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Thermal stability of soy protein isolate and hydrolysate ingredients

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Abstract

The objective of this study was to characterize the effects of pH, protein concentration and calcium supplementation on thermal stability, at 140 °C, of soy protein isolate (SPI) and soy protein hydrolysate (SPH) ingredients. Increasing pH between 6.4 and 7.5 led to significantly ($p < 0.05$) higher mean heat coagulation times (HCTs) at 140 °C, for all soy protein ingredients at 1.8, and 3.6% (w/v) protein. Increasing protein concentration from 1.8 to 7.2% (w/v) led to shorter HCTs for protein dispersions. Calcium supplementation up to 850 mg/L, except in the case of supplementation of SPI 1 with calcium citrate (CaCit), decreased HCT for soy protein ingredient dispersions, at pH 6.4 – 7.5. No significant differences ($p < 0.05$) were found in mean HCT for dispersions supplemented with calcium chloride ($CaCl_2$) and those supplemented with CaCit at 450, 650 and 850 mg/L Ca^{2+}, in the pH range 6.4–7.5.

Keywords: Soy protein isolate; Soy protein hydrolysate; Heat coagulation time; Calcium supplementation

1. Introduction

During processing, food products containing SPI/SPHs are subjected to various thermal treatments to inactivate antinutritional factors, remove allergenic determinants, increase digestibility and to obtain desired functionality, such as solubility or textural properties (Mills, Huang, Noel, Gunning, & Morris, 2001). Many factors influence the thermal behavior of SPI/SPHs including pH, ionic environment, protein concentration and hydrolysis (Li et al., 2007).

Renkema, Lakemond, de Jongh, Gruppen, and van Vliet (2000) reported a decrease in the thermal denaturation temperatures for aqueous dispersions of SPI (12% (w/w) protein) and glycinin (0.3% (w/w) protein) during heating from 20 to 115 °C on reducing the pH from 7.6 to 3.8. At pH 3.8, the charge distribution differs from that at pH 7.6, and this may in turn lead to differences in quaternary structure and protein stability.

The thermal properties of soy proteins have been reported to be markedly sensitive to changes in ionic strength. Renkema et al. (2000) reported on the influence of ionic strength on the heat denaturation temperature (~88 °C) of glycinin at pH 7.6. Mills et al. (2001) reported that β-conglycinin aggregates only appeared at $I \geqslant 0.1$ during DSC analysis (on heating from 15 to 105 °C). Furthermore, at higher ionic strength ($I = 1.0$) the aggregates appeared to associate into clumps. However, in heat denaturation studies with 11S globulin and SPI, Kinsella (1979) and Wang and Damodaran (1991) observed that high ionic

Abbreviations: CaCit, calcium citrate; DH, degree of hydrolysis; DSC, differential scanning calorimetry; HCT, heat coagulation time; RP-HPLC, reversed-phase high performance liquid chromatography; SDS–PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; SPH, soy protein hydrolysate; SPI, soy protein isolate; TNBS, trinitrobenzene sulfonic acid; UHT, ultra-high temperature.

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strength (0.5–2.0 M NaCl/$NaClO_4$) seemed to stabilize the quaternary structure against dissociation and denaturation during heating (50–90 °C, pH 7.0). The inclusion of calcium in the form of $CaCl_2$ (0–30 mM Ca^{2+}) was shown to induce aggregation in SPI (0.5 mg/ml) on incubation at room temperature (pH 7.0). These aggregates could be resolubilized by the inclusion of citrate salts, due to their calcium chelating effects (Nagano, Horotsuka, Mori, Kohyama, & Nishinari, 1992).

Protein concentration has been shown in many studies to have a considerable influence on the thermal stability of soy globulins. At β-conglycinin concentrations higher than 0.4% (w/v), the formation of soluble macroaggregates following heating to 100 °C, at 10 °C/min, was observed by Mills et al. (2001). These results corresponded with those of Nagano et al. (1992) who found on heating a 10% (w/w) 7S globulin solution in 35 mM potassium phosphate buffer, pH 7.6, from 40 to 100 °C at a heating rate of 0.5–2.0 °C, that macroaggregates with molecular weights of between 4 and 8 million, rising to 80 million for the very large species, were formed.

Hydrolysis also affects the thermal stability of soy protein ingredients. At pH 4.5, SPI hydrolysates displayed greater heat stability (when placed in a boiling-water bath for 20 min) than intact SPI. The observed increase in heat stability was attributed to greater solubility of the hydrolysates at pH 4.5. However, below pH 3.5, SPHs displayed lower heat stability in comparison to SPI. However, the solubility of the hydrolysates was reported to be significantly lower than that of SPI at pH 3.5 (Kim, Park, & Rhee, 1990).

