. Additionally, malnutrition among preschool children is quite prevalent in underdeveloped and developing countries [6, 7]. Some families in developing countries cannot afford the milk based infant formula, however they can choose soy-based infant formula with relative low cost.

Numerous studies on soy-based infant formula in the previous two decades focused on the bio-availability of compositions and the activities of anti-nutritional factors [8–10]. However, few studies were conducted about thixotropy and *in vitro* protein digestibility, which play important roles in the appraisal for the quality of soy protein isolate (SPI) for infant formula. Thixotropy of fluid foods can be

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adopted to evaluate the aspect of oropharyngeal swallowing [11]. Additionally, *in vitro* protein digestibility methods were recommended by the joint FAO/WHO expert consultation committee on protein quality evaluation [12]. These methods have shown good correlations with the results of *in vivo* methods that are time-consuming and expensive [12].

The objective of this study is to analyze subunit molecular weight distributions of SPI by SDS-PAGE and gel size exclusion chromatography and further to investigate the differences of thixotropy, viscosity, *in vitro* digestibility and immunoreactivity of SPI for infant formula produced in Chinese domestic companies and world famous SPI manufacturers such as Dupont and Fuji. This study will provide the manufacturers in developing countries with practical information about SPI for infant formula.

## Materials and Methods

### Materials

Soy protein isolate (model: special for infant formula) were purchased from Henan Anyang Miantianxue Food Co., Ltd. (China), Ha'erbin High-Tech (Hagaoke) Soybean Food Co., Ltd. (China), Guangzhou Luxin Trade Co., Ltd. (China), DUPONT Zhengzhou Protein Co., Ltd. (China), FUJI(Beijing) Technology Co., Ltd. (Japan), The Solae Co., Dupont (USA). Their protein contents on dry basis were 91.61, 94.86, 96.26, 96.83, 97.77 and 96.55 % in the order of Miantianxue, Hagaoke, Dupont Zhengzhou, Luxin, Fuji and Solae.

Soybean allergen Elisa Fastkit was purchased from Nippon Meat Packers, Inc. R&D Center. Bovine serum albumin (BSA) was purchased from Sinopharm Chemical Reagent Co., Ltd. Low molecular weight protein markers and  $\beta$ -mercaptoethanol (2-ME) were purchased from Shanghai Shengzheng Biotech. Co., Ltd. Trifluoroacetic acid and acetonitrile (chromatographic grade) were purchased from Sigma-Aldrich Co. All the other chemicals (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd.

### Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed on a discontinuous buffered system according to the method of Tang and Sun [13], using 12 % separating gel and 4 % stacking gel [13]. The original samples were respectively weighed into centrifugal tubes. Their masses were 3.30, 2.90, 3.02, 3.10, 2.68 and 2.67 mg in the order of Miantianxue, Hagaoke, Dupont Zhengzhou, Luxin, Fuji and Solae. The protein samples were solubilized in 2.5 mL of 0.125 M Tris–HCl buffer (pH 6.8), containing

1 % (w/v) SDS, 2 % (v/v) 2-mercaptoethanol (2-ME), 5 % (v/v) glycerol and 0.025 % (w/v) bromophenol blue, and heated for 5 min in boiling water. And then samples were centrifuged (10,000g, 10 min) before electrophoresis. Protein concentration was 1 mg/mL per sample. Samples were loaded at 10  $\mu$ L/channel and run at 25 mA constant current. After the electrophoresis, the gel was dyed in 0.25 % Coomassie blue (R-250) in the 50 % trichloroacetic acid and then destained in 7 % acetic acid (methanol:acetic acid:water, 227:37:236 (v/v/v)).

