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Effects of heating on hydrophobicity, viscosity, and gelling properties of soy products

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ABSTRACT

The co-product of soybean after oil extraction is the meal, which is rich in protein. From this meal, protein concentrate and protein isolate are prepared and are commercially available as functional ingredients. Thermal treatment is the most common step applied to foods during processing. Changes in structural and functional properties can be affected by thermal or chemical treatments. The objective of this study was to evaluate the effect of heat on surface hydrophobicity, gelling properties, and viscosity of soy meal (SM), soy protein concentrate (SPC), and soy protein isolate (SPI). The soy products were subjected to heat at varying temperatures and heating times. Viscosity of soy protein products treated with heat increased for SM when temperature and heating times increased, but decreased for SPC and SPI. This may be due to the polysaccharides present in SM that could form starch gelation and increase meal viscosity. The surface hydrophobicity of the soy products increased when the proteins were treated with heat, possibly due to heat exposing the hydrophobic amino acids buried within the protein molecule making them become more hydrophobic on the surface of the molecule. When 8% suspensions (protein basis) were heated at 100°C, all soy products formed firm gels, indicating that protein plays an important role in gel network formation. Precaution must be taken to maintain functionality when heat processing is applied to food systems that contain soy protein products as functional ingredients.

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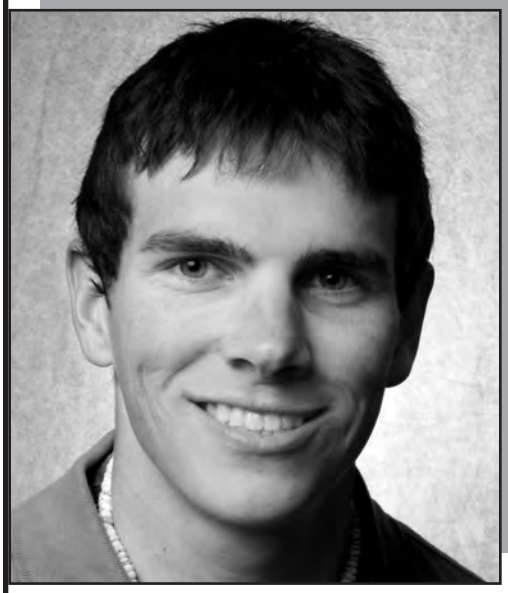
MEET THE STUDENT-AUTHOR

I am from Charleston, Ark., and a graduate of Charleston High School. At present, I am a senior honors student at the University of Arkansas majoring in food science. Through the Food Science Department, I have received the Carolyn S.Q. Sharp Scholarship from the Ozark Food Processors Association and a Mid-South Institute of Food Technologist Scholarship. My future plans include attending pharmacy school. I got the opportunity to work with my mentor, Dr. Navam Hettiarachchy, after participating in her Honors Proposal Development class. Under her guidance and the help of several of the protein chemistry lab M.S. and Ph.D. candidates, I learned a great deal about research and had the opportunity to work with many of the leading experts in the world of food science. I was able to present my research and compete in the Ozark Food Processors Association poster competition and came away with a third-place prize. My experiences at the University of Arkansas have not been limited to research. Other activities that I have participated in include serving as a Bumpers College Ambassador and a member of Alpha Gamma Rho Fraternity; holding a position on the executive board for Up 'til Dawn; competing on the U of A Cycling Team; serving as a Greek Life Facilitator; and being inducted into the Alpha Zeta and Gamma Beta Phi honor societies. Financial support of my project has been provided by the Dale Bumpers Agricultural, Food and Life Sciences Undergraduate Research award and a Silo Undergraduate Research Fellowship (SURF).



Robert S. Walnofer

INTRODUCTION

Soybeans (*Glycene max*) are an excellent source of protein. The use of soybean in the United States is expected to grow more than 10 % annually. Soy protein use is expected to reach nearly 50 million bushels by 2010 (USDA, 2004). Additionally, soybean is an important export product for U.S. processors. Soybean production has traditionally been one of the largest agricultural enterprises in Arkansas. Arkansas ranks eighth nationally in soybean production (ASPB, 2003). Due to its abundance and use as an inexpensive ingredient, soybean is also an important product in the food industry (Añón et al., 2001).