No studies to date appear to have analysed the heat stability of composite soy protein ingredient samples at 140 °C. Furthermore, no studies describe the comparative effects of ionic strength and protein concentration on the heat stability, at 140 °C, of SPH ingredients. Therefore, the objective of this study was to characterize the effect of pH, calcium, (i.e., $CaCl_2$ and CaCit) and protein concentration on the thermal stability properties of commercially available SPI and SPH ingredients.

2. Materials and methods

2.1. Materials

Two SPI (designated SPI 1 and SPI 2) and five SPH (designated SPH 1–5) ingredients were obtained from Dupont Protein Technologies (Paris, France) and were used without further purification. Molecular weight markers for polyacrylamide gel electrophoresis (PAGE) were from Sigma (Sigma Chemical, St Louis, MO, USA). Calcium chloride ($CaCl_2$) and calcium citrate (CaCit) were food-grade ingredients purchased from Merck, Sharp & Dohme, Inc. (Hoddesdon, Hertfordshire, UK).

Distilled water was used to prepare the aqueous protein solutions.

2.2. Compositional analysis

Moisture analysis was performed using IDF Standard 15B (1991). Samples (1.5 g) of SPI and SPH were dried to a constant weight at 70 °C under reduced pressure of 1000 mBar in a vacuum-heating oven (Model No: OVA031, Sanyo Gallenkamp plc, Middlesex, UK) for 24 h. The analysis was carried out in duplicate and values expressed as the mean ± SD.

Nitrogen content was determined using the macro-Kjeldahl procedure (IDF 20B, 1993). Protein content was calculated using a Kjeldahl factor of 6.25.

2.3. Degree of hydrolysis (DH) analysis

The trinitrobenzene sulfonic acid (TNBS) assay was used, in duplicate, as described by Spellman, McEvoy, O' Cuinn, & FitzGerald (2003). Unhydrolyzed SPI was used as 0% DH control.

2.4. Preparation of reconstituted protein ingredients

Reconstituted protein ingredients were prepared by adding appropriate amounts of SPI or SPH to distilled water at 70 °C to make 1.8, 3.6 and 7.2% (w/v) protein solutions. Subsequently, $CaCl_2$ or CaCit was added to the samples to achieve final calcium concentrations of 450, 650 and 850 mg/ml solution. Calcium supplemented samples were stirred for 1 h at room temperature, and then stored over a period of at least 15 h at 4 °C to ensure maximal hydration prior to heat stability analysis.

2.5. Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) of samples was performed using a Protean II xi Electrophoresis system, (Bio-Rad Laboratories, Herts, UK) according to the method of Laemmli (1970). SPI/SPH solutions (10 mg/ml protein) were prepared in SDS–PAGE sample buffer prior to electrophoresis.

2.6. Reversed-phase high performance liquid chromatography (RP-HPLC)

Samples were diluted to 1.8% (w/v) protein equivalent in H_2O, and filtered through 0.2 μm syringe filters (Whatman, Maidstone, Kent, UK). RP-HPLC was carried out according to the method of Flanagan and FitzGerald (2002).

2.7. Determination of heat stability

For heat stability studies, the pH of aliquots of the reconstituted aqueous soy protein ingredients (1.8, 3.6 and 7.2% (w/v) protein), following overnight hydration, were adjusted to between 6.4 and 7.5, using 0.1 M and 1 M HCl or NaOH. Samples were then allowed to equilibrate for at least 1 h at room temperature. The pH of the

samples was then re-measured and re-adjusted, if necessary, prior to heat stability analysis. Immediately after final pH adjustment, samples (2 ml) were placed in glass tubes (10 mm i.d. × 120 mm, AGB Scientific, Dublin, Ireland), sealed with silicone bungs, immersed in an oil bath thermostatically controlled at 140 °C (Elbanton BV, Kerkdriel, The Netherlands), with continuous rocking at motor speed setting 3. The heat coagulation time (HCT) was taken as the length of time in minutes that elapsed between placing the sample in the oil bath and the onset of coagulation (McSweeney, Mulvihill, & O'Callaghan, 2004). These analyses were performed in triplicate.