### Determination of the Molecular Weight of Hydrolysates

The molecular weight of hydrolysates was determined by gel size exclusion chromatography [14], with some modification. Each sample (protein concentration 0.5 mg/mL) in the acetonitrile/water/trifluoroacetic acid (45/55/0.1 v/v/v) was centrifuged at 5,590g for 10 min, and then the obtained supernatant was filtered with Millipore membrane (0.22  $\mu$ m). 10  $\mu$ L of each sample was loaded onto the column and analyzed in duplicate. A high performance liquid chromatography (Waters 600, USA) with a TSKgel 2000SWXL column (7.8  $\times$  300 mm, Tokyo, Japan) was eluted with acetonitrile/water/trifluoroacetic acid (45/55/0.1 V/V/V) at a flow rate of 0.5 mL/min and monitored at 220 nm. The temperature of column was set at 30 °C. A molecular-weight calibration curve was obtained using the following standards: acetyl-acetamide ( $M_w$  189 Da), acetamide-arginine ( $M_w$  451 Da), bacitracin ( $M_w$  1,450 Da), aprotinin ( $M_w$  6,500 Da), and cytochrome C ( $M_w$  12,500 Da). The reference curve was  $\lg Y = -0.2281X + 6.696$ ,  $R^2 = 0.991$ , where Y is the molecular weight of sample (Da), X is retention time (min). The results were processed using Millennium32 Version 3.05 software (Waters Corporation, Milford, MA, USA).

### Thixotropic Behavior

The thixotropy was measured according to the method of Zhang *et al.* [15], with some modification. The thixotropy was determined at 25 °C with a controlled stress rheometer AR-G2 (TA Instruments, England). The rheometer was equipped with a stainless steel plane having a diameter of 40 mm. For the investigation of the thixotropic property of aqueous SPI solutions, the flow curves were measured by increasing the shear rate from a minimum of 2 s<sup>-1</sup> to a maximum of 200 s<sup>-1</sup> and then decreasing the shear rate in the same equal steps. The duration of shear at each step was about 3 min. If the system investigated exhibits the thixotropy, a hysteresis loop could be obtained from these “upward” and “downward” curves developed during round-trip shear stress–shear rate paths, and the corresponding enclosed area could be used to evaluate the magnitude of the thixotropy. Triplicate

determinations were performed for each sample. The results were processed using the function of “Best fit using Stress vs. Rate” by rheology advantage data analysis software.

#### Immunoreactivity Determination

##### (a) Soy protein isolate allergen extraction

Weigh 1 g of the sample into a plastic centrifugal tube. Add 19 mL of the sample extracting medium for soybean into the tube. The next operation parameters were according to the method of Li *et al.* [16].

##### (b) Sandwich Enzyme-linked immunoabsorbent assay (ELISA)

The allergen content was measured according to the method of Li *et al.* [16]. The absorbance at 450 nm was determined on a microtiter automated ELISA plate reader (SH-1000 Lab Microplate reader, Corona Electric, Japan). The standard curve equation was as follows:  $Y=0.0469X+0.0372$ ,  $R^2=0.991$ , where Y is the soy allergen content (mg/gprotein), X is the absorbance of sample extraction solution at 450 nm [16]. The concentration of the proteins was determined by the Bradford method [17], using BSA as the standard. Each sample was analyzed in duplicate.

#### Sequential *In vitro* Digestibility of Protein Procedure

The *in vitro* digestibility of the proteins was evaluated by the method of Tang, with some modification [18]. Pepsin (Sigma, P7000, 1:10,000, 600–1000 units/mg) and trypsin (Genview, DH355-1, 1:250) were utilized for *in vitro* digestion investigation. Briefly, 10 mL of 1 % (w/v) SPI solutions adjusted to pH 1.5 with 1 M HCl were pre-incubated in a water bath at 37 °C for 3–5 min. Then, an amount of pepsin (20 mg of pepsin/mL of 0.1 M  $\text{KH}_2\text{PO}_4$  buffer, pH 2) was added, and the ratio of enzyme to protein substrate was 1:100 (u/w). The mixtures of pepsin and protein solution were mixed well and incubated at 37 °C at 0, 1, 5, 10, 20, 30, 60 and 120 min periods of time, aliquots of the mixtures were taken, and the pH was adjusted to 7 with 1 M NaOH to stop the enzymatic reactions. Additionally, the final pepsin-digested hydrolysates (pH 7.0) were further digested by the addition of trypsin at an enzyme:substrate ratio of 1:20 (u/w) (20 mg of trypsin/mL of 0.1 M Tris-HCl buffer, pH 7.0) at 37 °C for 0, 1, 5, 10, 20, 30, 60 and 120 min, respectively. Each sample was analyzed in triplicate.