Soy products are commercially available to the food industry in the form of flours, concentrates, and isolates. Soy protein has received substantial publicity because of the U. S. Food and Drug Administration's claim that 25 g per day of soy protein can reduce the incidence of heart disease. This is important because heart disease is the number one cause of premature death in the U.S. Numerous products in the grocery store contain soy protein as a functional ingredient. A functional ingredient is that property of a substance that exhibits any property other than nutrition. The use of these products in a wide variety of foods has been increasing due to their desirable nutritional, nutraceutical, and functional properties, such as high essential amino-acid contents, and good emulsifying, foaming, fat absorption, and gelling properties. Soy protein as a functional ingredient has been studied extensively (Kalapathy et al., 1996; Kalapathy et al., 1997; Qi et al., 1997; Wu et al., 1998; Wu et al., 1999; Xie et al., 1998a; Xie et al., 1998b). Soy protein products can be used in food as water-binding agents, to increase viscosity, and to form protein gel (Kinsella et al., 1985). Soy flours or soy meals are prepared from defatted ground seed and usually contain about 40-50% protein. These are mostly used in food products such as bakery products and cereals. Protein content of soy protein concentrates usually varies from 60% to near 90%. These protein concentrates are prepared from defatted soy flour by removing the oligosac-

charides, fiber, and part of the minerals. Protein isolates contain more than 90% protein. Protein isolates are prepared from defatted flour by separating protein from polysaccharides, fiber components, and other low molecular-weight compounds.

In order to increase its use in the food industry, modification of soy protein is widely used to improve functional properties. Structural and functional property changes in soy protein can be achieved by thermal, enzymatic, or chemical treatments (Kalapathy et al., 1996; Kalapathy et al., 1997; Qi et al., 1997; Sorgentini et al., 1995; Wu et al., 1998; Wu et al., 1999; Xie et al., 1998a; Xie et al., 1998b). Thermal modification is much preferred due to the use of fewer chemicals in the process. During food processing, thermal treatment is the most common step that may affect the properties of soy products in the food system. However, information on changes in physicochemical properties of soy products after thermal modifications and treatments is limited.

MATERIALS AND METHODS

Protein determination

Protein contents of soy meal (SM), soy protein concentrate (SPC), and soy protein isolate (SPI) obtained from Archer Daniels Midland Company (Decatur, Ill.) were determined by an Automatic Kjeldahl method (AACC, 1990). The Kjeldahl 2006 Digestor (Foss Tecator, Hoganas, Sweden) was used for digesting the soy products in concentrated sulfuric acid with Kjeldahl tablet® as catalyst at 420°C for 1 h, and the Kjeltec® 2300 Analyzer Unit (Foss Tecator, Hoganas, Sweden) was used to determine the protein contents of the digested soy products. The protein contents were automatically calculated using 6.25 as the protein conversion factor commonly used in soybean industries.

Molecular size determination

Molecular sizes of the soy products were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) based on the method of Laemmli (1970). The SDS-PAGE was carried out on a slab gel in an SDS-Tris-Glycine discontinuous buffer system. Protein solutions were prepared in non-reducing buffer solutions. Twelve microliters of the solution containing approximately 2 mg/mL of protein were loaded onto the gel performing at a constant current of 60 mA per gel for approximately 45 min. The gel was stained using a 0.1% Coomassie brilliant blue solution in acetic acid/ethanol/water (10/40/50,v/v/v) and de-stained in the same solvent in the absence of Coomassie brilliant blue. The approximate molecular sizes were determined by comparing the sample bands with Bio-Rad molecular size standard bands ranging from 6.5 to 200 kDa (Mysosin 200 kDa, β-galactosidase 116.25 kDa, Phosphorylase B 97.4 kDa, Serum albumin 66.2 kDa, Ovalbumin 45 kDa, Carbonic anhydrase 31 kDa, Trypsin inhibitor 21.5 kDa, Lysozyme 14.4 kDa, and Aprotinin 6.5 kDa) (Bio-Rad Laboratories, Hercules, Calif.).

Preparation of soy solutions

Soy meal, SPC, and SPI were suspended in deionized water (6%, based on protein content). Each suspension was heated in a water bath at 50/70/90°C for 30, 60, 90, and 120 min and then cooled to room temperature before viscosity and hydrophobicity determinations.

Viscosity determination

Viscosities of the thermally treated soy products were determined by a rotational rheometer (Haake VT 550, Germany) equipped with a MVDIN measuring spindle (radius = 19.36 mm, height = 58.08 mm) at room temperature (26°C). Samples (30 mL, 6% protein basis) were loaded into the cylindrical cup (radius = 21.0 mm). The samples were subjected to a constant shear rate (400 s^{-1}) and the viscosity was determined automatically using Rheowin Pro Data manager version 2.84 (Haake Mess Tech, Germany). All experiments were carried out in triplicates at room temperature.