2.8. Differential scanning calorimetry (DSC)

DSC measurements were performed using a PerkinElmer Model Pyris 1 calorimeter (PerkinElmer Life and Analytical Sciences, Milan, Italy). The calorimeter was calibrated using an indium standard. Protein samples for analysis were dissolved in distilled water (3.6% (w/v) protein) where approximately 20 mg of each solution was contained in stainless steel sample pans, which were sealed using o-rings (PerkinElmer Life and Analytical Sciences). A hermetically sealed stainless steel pan containing 19.3 mg distilled water was used as reference. Baselines were established using references and were subtracted from all thermal curves. Calorimetric measurements were carried out using independent duplicate analyses, under nitrogen at 30 psi. Reconstituted SPI 1 and SPH 3 (3.6% (w/v) protein) samples were heated from 30 to 140 °C (phase 1 heating), held for 1 min at 140 °C, cooled to 30 °C, and again reheated to 140 °C (phase 2 heating). The heating/cooling rate in all cases was 5 °C min^{-1}.

2.9. Statistical analysis

Significant differences were determined by non-parametric tests (SPSS version 13.0). Treatments were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Compositional analysis

The compositional characteristics of the SPI and SPH ingredients are summarized in Table 1. Moisture content varied between 3.55% for SPH 4 and 4.96% for SPH 5. Protein content varied between 75.96% for SPH 5 and 86.93% for SPH 3. The DH of the hydrolyzed samples varied between 1.37% for SPH 2 and 6.02% for SPH 5. Intrinsic [Ca^{2+}] values for samples varied between 0.2% for SPI 1, SPH 1 and SPH 3 and 3.0% for SPI 2, SPH 2 and SPH 4. Calcium phosphate (Ca_2PO_4) was combined with the ingredient samples SPI 2, SPH 2 and SPH 4 through a patented process that is claimed to minimize mineral sedimentation (Dupont Protein Technologies., 1999) in finished products and deliver a calcium to protein ratio (35:1) equivalent to cow's milk (Walstra & Jenness, 1984).

3.2. SDS–PAGE

The distribution of individual intact proteins and peptides in SPI and SPH was investigated by SDS–PAGE (Fig. 1). SPI samples (SPI 1 and SPI 2) displayed five major protein bands eluting at positions equivalent to molecular masses of approx. 85, 76, 60, 34, and 18 kDa (lane no.s 3, 4, 9 and 10). The first three high molecular weight bands

Table 1
Properties of soy protein isolate (SPI) and soy protein hydrolysate (SPH) ingredients

Sample code	Protein (%) (mean ± S.D.)	Moisture (%) (mean ± S.D.)	DH[a] (%) (mean ± S.D.)	Intrinsic[b] [Ca^{2+}] (%)	Reconstitution pH	Relevant technological data[b]
SPI 1	86.09 ± 0.28	4.59 ± 0.11	0	0.2	7.20	Isolated soy protein
SPI 2	80.94 ± 0.33	3.74 ± 0.28	0	3.0	6.99	Isolated soy protein Low in sodium Contains stabilized Ca_2PO_4 Less than 2% lecithin
SPH 1	86.68 ± 0.33	3.94 ± 0.01	1.44 ± 0.19	0.2	7.54	Isolated soy protein product Less than 2% lecithin
SPH 2	79.23 ± 0.20	4.19 ± 0.03	1.37 ± 0.51	3.0	7.63	Isolated soy protein product Contains stabilized Ca_2PO_4 Less than 2% lecithin
SPH 3	86.93 ± 0.23	3.52 ± 0.06	1.82 ± 0.18	0.2	7.07	Isolated soy protein product
SPH 4	80.21 ± 0.23	3.55 ± 0.23	1.92 ± 0.16	3.0	7.08	Isolated soy protein product Low in sodium Contains stabilized Ca_2PO_4 Less than 2% lecithin
SPH 5	75.96 ± 0.08	4.96 ± 0.04	6.02 ± 0.30	2.8	6.75	Isolated soy protein product

[a] Degree of hydrolysis.
[b] Details provided by the manufacturers.

