Physicochemical and functional property changes in soy protein isolates stored under high relative humidity and temperature

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Abstract The effects of water activity (*aw*) and temperature during storage on the physicochemical characteristics and functional properties of soy protein isolate (SPI) were investigated. SPI was stored with two different temperatures (25, 45 °C) and two levels of water activity (0.25, 0.75) for 224 days. During the 224-day storage period, all the samples showed decreases in gel hardness, emulsifying stability, foaming properties, viscosity, solubility, and color alteration, but increased in surface hydrophobicity (R_{So}). These alterations were stronger when stored at 45 °C than at 25 °C and in 0.75 *aw* than 0.25 *aw*, and most pronounced at 45 °C and 0.75 *aw*. Our results revealed that storage conditions - temperature and water activity - will indeed affect the functional properties of soy protein isolates.

Keywords Soy protein isolate · Storage · Water activity · High temperature · Functional property

Introduction

Thousands tons of soy protein isolate (SPI) are used worldwide as a functional ingredient in the food industry. The main

functional properties of soy proteins are hydration capacity, solubility, colloidal stability, gelation, emulsification, foaming and adhesion/cohesion (Morr 1990). The functional properties of proteins are controlled largely by their chemical and physicochemical properties, and these in turn are influenced by their composition and conditions of processing and storage (Hsu and Fennema 1989; Martins and Netto 2006; Schmidt et al. 1984).

Kehrberg and Johnson (1975) found that the browning rate of dried sweet cheese whey powder stored at room temperature increased with increasing moisture content over the range of 3.6–5.5 %. Hsu and Fennema (1989) monitored the changes in functional properties and development of browning in dry whey protein concentrate (52 %) for 6 months and found that storage temperature and time were most important and water activity was secondary.

The two major physicochemical factors that alter the rate of the Maillard reaction are temperature and water content as a function of water activity (*aw*). The Maillard reaction has an activation energy of 25–40 kcal/mol, suggesting that reaction rates increase by 3- to 8-fold for every 10 °C rise in temperature (Q10) (Labuza and Saltmarch 1981). When *aw* is higher than the monolayer moisture content (*aw* ~0.2), the rate of browning increased approximately 2–3 times for every 0.1 *aw* increase. If temperature and *aw* effects are combined, storage at 35 °C and 50 % RH could increase the rate up to 600-fold in comparison to the more favorable conditions of 20 °C and 20 % RH. Thus, conditions that occur in many food storage environments may be detrimental to the products (Davies et al. 1998). However, in many food studies, storage conditions are not given consideration as a factor that could influence results.

Water activity is known to be a fundamental parameter for the stability of foods, so the knowledge of safe moisture values for protein isolates would be useful shelf-life

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information. Pinto et al. (2005) studied the effects of one year of storage at different temperatures and one month of storage at different water activities on the content and profile of isoflavones and antioxidant activity of SPI. They found that storage for up to one year at temperatures from -18 to 42 °C had no effect on the total content of isoflavones, but the profile changed drastically at 42 °C, with a significant decrease in the percentage of malonylglucosides and a proportional increase in alpha-glucosides.

The objectives of this study were to determine the effects of storage temperature and water activity on the physicochemical and functional properties of soy protein isolates. This information would be useful for improving soy products used in food.

Material and methods

Materials

Soy protein isolate (SPI) ADM974 was provided by Archer Daniels Midland Company (Decatur, IL, USA). The protein isolate had $4.8\% \pm 0.32$ moisture content, $92.00\% \pm 0.33$ crude protein, $0.01\% \pm 0.01$ crude fat, and $3.35\% \pm 0.01$ ash. In order to obtain samples with the desired aw, 300 g of the original SPI with an aw of 0.25 (SPI-0.25) was sprayed evenly with 30.6 g water (12.6 % final moisture content) and mixed well. The moistened SPI was then sealed in the pouch and stored at 5 °C for several days to assure that moisture equilibrium occurred throughout the sample, thus obtaining an SPI having an aw of 0.75 (SPI-0.75). The water activity of SPI-0.75 was then determined with a water activity and moisture meter (Hygrolab 2, Rotronic AG, Bassersdorf, Switzerland). Measurements were done in triplicate at 25 °C.