Hydrophobicity determination

Surface hydrophobicity of the thermally treated soy products was determined by using an 8-anilino-1-naphthalene sulfonate (ANS) method adopted from Hayakawa and Nakai (1985). Concentrations ranging from 0.0005 to 0.003% (protein basis) were prepared by serially diluting the solution in 0.01 M phosphate buffer (pH 7). Ten microliters of 8 mM ANS (in 0.01 M phosphate buffer pH 7) were added to 2.0 mL of soy product solution. Fluorescence intensity of the ANS-protein conjugates was measured with a Shimadzu Model RF-1501 Spectrofluorophotometer (Shimadzu Corporation, Kyoto, Japan) at excitation and emission wavelengths of 390 nm and 470 nm, respectively. The slope of the fluorescence intensity versus the soy product concentration was calculated by linear regression and was used as an index of the soy product hydrophobicity.

Gelling property determination

Gelling properties of the soy product solutions in water were determined by a slightly modified method of Coffmann and Garcia (1977) as described by Sathe et al. (1982). A series of concentrations of soy product suspensions from 2 to 20% w/v with 2% increments were prepared in 5 mL deionized water to determine the least or lowest gelation concentration of soy protein products in water. The test tubes containing these suspensions were then heated in a boiling water bath for 1 h followed by rapid cooling under running cold tap water. The test

tubes were further cooled for 30 min at 4°C, and the cooled suspension in the tubes was considered to form a firm gel if the suspension of inverted test tube did not slip or spill.

Statistical analysis

Data were analyzed for variance with multiple mean comparisons using JMP 5 software package (SAS Inst., 2002). The significance of means was determined by the Tukey Honestly Significant Difference (HSD) procedure at $P \leq 0.05$.

RESULTS AND DISCUSSION

Protein Content

Before functional protein analysis, protein contents of soy products had to be determined because protein of these products plays an important role in food systems (Table 1). From the Kjeldahl analysis, the protein contents in SM, SPC, and SPI were 51.2%, 65.2%, and 84.7%, respectively. The SM of 51% was above the expected range of 40-50% and the SPI of 85% was under the expected range of slightly >90% due to the samples being commercially produced compared with lab-scale soy protein isolate. Because protein isolates are prepared from defatted flour by removing the polysaccharides and other low molecular-weight compounds, the residual polysaccharides and fiber could have influenced protein levels.

Molecular Size

An electrophoretogram obtained using SDS-PAGE electrophoresis showed the molecular sizes of the proteins in SM, SPC, and SPI (Fig. 1). Soy products consisted of more than one type of protein with varying molecular size. SDS-PAGE was used because it promotes a separation based on the size of protein molecules. Based on the molecular size, the protein molecules move in an electric field and different size of proteins are separated. The major bands of all soy products ranged from 14.4-35 kDa as compared by Bio-Rad molecular size standard (Fig. 1). The SPI, SPC, and SM showed similar bands located at 35, 22, and 14.4 kDa, even though lighter bands at 14.4 kDa were observed for SPC and SPI in comparison to SM. The SM had larger amounts of proteins at 14.4 kDa and < 6.5 kDa than those of SPI and SPC, while more proteins with the molecular size of > 200.0 kDa were observed in SPC and SPI. This is important because the molecular size of a protein plays a role in gel formation in that disulfide linkages form cross-links, and the cross-linking of protein molecules forms gel. The larger the protein, the firmer the gel.