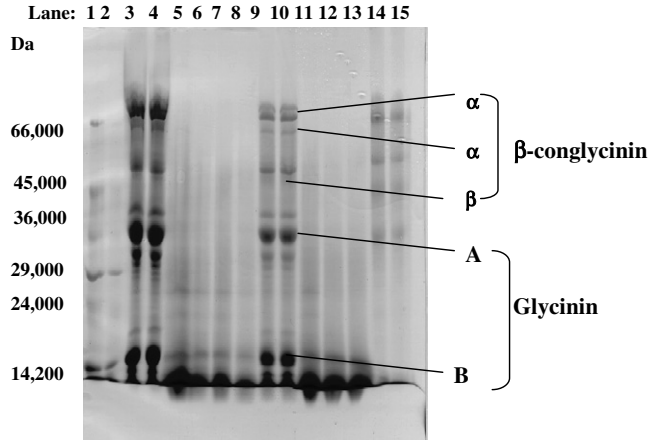

Fig. 1. SDS–PAGE Commassie blue stained profiles of commercial soy protein isolate (SPI) and soy protein hydrolysate (SPH) ingredients: 7S subunits (α′, α and β) and 11S polypeptides (A and B) are indicated. Low molecular weight markers (lanes 1–2), SPI 1 (lanes 3–4), SPH 1 (lanes 5–6), SPH 2 (lane 7), SPH 3 (lane 8), SPI 2 (lanes 9–10), SPH 4 (lane 11), SPH 5 (lanes 12–13), high molecular weight markers (lanes 14–15).

at approximately 85, 76 and 60 kDa correspond to the subunits of β-conglycinin (7S globulin). The observed molecular masses are in general agreement with the values for the α (85 kDa), α′ (76 kDa) and β (60 kDa) subunits as given by Beachy, Jarvis, and Barton (1981) and Kim et al. (1990). The two other bands having molecular masses of approximately 34 and 18 kDa correspond to the acidic (34 kDa) and basic polypeptides (18 kDa), respectively of glycinin (11S globulin), (Kim et al., 1990). Considerable differences in the electrophoretic patterns between the SPI and SPH samples were observed; bands representing intact α, α' and β subunits (85, 76 and 60 kDa) and the11S-glycinin, acidic polypeptide (34 kDa) were not observed in the hydrolysates (lanes 5–8 and lanes 11–13). However, the hydrolyzed samples contained some intact 11S glycinin, basic polypeptide (18 kDa). This may be due to a difference in compactness of structures between the two polypeptides; i.e., basic polypeptides are more hydrophobic (Beachy et al., 1981) and hence may be more compact, making them less accessible to proteolytic attack. This was also observed by Kim et al. (1990) when SPI was hydrolyzed with Alcalase™, α-chymotrypsin, or trypsin. They also reported a decrease in the intensities of the bands with increasing DH. In this study, the basic polypeptide fraction (18 kDa), i.e., in the hydrolyzed samples, also seemed quite resistant to enzyme attack. Adler-Nissen (1976) reported that the relative resistance of different proteins to proteolysis is generally explained by the compact tertiary structure of the protein, which protects most of the peptide bonds from enzyme digestion.

Distinct differences in the 27–30 kDa molecular mass region are evident between SPH 3 (lane 8) and SPH 5 (lanes 12 & 13), and the other hydrolyzed samples. While SPH 1 (lanes 5 & 6), SPH 2 (lane 7), and SPH 4 (lane 11) contain Commassie blue stained bands corresponding to approximate molecular masses of 27 and 30 kDa, probably representative of degradation products of larger proteins, these bands are not present in SPH 3 and SPH 5. These differences are probably related to the higher DH values i.e., 1.82 and 6.02%, respectively, for SPH 3 and SPH 5, compared to DH values of 1.44, 1.37, and 1.92%, respectively, for SPH 1, SPH 2 and SPH 4 (Table 1).

3.3. Reversed-phase HPLC profiles of soy protein ingredient samples

Reversed-phase HPLC profiles for various samples (Fig. 2) demonstrate considerable variation in peptide composition of soy protein isolate and hydrolysate ingredients. Some differences were observed in the RP-HPLC profile of the two SPI samples (Fig. 2a and b), i.e. peaks with a retention time within the range 38–48 min were more numerous for SPI 2. However, peaks with a retention time of 51 and 57 min. were observed in SPI 1, which were not present in SPI 2. This may be due to differences in the manufacturing procedure, as SPI 2 was manufactured using a process to incorporate calcium with this protein ingredient (Dupont Protein Technologies, 1999). It was seen that SPH 5 had a high content of hydrophilic peptide material, i.e., peptide material eluting at low acetonitrile concentration (Fig. 2g). It is presumed that the DH (6.02%) of this sample compared to that of the other samples (all with DHs in the region of 0.00–2.04%, Table 1) may have given rise to the higher content of short-chain, hydrophilic peptide material. Peaks eluting between 50 and 60 min for SPI 1 and SPI 2 (Fig. 2a and b) presumably correspond to intact soy protein since peaks in this region are absent in the SPH samples (Fig. 2c–f).