#### Nitrogen Release During Digestion

The % nitrogen release during digestion process was determined by the method of Tang, with some modification [18]. 10 mL of 10 % (w/v) trichloroacetic acid (TCA) were added into 10 mL of the digested mixtures

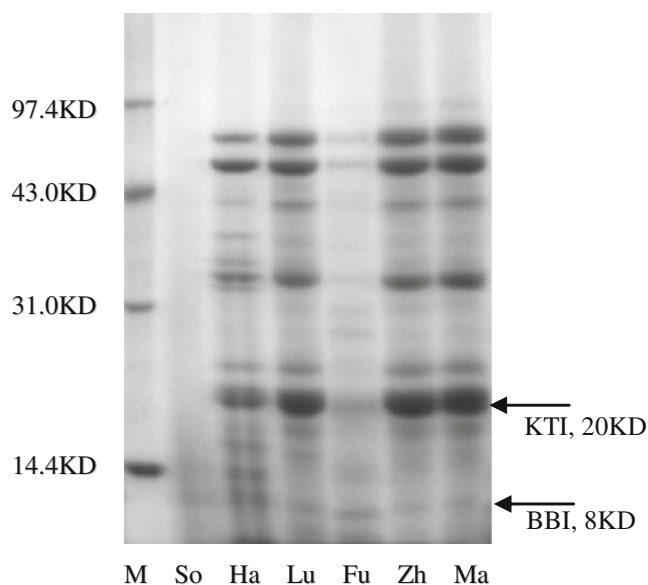


**Fig. 1** SDS-PAGE of SPIs (Lane distribution M: marker; So: Dupont Solae; Ha: Hagaoke; Lu: Luxin; Fu: Fuji; Zh: Dupont Zhengzhou; Ma: Mantianxue)

and the final concentration of TCA reached to 5 % (w/v). The mixtures were then centrifuged (8,000g, 30 min) to obtain the precipitates. After washed with 10 mL of TCA (10 %, w/v), the precipitates were obtained again by centrifugation at the same parameters. The N content of the samples was determined by Kjeldahl method ( $N \times 6.25$ ). The % N release during the digestion was calculated as  $\% \text{ N release} = (N_0 - N_t) \times 100 / N_{\text{tot}}$ , where t is the digestion time (min),  $N_t$  (mg) is the TCA-insoluble N after digestion for t (min),  $N_0$  (mg) is the TCA-insoluble N in the protein sample, and  $N_{\text{tot}}$  (mg) is the total N of protein sample. Triplicate determinations were performed for each sample.

**Table 1** Percentage of molecular weights of Fuji and Solae soy protein isolate

| Molecular weight (Mw/Da) | Molecular weight distribution (%) |             |
|--------------------------|-----------------------------------|-------------|
|                          | Solae                             | Fuji        |
| >10,000                  | 6.33±0.43a                        | 15.73±0.51b |
| 10,000–5,000             | 11.12±0.61a                       | 20.22±0.74b |
| 5,000–3,000              | 13.40±0.23a                       | 14.58±0.36a |
| 3,000–2,000              | 10.01±0.05a                       | 11.42±0.09a |
| 2,000–1,000              | 17.51±0.72a                       | 16.29±0.21a |
| 1,000–500                | 21.20±0.90a                       | 11.63±0.26b |
| 500–180                  | 16.59±0.27a                       | 6.49±0.08b  |
| <180                     | 3.84±0.05a                        | 3.64±0.07a  |

Means with different letters in the row are significantly different at the 5 % level. (n=2)

**Table 2** Analysis of rheology results by rheology advantage data analysis software

|                                  | Mantianxue | Hagaoke                 | Luxin                   | Dupont Zhengzhou | Fuji                   | Solae                   |
|----------------------------------|------------|-------------------------|-------------------------|------------------|------------------------|-------------------------|
| a: yield stress (Pa)             | 3.666      | $-2.329 \times 10^{-3}$ | $-8.684 \times 10^{-2}$ | 7.430            | $1.808 \times 10^{-2}$ | $-3.175 \times 10^{-3}$ |
| b: viscosity (Pa.s)              | 5.966      | $1.342 \times 10^{-2}$  | $2.278 \times 10^{-2}$  | 2.729            | $1.715 \times 10^{-3}$ | $2.944 \times 10^{-3}$  |
| c: rate index                    | 0.4367     | 0.9466                  | 0.9858                  | 0.5502           | 1.119                  | 1.042                   |
| thixotropy (Pa.s <sup>-1</sup> ) | 2384       | 150.4                   | 193.3                   | 2290             | 114.4                  | 109.6                   |
| standard error                   | 10.29      | 10.20                   | 9.245                   | 7.933            | 24.21                  | 12.32                   |

Herschel-Bulkley model equation  $\text{stress} = a + b \times \text{rate}^c$  Standard error was from the curve fitting. ( $n=3$ )

### Statistical Analysis

Data were analyzed using SPSS for Windows (version 15, SPSS Inc. Chicago, IL, USA) following a one-way analysis of variance (ANOVA) linear model. Mean comparisons were performed using the Duncan test, and the significant level was established for  $P < 0.05$ .