Viscosity

The viscosity of a product is simply its resistance to flow, which is an important factor in food processing. The determination of viscosity is important for the type of product application and to design needed equipment. Highly viscous products have a thicker solution and can cause clogging of narrow tubes and piping in a production facility. The control was determined by recording the viscosity of non-treated samples of each protein type. The viscosities of the SM heated at 50°C for up to 90 min and at 70°C for up to 60 min did not significantly differ in comparison to untreated SM ($P > 0.05$) (Table 2). This result indicated that these treatments were not enough to cause changes in the viscosity of SM. Yet when SM was heated longer and at higher temperature (up to 120 min at 50°C, up to 90 min at 70°C, or only 30 min at 90°C), its viscosity was significantly higher ($P < 0.0001$) than unheated SM. The viscosities of heat treatments at 90°C were much higher than those of heat treatments at 50 and 70°C. This result clearly showed that heating time and temperature affect the viscosity of SM in water. On the other hand, the viscosity of SPC and SPI treated with heat showed the opposite results. The results for SPC showed that SPC treated at 50°C and 90°C across all the heating times had significantly lower viscosity in comparison to untreated SPC ($P < 0.0001$). However, even though the viscosities of SPC treated at 70°C for up to 60 min were significantly lower than control ($P < 0.0001$), there were no significant differences between the viscosities of SPC treated up to 90 and 120 min and that of untreated SPC ($P > 0.05$). The results for SPI were quite similar to those for SPC. However, the viscosities of SPI treated with heat across all the temperatures and for all the heating times were significantly lower than that of untreated SPI ($P < 0.0001$). Heating the SPI at 90°C for any time greatly decreased its viscosity in water suspension. The results indicated the viscosity of soy products was affected by the protein-polysaccharide ratio. When the polysaccharide content was high, as occurred in SM, the polysaccharide affected the viscosity more than the protein by forming starch gelation that decreased the ability of the suspension to flow and increased viscosity. When there was no polysaccharide in the soy product, which happened in SPI, the protein characteristics considerably affected the viscosity of its suspension in water. This may be due to protein denaturation. At high temperature, the protein is denatured and its structure is opened up to expose the hydrophobic residues, along with some hydrophilic residues of protein. This unfolded protein with more hydrophilic amino acids on the surface of the molecules probably could interact more with water mol-

ecules by forming hydrogen bonds that in turn could cause the increase of its viscosity due to the hydration of the protein molecules.

Hydrophobicity

The surface hydrophobicity was determined from a linear relationship between the protein concentrations and fluorescence intensity. By plotting the line of regression, the surface hydrophobicity was expressed as the slope of fluorescence intensity versus protein concentration. The surface hydrophobicities of untreated soy protein products (control) showed that the surface hydrophobicity of SM was significantly lower than those of SPC and SPI ($P < 0.0001$) (Table 3). This could be due to proteins in SPC and SPI undergoing partial denaturation during preparation that could open up some buried hydrophobic residues to the surface of the protein molecules. Overall, surface hydrophobicities of the SM and SPC treated with heat across all temperatures were significantly higher than those of untreated samples with the exception of SPC at 70°C with up to 120 min heating ($P < 0.0001$). For SM, the higher the temperatures applied, the higher the surface hydrophobicities of the protein. This was due to the increase in degree of denaturation. This result exhibited that when more heat was applied to the protein, this could cause more protein to be unfolded and expose more hydrophobic amino acids of the protein to the surface of the protein molecules. At lower temperature (50°C and 70°C), longer heating time was needed by SM to unfold more of its protein molecules, which in turn increased the surface hydrophobicity value of this soy protein product. Similar results were obtained for SPC, which had a significantly higher surface hydrophobicity value for a heat-treated sample than that for untreated sample (control) ($P < 0.0001$). However, unlike SM, different temperature and heating time treatments conducted on SPC did not show considerable effects on the surface hydrophobicity, probably due to maximal hydrophobic residues that had been exposed to the surface of the proteins even at low temperature (50°C). Unlike SM and SPC, surface hydrophobicities of SPI treated at 50°C, particularly for longer heating time (up to 60 min and longer), were significantly lower than that of untreated sample ($P < 0.0001$). This was probably due to hydrophobic interaction between proteins, which may be thermodynamically favorable at this temperature, reducing the amount of hydrophobic residues on the surface of the protein structure. However, when the sample was heated at higher temperature (90°C), the surface hydrophobicities increased significantly, with the exception of heating time up to 60 min ($P < 0.0001$). When hydrophobicity values of the soy protein products treated at 90°C were observed, all types of the soy products showed the same pattern over the heating times. Even though this result is not clearly understood, these fluctuation values could be caused by protein-protein interactions either between hydrophobic residues or hydrophilic residues, depending on the time duration of temperature applied to the protein.