3.4. Effect of pH on heat coagulation time (HCT)

Fig. 3a–c shows the effect of pH on HCT at 140 °C of 1.8, 3.6 and 7.2%, (w/v) dispersions of different SPI and SPH ingredients. As pH increases, HCT increased significantly particularly for dispersions at 1.8% (w/v) protein. For example, SPI 1 had a mean HCT at pH 6.4 of 14.5 ± 0.3 min, compared to 108.9 ± 0.3 min at pH 7.5 (Fig. 3a). At pH 7.5, 1.8% (w/v) dispersions of SPH 1 were minimally stable (23.16 ± 1.53 min), SPI 2 and SPH 3 had intermediate heat stability (77.05 ± 1.45 and 66.97 ± 0.26 min, respectively) and SPI 1, SPH 2 and SPH4 had highest heat stability (108.93 ± 0.28, 118.23 ± 2.24 and 138.63 ± 1.21 min, respectively).

The trend in these HCT results are consistent with those reported by Mohamed and Xu (2003), where native soy protein was shown to be more heat stable at alkaline pH (pH 9.0) than at acidic pH (pH 3.8). Renkema et al. (2000) also found that lowering pH from 7.6 to 3.8 caused the denaturation temperature of glycinin and SPI to shift to lower values. Similar results were reported by Hermansson (1978) for SPI and by Iwabuchi, Watanabe, and Yamauchi (1991) and Nagano et al. (1992) for β-conglycinin.