## Results and Discussion

### SDS-PAGE and Molecular Weight Distribution

Figure 1 showed a sharp decrease in molecular weight obtained from Solae and Fuji, in comparison to the other four SPIs. By comparing the densities of bands, the high and middle molecular weight subunit bands of Hagaoke were lighter than those of the other three SPIs, however the low molecular weight subunit bands of Hagaoke were deeper than those of the other three SPIs. These data indicated that Solae, Fuji and Hagaoke were treated by enzymatic hydrolysis. Furthermore, the degree of hydrolysis of Solae was higher than those of Fuji and Hagaoke. The result of molecular weight distribution (Table 1) also verified the above phenomenon. The total percentages of  $M_w$  between 10,000–5,000 and  $>10,000$  of Fuji accounted for 35.95 %, however, these percentages of Solae only reached 17.45 %. The percentages of  $M_w$  1,000–180 of Solae and Fuji were 37.79 and 18.12 %, respectively. This subunit distribution tendency was consistent with the statements of El-Agamy [19] and Terracciano *et al.* [20]. They stated that soy protein special for infant formula was hydrolyzed by proteolytic enzymes to develop a number of soy protein hydrolysates. The products had been classified according to the degree of protein hydrolysis as extensively or partially hydrolyzed protein products [19, 20].

### Thixotropy and Viscosity of Soy Protein Isolate

Thixotropy of a polymer solution is quantified by the solution's ability to regain its gel structure when the solution is allowed to rest for a longer period of time, which is usually

attributed to the breakdown of network links formed by the associations between polymer chains under shear [21]. To characterize the thixotropic property of aqueous SPI solutions, the “upward” and “downward” curves developed during round-trip stress–shear rate paths, and the areas of corresponding hysteresis loops were determined in Table 2. Six kinds of SPI solutions followed Herschel-Bulkley model. As can be seen, the hysteresis area of enzymatic hydrolyzed SPIs was remarkably smaller than that of the other three SPIs and the viscosity of hydrolyzed SPIs presented the similar tendency to thixotropy. Thixotropy of Fuji and Solae reduced around by 95 %, compared with those of Mantianxue and Dupont Zhengzhou. Low thixotropy value shows low difficulty or shear stress in swallowing for infants [11]. Viscosity, or resistance to flow, increases with polymer molecular weight. In other words, the ability of a polymer to flow decreases with increasing molecular weight [22]. Therefore, enzymatic hydrolysis may reduce the molecular weight, resulting in viscosity decreasing.

### Allergen Content of Soy Protein Isolate

The allergen contents of SPIs were 27.63, 18.76, 29.29, 28.15, 11.07 and 4.43 mg/g protein in the order of Mantianxue, Hagaoke, Luxin, Dupont Zhengzhou, Fuji and Solae. The allergen content of Fuji and Solae dropped by 60 and 84 % respectively, in comparison to that of Mantianxue. Figure 2 indicates that enzymatic hydrolysis was a valid method to decrease immunoreactivity of SPIs.

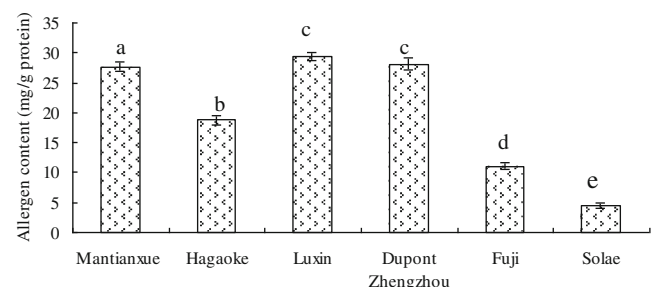


**Fig. 2** Allergen content of SPIs. Means with different letters in the column are significantly different at the 5 % level. ( $n=2$ )