Storage experiment

Samples (300 g) of SPI-0.25 and SPI-0.75 were separately packaged in sealed laminated pouches (30×20 cm, Ny + LLDPE). The pouches were incubated separately at 25 and 45 °C in different incubators for 224 d and removed every 28 d. SPI samples were examined with respect to color, gel hardness, emulsifying stability index, foaming properties, viscosity, nitrogen solubility index, and surface hydrophobicity.

Color analysis

The color of the SPI samples was measured by the modified method of Shih et al. (2009). The Hunter color of the SPI samples was determined with a colorimeter (Nippon Denshoku Kogyo Co., Tokyo, Japan). The reported values are the averaged values of four duplicate tests for each sample. Results were expressed as tri-stimulus values (L: lightness

[0 = black, 100 = white], a: -a = greenness, +a = redness, and b: -b = blueness, +b = yellowness) of the Hunter color scale. The instrument was set at the reflectance mode. A numerical total color difference (ΔE) was calculated as

$$\Delta E = \left[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2 \right]^{1/2}$$

where L_0 , a_0 , and b_0 are the Lab values of reference samples, which herein are the control sample's Lab values.

Gel hardness

Gels were prepared according to Martins and Netto (2006) with some modifications. Suspensions (pH 7.0) containing 100 ml of 20 % protein in a 100 ml beaker were stirred for 10 min. The beakers were sealed, heated at 90 °C for 15 min in a water bath, and then cooled to 25 °C. Samples were then stored for 24 h in a refrigerator at 6 °C before being analyzed for texture. Gel texture profiles were determined using a TA.XT-2 Texture Analyser (Stable Microsystems, Surrey, UK). The analyses were carried out at 25 °C. Gels were compressed twice to 60 %. The operating apparatus was a cylindrical probe having a 10 mm diameter (p/10), test speed was 1.0 mm/s, and the applied force was 25 kg. Hardness, defined according to Bourne (1982), was measured.

Emulsifying stability index

The emulsifying properties of SPI samples were determined by the turbidometric method of Guo et al. (2007). To prepare the emulsions, 20 ml of pure soybean oil and 20 ml of each sample solution (0.5 % protein) in 0.1 M phosphate buffer (pH 7.0) were mixed together in a 100 ml beaker at 25 °C and homogenized with a Polytron homogenizer (Model PT 1200E, equipped with a high-foam PT-DA 1205/2EC-E generator) (Kinematica AG, Luzern, Switzerland) at maximum speed ($\sim 20,000$ rpm). The emulsion was immediately transferred into a 15 ml plastic beaker (dia. 3.0 cm). Aliquots of freshly prepared emulsion (50 ml) were taken 0.5 cm from the bottom of the beaker and dispersed into 5 ml of 0.1 % sodium dodecyl sulfate (SDS) solution. Absorbance was measured at 500 nm against a 0.1 % SDS solution blank in a spectrophotometer. Emulsions were kept undisturbed for 10 min at 25 °C and then 50 ml aliquots were taken 0.5 cm from the bottom of the beaker and dispersed into 5 ml of 0.1 % SDS solution. Absorbance was measured at 500 nm as previously described. Emulsion stability was expressed as an index, ESI (%), of the homogenized samples, defined as

$$ESI = (A_{10}/A_0) \times 100\%$$

where A_{10} and A_0 represent the absorbance at 500 nm after 10 min and time zero, respectively.

Foaming properties

The foaming properties [foaming capacity (FC), foam maximum density (FMD) and foam drainage stability (FS)] of the samples were evaluated by the high speed agitation method described by Agyare et al. (2009). First, 0.4 g SPI sample was dissolved in 20 ml of distilled water. The sample was then introduced into a 100 ml plastic measuring cylinder and blended with a Polytron homogenizer (Model PT 1200E equipped with a high-foam PT-DA 1205/2EC-E generator) (Kinematica AG, Luzern, Switzerland) at maximum speed (~20,000 rpm). The total volume of foam generated was measured immediately and FC was computed according to Motoi et al. (2004). FC was used to calculate FMD using the formula of Ru'iz-Henestrosa et al. (2007a). Foam was allowed to stand undisturbed for 10 min and the volume of liquid that was then drained from the foam was used as the indicator of foam drainage stability (FS) (Mimouni et al. 1999).