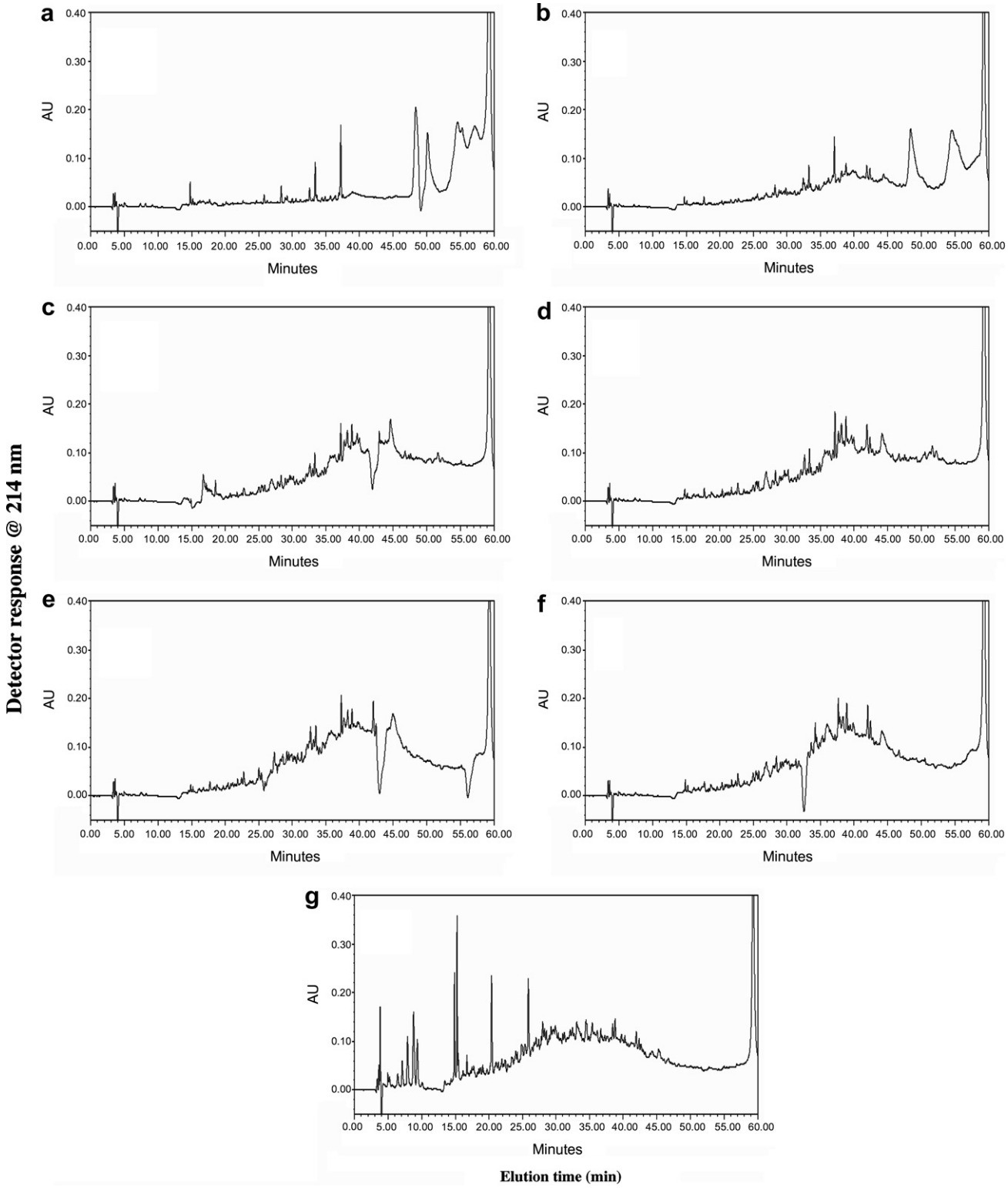

Fig. 2. Reversed-phase HPLC profiles of soy protein isolate (SPI) and soy protein hydrolysate (SPH) ingredient samples, (a) SPI 1, (b) SPI 2, (c) SPH 1, (d) SPH 2, (e) SPH 3, (f) SPH 4 and (g) SPH 5.

Significant differences in HCT for 3.6% (w/v) protein dispersions due to variation in sample pH were only observed where sample pH was greater than 6.9 (Fig. 3b). At 7.2% (w/v) protein (Fig. 3c), no significant difference in HCT was observed due to variation of sample pH in the range pH 6.4–7.5.

In general, no statistically significant differences were found in sample dispersion HCT as a function of hydrolysis,

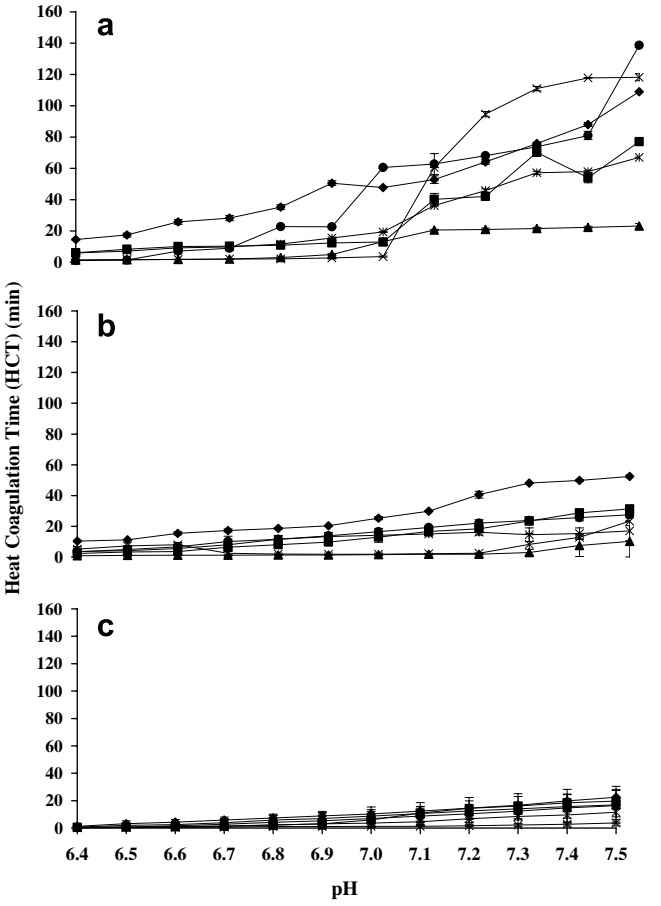

Fig. 3. Heat coagulation time (HCT, min) at 140 °C as a function of pH for soy protein isolate (SPI 1(♦) and SPI 2(■) and hydrolysate (SPH 1(▲), SPH 2(X), SPH 3(✱) and SPH 4(●) ingredients at (a) 1.8 (b) 3.6 & (c) 7.2% (w/v) protein. (Data points plotted are means ± S.D. of independent triplicate determinations).