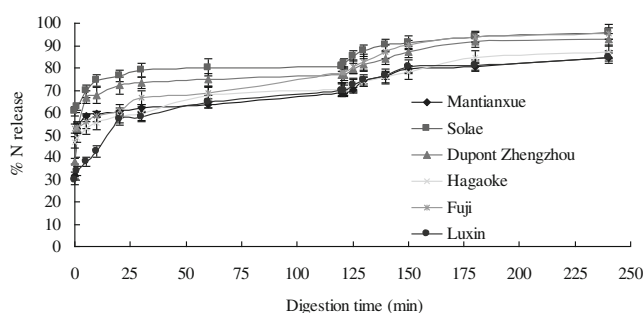


**Fig. 3** *In vitro* digestibility of SPIs ( $n=3$ )

The reducing efficiency of immunoreactivity would be closely related to the type of enzyme and degree of hydrolysis. This opinion was in accordance with the findings of Peñas *et al.* [23]. They stated that the combination of both enzymatic hydrolysis and high pressure treatment reduced the immunoreactivity of soy whey. Besides that, the best condition for the proteolysis and immunoreactivity depends on the type of enzyme used. Lee *et al.* [24] investigated the effect of peptic and chymotryptic hydrolysis on the immunoreactivity of 11S soybean globulin, which is the primary soybean allergen composed of acidic and basic polypeptides. They found that acidic polypeptides were effectively hydrolyzed, while basic polypeptides were more resistant to hydrolysis. Astwood *et al.* [25] and Moreno [26] reported a high stability (60 min) of beta-conglycinin (beta-subunit) and Kunitz-trypsin inhibitor against peptic digestion (pH 1.2). Soybean lectin was stable for 15 min, while the alpha-subunit of beta-conglycinin and Gly m Bd30K were completely abolished after 2 min and 30 s, respectively. In a word, various allergens of SPI exhibited different stability against peptic and chymotryptic digestion.

The molecular basis of changes in allergenicity is inactivation or destruction of epitope structures or formation of new epitopes or better access of cryptic epitopes by denaturation of native allergen. Thus, immunoreactivity may be unchanged, decreased or even increased by food processing [27].

#### *In vitro* Protein Digestibility of Soy Protein Isolate

The *in vitro* protein digestibility of SPIs was evaluated by TCA-soluble nitrogen release during digestion of pepsin and

trypsin in simulated gastric fluid. Figure 3 shows a typical profile of the nitrogen release of SPIs during sequential pepsin and trypsin digestion, as a function of digestion time, and the *in vitro* digestibility (% N release) at the end of pepsin (and trypsin) digestion for various SPIs is summarized in Table 3. The *in vitro* protein digestibility of Solae at the end of pepsin and trypsin digestion considerably increased by 11.7 and 11.3 %, respectively, in comparison to those of Mantianxue.

Firstly, the starting point variances in various SPIs may be ascribed to different solubilities at pH 1.5. Enzymatic hydrolysis could improve SPI solubility and widen SPI soluble pH scope. Therefore, the original digestion points of Fuji, Solae and Hagaoke are significantly higher than those of the other three SPIs. Secondly, during the digestion phase of pepsin, SPIs indicated specific linear hydrolysis time and slopes. The linear time of Mantianxue, Solae, Dupont Zhengzhou, Hagaoke, Fuji and Luxin was 1, 10, 5, 20, 30 and 20 min, respectively. The linear slopes of Mantianxue, Dupont Zhengzhou and Luxin were strikingly higher than those of the other three SPIs. Fuji showed the lowest linear slope. The discrepancy among SPIs may result from the distinctions in the susceptibility of their unique protein constituents to pepsin digestion [18]. Thirdly, upon further digestion by trypsin, the % N release of the pepsin-digested hydrolysates (with pH adjusted to 7) of SPIs increased continuously, but developed in particular ways (Fig. 3). The linear time of Mantianxue, Solae, Dupont Zhengzhou, Hagaoke, Fuji and Luxin was 30, 20, 60, 30, 30 and 30 min, respectively. The linear slopes did not exhibit significant differences between Mantianxue and Luxin. Similarly, Solae and Fuji did not present obvious variances in the linear slope. The latter two were considerably higher than the former two. Dupont Zhengzhou owned the lowest linear slope and the longest linear time. SDS-PAGE (Fig. 1) showed the presence of Kunitz and Bowman-Birk trypsin inhibitors (speculated by comparison with the literature [28]) in the six kinds of SPI. Additionally, the trypsin inhibitor activity existed in SPIs. The presence of trypsin inhibitors in beans [18] may account for the differences of trypsin digestion pattern among the above SPIs. Trypsin inhibitors interfere with the physiological functions of trypsin, which consist of both proenzyme activation and