Viscosity

The viscosity of SPI was measured in 20 % (w/w) dispersions in distilled water. Viscosity was measured at 25 °C in a Brookfield Viscometer RVT using a Helipath stand, 93-spindle series, at 10 rpm. Values are expressed as centipoises (cp).

Nitrogen solubility index

The NSI of SPI samples was measured by method modified from Pinto et al. (2005). Five grams of each sample were dispersed in 200 ml of water by stirring in a water bath at 30 °C for 120 min, then centrifuged at 400 g for 10 min. Total and soluble nitrogen (supernatant) were determined with the micro-Kjeldahl method (AOAC 2000) in triplicate using a conversion factor of 6.25. The correlation between total and soluble nitrogen fractions allows the calculation of solubility with the formula

$$\text{NSI}(\%) = (\text{NS}/\text{NT}) \times 100\%$$

where NS is soluble nitrogen and NT is total nitrogen.

Surface hydrophobicity (RSo)

The hydrophobicity of SPI was determined with a hydrophobicity fluorescence probe ANS (8-anilino-1-naphthalene sulfonic acid) according to Zhang et al. (2005). Samples of SPI were diluted to 1 mg/ml in 0.01 M phosphate buffer at pH 7.0 and serially diluted with the same buffer to obtain protein concentrations of 0.00625–0.1 mg/ml. Then, 10 µl of ANS (8.0 mM in 0.1 M phosphate buffer, pH 7) was added to 2 ml of the sample. Fluorescence intensity (FI) was measured with a Varioskan Flash spectral scanning multimode reader (Thermo Fisher Scientific, Waltham, MA) at wavelengths of 390 nm (excitation) and 515 nm (emission). The original SPI

Table 1 Change in SPI colors under different treatments and storage times ^a

Treatment		Days					
Temp.	Aw	0	28	56	112	168	224
a value							
25 °C	0.25	0.57A a	0.56A b	0.55A c	0.56A b	0.52B b	0.57A b
45 °C	0.25	0.57A a	0.57A b	0.59A b	0.58A b	0.61B a	0.64 Ba
25 °C	0.75	0.57A a	0.54B b	0.50C d	0.40D c	0.42D c	0.31E c
45 °C	0.75	0.57A a	0.82B a	0.98C a	1.14D a	-	-
b value							
25 °C	0.25	13.90A a	13.99A c	13.95A d	13.88AB d	13.78B c	13.90A c
45 °C	0.25	13.90A a	14.06B c	14.27C c	14.38C c	14.39C b	14.59D b
25 °C	0.75	13.90A a	14.31B b	14.56C b	14.83D b	14.88D a	15.36E a
45 °C	0.75	13.90A a	15.61B a	16.15C a	16.37D a	-	-
ΔE							
25 °C	0.25	0A a	0.10B c	0.16BC d	0.11BC d	0.20C c	0.32D c
45 °C	0.25	0A a	0.16B c	0.43C c	0.48C c	0.54C b	0.69D b
25 °C	0.75	0A a	0.67B b	0.76BC b	0.99CD b	1.11D a	1.50E a
45 °C	0.75	0A a	1.74B a	2.30C a	2.54D a	-	-

^a mean ($n = 3$), A-E means in the same row with different superscripts are significantly different ($p < 0.05$), a-d means in the same column with different superscripts are significantly different ($p < 0.05$)

Table 2 Change in SPI gel hardness under different treatments and storage times (g)^a

Treatment		Days					
Temp.	AW	0	28	56	112	168	224
25 °C	0.25	323A a	296AB a	273BC a	281ABC a	242C a	166D a
45 °C	0.25	323A a	282B a	250C a	141D b	143D b	140D b
25 °C	0.75	323A a	92D c	80D c	79D d	125C b	160B a
45 °C	0.75	323A a	150B b	145B b	111C c	-	-

^a mean ($n = 3$), A-D means in the same row with different superscripts are significantly different ($p < 0.05$), a-d means in the same column with different superscripts are significantly different ($p < 0.05$)

sample was used as the control. The degree of hydrophobicity was expressed as a ratio of the fluorescence intensity of the treated sample to that of the control. The initial slope of FI versus protein concentration was used as an index for protein hydrophobicity.