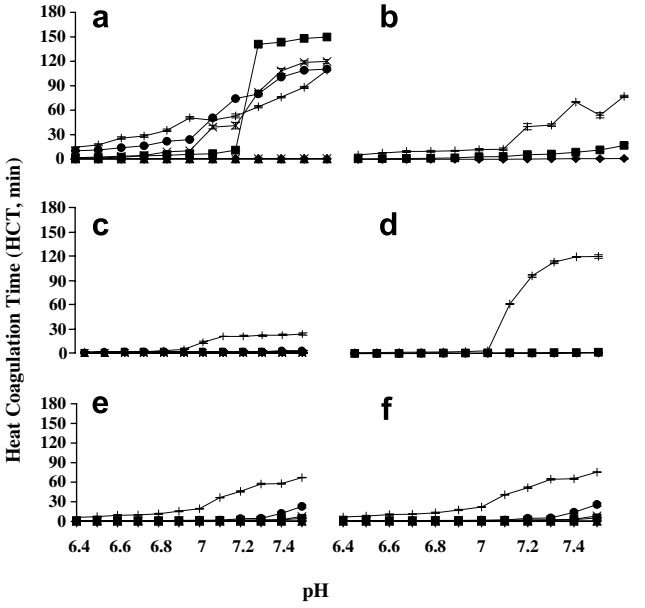

Fig. 4. Heat coagulation time (HCT, min) at 140 °C, as a function of pH and calcium supplementation level (+ 0 mg Ca^{2+}/L, ✱ 450 mg Ca^{2+}/L (chloride salt), ● 450 mg Ca^{2+}/L (citrate salt), ♦ 650 mg Ca^{2+}/L (chloride salt), x 650 mg Ca^{2+}/L (citrate salt), ♦ 850 mg Ca^{2+}/L (chloride salt), and ■ 850 mg Ca^{2+}/L (citrate salt), for aqueous solutions (1.8% (w/v)) of commercially available soy protein isolate (SPI) ingredients, (a) SPI 1, (b) SPI 2 and soy protein hydrolysate (SPH) ingredients, (c) SPH 1, (b) SPH 2, (c) SPH 3 and (d) SPH 4. (Values plotted are means ± S.D. of independent triplicate analysis).

i.e., when comparing the HCTs of the SPI and SPH samples. This result is in contrast to the findings reported by Kim et al. (1990) where, at pH 4.5, SPI hydrolysates displayed greater heat stability than intact SPI, when heated in a boiling-water bath for 20 min. However, the lack of comparability may be attributed to the fact that measurement of heat stability was performed in a different pH region (6.4–7.5) in the present study.

3.5. Effect of protein concentration on HCT

Comparison of results of mean HCT values for 1.8, 3.6 and 7.2% (w/v) soy protein/hydrolysate solutions across the pH range studied (6.4–7.5) indicates that increasing protein concentration results in significantly reduced heat stability. This is probably due to the resultant increase in protein–protein interactions, promoting aggregation in the presence of heat. For example, for SPI1, at pH 7.5, the mean HCT at 140 °C decreased from 108.90 ± 0.28 min at 1.8% (w/v) protein (Fig. 3a) to 52.47 ± 0.95 min at 3.6% (w/v) protein (Fig. 3b) and to 22.48 ± 4.97 min at 7.2% (w/v) protein (Fig. 3c). As protein concentration increases, protein–protein interactions become dominant, promoting heat induced coagulation (Wagner, Sorgentini, & Añón, 1992).

3.6. Effect of addition of calcium salts on heat coagulation time

The intrinsic [Ca^{2+}] in the test samples is summarized in Table 1. Calcium supplementation of aqueous solutions (1.8% (w/v) protein) significantly decreased mean HCT at each pH value over the pH range studied (pH 6.4–7.5) for the SPI (with an exception in the case of supplementation of SPI 1 with CaCit) and the SPH ingredients, (Fig. 4a–f). For example, in the case of SPI 2 at pH 6.4, the mean HCT was reduced from 6.1 ± 0.9 min for un-supplemented aqueous solution to 0.4 ± 0.2 min for the aqueous solution supplemented to 850 mg/L Ca ($CaCl_2$) and 1.1 ± 0.1 min for the solution supplemented to 850 mg/L Ca (CaCit), (Fig. 4b). At pH 7.5, the mean HCT for SPI 2 was reduced from 77.1 ± 1.5 min for the un-supplemented aqueous solution to 1.5 ± 0.8 min for the solution supplemented to 850 mg/L Ca ($CaCl_2$) and to 17.4 ± 2.3 min for the aqueous solution supplemented to 850 mg/L Ca (CaCit) (Fig. 4b). These results are probably due to shielding of the negatively charged surface by Ca^{2+} inducing a reduction in electrostatic repulsion between protein molecules thus enhancing their aggregation (Iwabuchi et al., 1991; Li et al., 2007; McCrae C.H., 1995). Due to the charge distribution at low ionic strength, molecules can aggregate only when they

Table 2
Summary of the prominent endothermic denaturation transitions observed during differential scanning calorimetry of soy protein isolate (SPI 1) and hydrolysate (SPH 3) samples at 1.8 % (w/v) protein

Sample	Peak no.	Heating phase[a]	Onset temp. (°C)	Delta H (J/g)	Max peak temp. (°C)	Peak breadth (°C)	End temp. (°C)
SPI 1 (30–140 °C)	1	1	75.99	661.13	91.4	33.08	109.07
	2	2	75.98	12.22	76.59	2.43	78.41
	3	2	87.25	291.7	98.88	30.19	117.44
SPH 3 (30–140 °C)	1	1	78.80	92.15	83.46	15.46	94.26
	2	1	116.2	11.53	118.46	3.71	119.91

The heating/cooling rate was 5 °C min^{-1}. Phase 1 refers to the initial heating of samples from 30 to 90 °C. Phase 2 refers to the second heating of samples from 30 to 90 °C.