**Table 3** *In vitro* protein digestibility of SPI at digestion time of 0, 120 and 240 min (%)

| Digestion time (min) | 0           | 120         | 240         |
|----------------------|-------------|-------------|-------------|
| Mantianxue           | 31.82±1.32a | 68.94±3.11a | 84.85±3.05a |
| Solae                | 60.49±2.07b | 80.64±2.43b | 96.13±2.73b |
| Dupont Zhengzhou     | 38.20±1.35c | 70.40±3.12a | 87.09±3.05c |
| Hagaoke              | 46.65±2.57d | 76.97±3.42c | 92.66±2.69d |
| Fuji                 | 53.01±2.11e | 77.82±2.17c | 95.42±3.17b |
| Luxin                | 29.69±1.78a | 69.98±2.65a | 84.82±2.65a |

Means with different letters in the row are significantly different at the 5 % level. ( $n=3$ )

hydrolysis of dietary proteins in the gut. Because of this double action, trypsin inhibitors are the main components claimed to be responsible for the poor digestibility of dietary proteins [29]. Overall, enzymatic processing may decrease the amount of high molecular weight subunits, which are partially cleaved into small proteic compounds, thereby helping the action of pepsin and trypsin [30].

## Conclusions

The discrepancies between subunit molecular weight distributions were analyzed by SDS-PAGE and gel size exclusion chromatography, demonstrating that Solae, Fuji and Hagaoke were treated by proteolytic enzymes. The thixotropy of Fuji and Solae markedly reduced around by 95 %, in contrast to those of Mantianxue and Dupont Zhengzhou. The allergen content of Fuji and Solae considerably decreased by 60 and 84 % respectively, compared with that of Mantianxue. The *in vitro* protein digestibility of Solae at the end of pepsin and trypsin digestion strikingly increased by 11.7 and 11.3 %, respectively, in comparison to those of Mantianxue. Connecting subunit molecular weight distributions with the above qualities of SPIs, the results indicated that suitable enzymatic hydrolysis was an optimal approach to improve some physical properties, immunoreactivity and *in vitro* protein digestibility of SPIs for infant formula. The lower thixotropy exhibits low difficulty or shear stress in swallowing for infants. The lower immunoreactivity will enhance the security of SPI for CMA babies.