Statistical analysis

Data were analyzed by an analysis of variance (ANOVA) using a general linear model. Duncan's multiple range test was used to determine the differences among samples. Significant levels were defined as probabilities of ≤ 0.05 . All processing treatments were done in triplicate.

Results and discussion

Color analysis

The color analysis results are presented in Tables 1. The L value of the original SPI was 89.05. The range of L values that changed during storage were 89.04–89.37 for SPI-0.25 and 89.04–89.56 for SPI-0.75, significantly higher than SPI-0.25 (data not shown). The a values in the samples that were stored at 45 °C increased 12 % (SPI-0.25) to 100 % (SPI-0.75) (Table 1). In contrast, samples stored under high aw exhibited 46 % decreases in a values at 25 °C and 100 % increases at 45 °C. The b values were increased by 0 % (SPI-0.25, 25 °C)

to 18 % (SPI-0.75, 45 °C). The ΔE values increased 69 % (SPI-0.25) to 254 % (SPI-0.75) in samples stored at 45 °C.

Color changed significantly even after 28 d at 25 °C and low water activity. The results indicate that aw, temperature, and storage time all have significant effects on the Maillard reaction, although aw had a more pronounced effect on the Maillard reaction than temperature or storage time. The general tendency for browning to increase with increasing aw was also observed by Hsu and Fennema (1989); Kehrberg and Johnson (1975), and Labuza and Saltmarch (1981). Davies et al. (1998) studied SPI stored at different aw and temperatures and with or without glucose and found that in the absence of glucose only soy protein underwent browning. Otherwise, the rate of browning increased with increasing temperature and aw.

Gel hardness

Protein gels were composed of three-dimensional matrices or intertwined networks partially associated with polypeptides having entrapped water. The ability of proteins to form gels and provide structures for holding water, flavor, sugar, and other constituents of food are useful in food applications and developing new products, providing an added dimension to protein functionality. Table 2 shows the changes in SPI gel hardness under different treatments and storage times. Our results showed a decrease of almost 50 % in hardness of all of samples stored for 224 d, although hardness values changed significantly after only 28 d of storage in the high aw groups.

Table 3 Changes in the emulsifying stability index of SPI under different treatments and storage times^a

Treatment		Days					
Temp.	AW	0	28	56	112	168	224
25 °C	0.25	65.44A a	53.97AB ab	54.23AB a	48.72AB a	30.03B ab	30.22B b
45 °C	0.25	65.44A a	63.44A a	45.69AB a	54.61AB a	40.97B a	59.05AB a
25 °C	0.75	65.44A a	34.66B bc	22.84B b	29.48B ab	14.30B b	15.83B c
45 °C	0.75	65.44A a	14.39B c	14.39B b	14.99B b	-	-

^a mean ($n = 3$), A-B means in the same row with different superscripts are significantly different ($p < 0.05$), a-c means in the same column with different superscripts are significantly different ($p < 0.05$)

Table 4 Changes in SPI forming properties under different treatments and storage times ^a

Treatment		Days					
Temp.	AW	0	28	56	112	168	224
Foam maximum density							
25 °C	0.25	0.33A a	0.32A a	0.34A a	0.33A a	0.16B ab	0.13B b
45 °C	0.25	0.33A a	0.35A a	0.31A a	0.28A ab	0.20B a	0.17B a
25 °C	0.75	0.33A a	0.28A ab	0.25A b	0.29A a	0.13B b	0.12B c
45 °C	0.75	0.33A a	0.13B b	0.17B c	0.16B b	-	-
Foam stability							
25 °C	0.25	70A a	76A a	51B b	49BC b	36BC a	33C b
45 °C	0.25	70A a	46BC b	54B b	36CD c	42BCD a	33D b
25 °C	0.75	70A a	12D c	22CD c	38BC c	44B a	40BC a
45 °C	0.75	70A a	16B c	62C a	92C a	-	-