[a] Heating phase 1: Initial heating of ingredient dispersions from 30 to 140 °C at a heating rate of 5 °C min^{-1}. Heating phase 2: Second heating of ingredient dispersions from 30 to 140 °C at a heating rate of 5 °C min^{-1}.

are specifically oriented, however, at higher ionic strength, aggregation can occur more randomly (Schokker, Singh, Pinder, & Creamer, 2000).

In contrast to the above results, Hashizume, Nakamura, and Watanabe (1975) found that 11S globulins were stabilized against thermal aggregation on heating, up to 80 °C, by high ionic strength solutions, whereas at low ionic strength aggregation occurred rapidly. However, the present study was performed at 140 °C. 7S globulins were reported to be more stable at low ionic strength, while aggregation was accelerated at high ionic strength (Mills et al., 2001). However, in a heat denaturation study of soy globulins in soybean seeds, Hermansson (1978) and Kinsella (1979) reported that increasing NaCl concentrations from 0.05 to 2.0 M increased the temperature of denaturation of 7S from 77 to 100 °C and of 11S from 92 to 113 °C at pH 7.0. Wang and Damodaran (1991) also reported the stabilization of SPI ingredients against denaturation at 0.5–2.0 M $NaCl/NaClO_4$ during heating at 50–90 °C (pH 7). No significant differences were found in mean HCT for protein/hydrolysate dispersions supplemented with calcium chloride ($CaCl_2$) and those supplemented with CaCit at 450, 650 and 850 mg/L Ca^{2+}, in the pH range 6.4–7.5.

In the present study, the results obtained with SPI 1 proved to be an exception in that at pH $\geqslant$ 6.5, supplementation to 450, 650 and 850 mg/L Ca (CaCit) significantly increased HCT, at 140 °C. At, pH 7.5, supplementation with CaCit increased the mean HCT, from 108.93 ± 0.3 min for un-supplemented aqueous solutions to 119.7 ± 2.3 and 149.8 ± 1.0 min for aqueous solutions supplemented to 650 and 850 mg/L Ca^{2+}, respectively (Fig. 4a). The reason for this is unclear and warrants further study.

3.7. Summary of the prominent denaturation transitions observed during differential scanning calorimetry (DSC)

A summary of the results obtained for one SPI and one SPH ingredient at 3.6% (w/v) protein can be seen in Table 2. Upon initial heating between 30 and 140 °C (phase 1), one denaturation transition was observed for SPI 1 with onset, maximum peak and end temperatures occurring at 75.99, 91.4 and 109.07 °C, respectively, with an enthalpy input of 661.13 J/g. For SPH 3, two denaturation transitions were observed during heating phase one (Table 2). In order to eliminate the influence of the thermal history of the samples, transition temperatures are usually determined in the second heating phase (Tang, Choi, & Ma, 2007). During the second heating phase between 30 and 140 °C (phase 2), maximum peak temperatures observed in the profile of SPI 1 in this study were close to those attributed to β-conglycinin (76.7 °C) and glycinin (94.1 °C) components by Tang et al. (2007) using 2.0 mg SPI (97.0% protein) and 10 μl of 0.05 M phosphate buffer (pH 7.0) in hermetically sealed aluminium pans heated from 25 to 110 °C at a rate of 5 °C/min. No denaturation transitions were observed for SPH 3 during the second heating phase (Table 2). It can, therefore, be assumed that the transition temperatures observed in the present study relate to the denaturation temperatures of 7S/11S globulins in the case of SPI 1, and the 11S globulin basic polypeptide fraction in the case of SPH 3, as SDS–PAGE electrophoresis showed that all 7S globulin and 11S acidic polypeptide subunits had been hydrolyzed in the SPH 3 sample (Fig. 1).

This study appears to be the first with regard to analysis of the effects of ionic strength and protein concentration on the thermal stability, at 140 °C, of SPH ingredients. The results herein should be relevant in understanding the behavior of soy protein ingredients during the manufacture of UHT and retort treated soy protein ingredient based infant and consumer products.

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