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**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Bhatia J, Greer F, the Committee on Nutrition (2008) Use of soy protein-based formulas in infant feeding. *Pediatrics* 121(5):1062–1068
- Canadian Paediatric Society (2009) Concerns for the use of soy-based formulas in infant nutrition. *Paediatr Child Health* 14(2):109–113, PMID: PMC2661347
- Allesa MS, Scholtens PAMJ, Bindels JG (2004) Current trends in the composition of infant milk formulas. *Curr Paediatrics* 14(1):51–63
- American Academy of Pediatrics, Committee on Nutrition (2000) Hypoallergenic infant formulas. *Pediatrics* 106(2):346–349
- Klemola T, Vanto T, Juntunen-Backman K, Kalimo K, Korpela R, Varjonen E (2002) Allergy to soy formula and to extensively hydrolyzed whey formula in infants with cow's milk allergy: a prospective, randomized study with a follow-up to the age of 2 years. *J Pediatr* 140(2):219–224
- Osundahunsi OF, Aworh OC (2002) A preliminary study on the use of tempe-based formula as a weaning diet in Nigeria. *Plant Foods Hum Nutr* 57(3–4):365–376
- Fernandez DR, Vanderjagt DJ, Williams M, Huang YS, Chuang LT, Millson M, Andrews R, Pastuszyn A, Glew RH (2002) Fatty acid, amino acid, and trace mineral analyses of five weaning foods from Jos, Nigeria. *Plant Foods Hum Nutr* 57(3–4):257–274
- Pandelova M, Lopez WL, Michalke B, Schramm KW (2012) Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *J Food Compos Anal* 27(2):120–127
- Hernández-Ledesma B, Hsieh CC, de Lumen BO (2009) Lunasin and Bowman-Birk protease inhibitor (BBI) in US commercial soy foods. *Food Chem* 115(2):574–580
- Jovaní M, Barberá R, Farré R (2003) Effect of lactoferrin addition on the dialysability of iron from infant formulas. *J Trace Elem Med Biol* 17(2):139–142
- Igarashi A, Kawasaki M, Nomura SI, Sakai Y, Ueno M, Ashida I, Miyaoka Y (2010) Sensory and motor responses of normal young adults during swallowing of foods with different properties. *Dysphagia* 25(3):198–206
- Abdel-Aal ESM (2008) Effects of baking on protein digestibility of organic spelt products determined by two *in vitro* digestion methods. *LWT-Food Sci Technol* 41(7):1282–1288
- Tang CH, Sun X (2011) A comparative study of physicochemical and conformational properties in three vicilins from *Phaseolus* legumes: implications for the structure-function relationship. *Food Hydrocolloids* 25(3):315–324
- Zhang LM, Kong T, Hui PS (2007) Semi-dilute solutions of hydroxypropyl guar gum: viscosity behaviour and thixotropic properties. *J Sci Food Agric* 87(4):684–688
- Tang CH, Peng J, Zhen DW, Chen Z (2009) Physicochemical and antioxidant properties of buckwheat (*Fagopyrum esculentum* Moench) protein hydrolysates. *Food Chem* 115(2):672–678
- Li HJ, Zhu KX, Zhou HM, Peng W (2012) Effects of high hydrostatic pressure treatment on immunoreactivity and structural properties of soybean protein isolate for infant formula. *Food Chem* 132(2):808–814
- da Silva MAO, Arruda MAZ (2006) Mechanization of the Bradford reaction for the spectrophotometric determination of total proteins. *Anal Biochem* 351(1):155–157
- Tang CH (2007) Functional properties and *in vitro* digestibility of buckwheat protein products: influence of processing. *J Food Eng* 82(4):568–576
- El-Agamy EI (2007) The challenge of cow milk protein allergy. *Small Ruminant Res* 68(1–2):64–72
- Terracciano L, Isoardi P, Arrigoni S, Zoja A, Martelli A (2002) Use of hydrolysates in the treatment of cow's milk allergy. *Ann Allergy Asthma Immunol* 89(6 suppl 1):86–90
- Wei YP, Wang CS, Wu JSB (2001) Flow properties of fruits fillings. *Food Res Int* 34(5):377–381
- Gupta P, Elkins C, Long TE et al (2005) Electrospinning of linear homopolymers of poly(methyl methacrylate): exploring relationships between fiber formation, viscosity, molecular weight and concentration in a good solvent. *Polymer* 46(13):4799–4810
- Peñas E, Préstamo G, Polo F, Gomez R (2006) Enzymatic proteolysis, under high pressure of soybean whey: analysis of peptides and the allergen Gly m 1 in the hydrolysates. *Food Chem* 99(3):569–573
- Lee UW, Keum EH, Lee SJ, Sung DE, Chung DH, Lee SI, Oh S (2007) Immunoreactivity of proteolytic hydrolysates on the soybean 11S globulin. *J Food Sci* 72(3):C168–C172
- Astwood JD, Leach JN, Fuchs RL (1996) Stability of food allergens to digestion *in vitro*. *Nat Biotechnol* 14(10):1269–1273
- Moreno FJ (2007) Gastrointestinal digestion of food allergens: effect on their immunoreactivity. *Biomed Pharmacother* 61(1):50–60

27. Besler M, Steinhart H, Paschke A (2001) Stability of food allergens and immunoreactivity of processed foods. *J Chromatogr B* 756(1–2):207–228
28. Amnuaycheewa P, de Mejia EG (2010) Purification, characterisation, and quantification of the soy allergen profilin (Gly m 3) in soy products. *Food Chem* 119(4):1671–1680
29. Bajpai S, Sharma A, Gupta MN (2005) Removal and recovery of antinutritional factors from soybean flour. *Food Chem* 89(4):497–501
30. Correia I, Nunes A, Barros AS, Delgadillo I (2010) Comparison of the effects induced by different processing methods on sorghum proteins. *J Cereal Sci* 51(1):146–151

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