^a mean ($n = 3$), A-B means in the same row with different superscripts are significantly different ($p < 0.05$), a-c means in the same column with different superscripts are significantly different ($p < 0.05$)

Surface properties

Emulsifying stability index (ESI)

There was a decrease of approximately 50 %- 75 % in the emulsifying stability index (ESI) in all samples stored for 224 d except SPI-0.25 stored at 45 °C (Table 3). At 45 °C, the ESI of SPI-0.25 decreased until 168 d, when it began increasing until reaching its initial value after 224 d. This is an unusual result for which we have no explanation. The result was in contrast to the findings of Hsu and Fennema (1989) that at any given aw, the ESI of samples stored in air at 5 or 20 °C increased significantly (95 % level of confidence) during six months of storage.

Foaming properties

There were no significantly different of all the foaming capacity of the samples, and the range were from 15 to 16. There was a decrease of approximately 50 % ~ 61 % in the foam maximum density (FMD) in all samples stored for 224 d, but this occurred in SPI-0.75 samples after only 28 d at 45 °C

(Table 4). Since the interfacial behavior of proteins depends on their physical, chemical, and conformational properties (size, shape, amino acid composition, etc.), the composition of SPI might changed which affect the foam maximum density (FMD). Ru'iz-Henestrosa et al. (2007b) analyzed the effect of different condition on foam properties of soy globulins (7S and 11S at 0.1 wt%) and found the 7S globulin had higher foam maximum density (FMD) then 11S globulin at pH 7 and 0.05 M.

There was a decrease of 43 %- 50 % in the foam stability (FS) in all samples stored for 224 d except for SPI-0.75 at 45 °C, which decreased to 16 % after 28 d of storage and then increased to 92 % (Table 4). The FS of SPI-0.75 stored at 45 °C decreased from 70 to 16 after 28 d of storage and then increased to 92 after 112 d. This is an unusual result for which we have no explanation. Our results are in contrast to the finding of Hsu and Fennema (1989) that the effects of aw, storage temperature, and storage time on FS of WPC are all significant and that FS improved during three months of storage. This change was most dramatic at 40 °C. Furthermore, FS values after six months were inversely related to aw (as they were initially).

Table 5 Change in SPI viscosity under different treatments and storage times (cp) ^a

Treatment		Days					
Temp.	AW	0	28	56	112	168	224
25 °C	0.25	70.27A a	71.77A a	67.96A a	64.05A a	68.77A a	64.43A a
45 °C	0.25	70.27A a	56.40BC b	57.98B b	50.98C b	66.80A a	61.95A a
25 °C	0.75	70.27A a	37.97B c	29.95C c	36.53B c	23.60D b	13.53E b
45 °C	0.75	70.27A a	3.80B d	3.33B d	2.58B d	-	-

^a mean ($n = 3$), A-E means in the same row with different superscripts are significantly different ($p < 0.05$), a-d means in the same column with different superscripts are significantly different ($p < 0.05$)

Viscosity

The decrease in viscosity changed during storage by 8 % (25 °C) to 27 % (45 °C) for SPI-0.25 and 81 % (25 °C) to 96 % (45 °C) for SPI-0.75, significantly higher than SPI-0.25 (Table 5). Viscosity decreased rapidly from 70.27 cp to 3.80 cp for SPI-0.75 after 28 d of storage at 45 °C. This means that viscosity decreased as aw increased. Therefore, the effect of aw on viscosity is more significant than temperature.

There may also have been an interaction between temperature and aw because the greatest decrease was in the viscosity of the SPI-0.75 sample stored at 45 °C.

Nitrogen solubility index

Generally, higher water activity values resulted in greater reductions in nitrogen solubility index (NSI) values (Table 6). The decrease in the NSI of isolates stored at 25 °C occurred gradually, showing a solubility loss of only 37 % for the SPI-0.25 after 224 d of storage. The reduction was more pronounced during the first 28 days of storage for samples stored at 45 °C, reaching 66 % for SPI-0.25 and 81 % for SPI-0.75, but variation after this period was small. In other words, higher aw values result in lower NSI values, and therefore the effects of aw on NSI are greater than temperature.

Our results show that SPI quality decreases gradually during long periods of storage, in an intensity dependent on the conditions, mainly temperature and relative humidity. We found that storage at 45 °C led to a significant decrease in NSI for all tested samples (including SPI and defatted soy flours), similar to the results of Pinto et al. (2005) but to a higher degree than for SPI. Our results indicate that prolonged storage at higher temperatures and aw may cause protein denaturation, and as a consequence protein insolubilization, which can limit product utilization.

Surface hydrophobicity

Generally, surface hydrophobicity (RSo) increased as water activity increased (Table 7). The increase in RSo of the

Table 7 Change in SPI surface hydrophobicity (RSo) under different treatments and storage times (%)^a

Treatment		Days					
Temp.	AW	0	28	56	112	168	224
25 °C	0.25	0A a	1.09B c	1.36B c	1.50B c	4.22C b	5.23D b
45 °C	0.25	0A a	1.14B c	1.18B c	1.26B c	2.48C c	3.84D c
25 °C	0.75	0A a	2.61B b	3.57C b	4.12C b	9.10D a	12.31E a
45 °C	0.75	0A a	4.76B a	6.13B a	6.98B a	-	-

^a mean ($n = 3$), A-E means in the same row with different superscripts are significantly different ($p < 0.05$), a-c means in the same column with different superscripts are significantly different ($p < 0.05$)

isolates stored at 25 °C occurred gradually, showing an increase from 0 % to 5.23 % for SPI-0.25 after 224 d of storage. This increase was more pronounced during the first 28 d of storage at 45 °C, being 0 %-2.61 % for SPI-0.25 and to 4.76 % for SPI-0.75. It continued increasing significantly until the end of storage. Hydrophobicity is one of the most important structure- related factors influencing the functional properties of proteins, and surface hydrophobicity is significantly correlated with protein gelation properties (Hua et al. 2005). The denaturation of proteins is known to expose hydrophobic groups and thus increase surface hydrophobicity Ibrahim et al. (1993) reported that dehydrated whey proteins stored at 80 °C for 7 d underwent changes in surface hydrophobicity and conformation.

Discussion

The undesirable alterations that occur to SPI during storage might be the result of aggregation or Maillard reactions via changes in protein structure, protein-protein interactions, oxidation, and other unknown factors (Davies et al. 1998; Martins and Netto 2006). The decrease in solubility (NSI) and other functional properties during storage occurred due to changes in the protein structure followed by aggregation, because the high moisture content along with high temperatures favors aggregation reactions (De Graaf 2000). Rector

Table 6 Change in SPI nitrogen solubility index under different treatments and storage times (%)^a

Treatment		Days					
Temp.	AW	0	28	56	112	168	224
25 °C	0.25	56.81A b a	61.02A a	51.51B a	55.29B a	41.73C a	35.82C a
45 °C	0.25	56.81A a	37.18BCb	34.34BCb	40.34B a	30.26C b	19.13D b
25 °C	0.75	56.81A a	19.11B c	19.41B c	17.83B b	12.59B c	11.00B c
45 °C	0.75	56.81A a	10.92B d	10.14B d	12.14B b	-	-

^a mean ($n = 3$), A-D means in the same row with different superscripts are significantly different ($p < 0.05$), a-d means in the same column with different superscripts are significantly different ($p < 0.05$)

et al. (1991) also observed the polymerization of WPC after 7 d of storage at 80 °C. Aggregation mechanisms in solid-state proteins include non-covalent aggregation (mainly via hydrophobic interactions), aggregations by disulphide bonds, and non-reducible cross-links (Martins and Netto 2006).

We found that the RSo of SPI was gradually increased during the storage period. However, the Maillard reaction may also have contributed to the reduction in functional properties, which we also found in this study. This is supported by the report of Davies et al. (1998) that the Maillard reaction is important in the deterioration of SPI during storage.

Conclusions

We suggest that changes in protein structure may occur during the storage of isolates, and there is possibly an aggregation reaction or a Maillard reaction that results in alterations of the functional properties of SPI. Relative humidity, temperature, and storage time are shown to negatively affect the functional properties of protein isolates such as gel hardness, surface properties, viscosity, and solubility, all of which are important characteristics regarding the quality and use of these products as ingredients in the food industry.

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