Gelation

The lowest solution concentrations required to form gels for SM, SPC, and SPI were 14%, 10%, and 10% (weight basis), respectively. To determine the heating time needed by the soy protein products to form a firm gel at the lowest solution concentration, the soy product suspensions were heated at 70, 80, 90, and 100°C for 10, 20, 30, 40, 50, and 60 min. SM formed a firm gel by heating 14% (weight basis) of SM at 80°C for 30 min, and at 90°C and 100°C for 10 min, but it did not form gel at 70°C even for 60 min heating. On the other hand, 10% (weight basis) of SPC and SPI formed gel after heating at 70°C for 20 min and 10 min, respectively, and needed only 10 min for both to form gel when heated at 80°C and above. When suspensions were made on the basis of protein content, 8% (protein basis) for all soy products could form gel when the suspensions were heated at 100°C for 1 h. This result indicated that even though polysaccharide is present in a significant amount in SM for gel formation, the proteins of the soy products play a more important role in the formation of a gel network when their suspensions in water are heated.

ACKNOWLEDGMENTS

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Table 1. Protein contents of soy meal, soy protein concentrate, and soy protein isolate.[z]

Protein type	Protein content (%)
Soy meal	51.2 ± 0.6c
Soy protein concentrate	65.2 ± 0.7b
Soy protein isolate	84.7 ± 0.9a

[z]Values are means ± standard deviations of three replications; mean values with different lower cases in the same column are significantly different ($P < 0.05$).

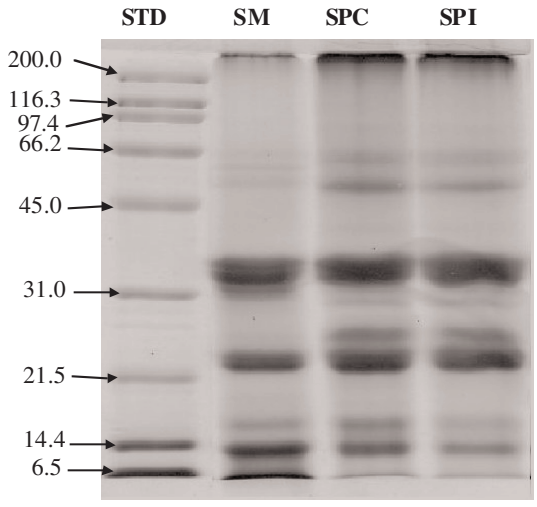

Fig 1. Electrophoretogram of soy meal (SM), soy protein concentrate (SPC), soy protein isolate (SPI), and Bio-Rad standard (Std).

Table 2. Viscosities (cps) of soy meal, soy protein concentrate, and soy protein isolate treated with heat at varying temperatures and heating times.[z]

		Temperature (°C)												
		50				70				90				
Protein type[y]	Control	30[x]	60	90	120	30	60	90	120	30	60	90	120	P-Value
SM	0.0095 cFGH	0.0093 cGH	0.0075 cH	0.0080 cH	0.0113 cEFG	0.0113 cEFG	0.0122 cEF	0.0124 cE	0.0129 cE	0.0384 bD	0.0506 bB	0.0472 aC	0.0804 aA	<.0001
SPC	0.0613 aA	0.0496 aFG	0.0507 aEFG	0.0512 aEFG	0.0535 aDE	0.0535 aDE	0.0574 aBC	0.0635 aA	0.0607 aAB	0.0485 aG	0.0551 aCD	0.0359 bH	0.0527 bDEF	<.0001
SPI	0.0458 bA	0.0385 bC	0.0400 bBC	0.0406 bBC	0.0265 bF	0.0265 bF	0.0406 bB	0.0357 bD	0.0332 bE	0.0143 cG	0.0092 cH	0.0091 cH	0.0075 cH	<.0001
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

[z]Values are means of three replications; mean values with different upper cases in the same row and different lower cases in the same column are significantly different (P < 0.05).

[y]SM = soy meal; SPC = soy protein concentrate; SPI = soy protein isolate.

[x]Heating times (min).

Table 3. Surface hydrophobicities of soy meal, soy protein concentrate, and soy protein isolate treated with heat at varying temperatures and heating times.[z]

Protein type[y]	Control	Temperature (°C)												P-Value
		50				70				90				
		30[x]	60	90	120	30	60	90	120	30	60	90	120	
SM	35194 cH	45909 cG	46244 cG	54439 cF	42688 cG	71226 cE	73729 cE	73269 cE	78158 bD	100421 bB	92815 bC	131772 bA	91181 bC	<.0001
SPC	89463 aI	119603 aD	127381 aC	131282 aB	118383 aDE	116105 aE	125996 aC	112437 aFG	76611 bJ	115271 aEF	98775 aH	153488 aA	111106 aG	<.0001
SPI	81578 bD	79666 bDE	61401 bF	74020 bE	66133 bF	92781 bC	89310 bC	78051 bDE	81851 aD	87835 cC	76880 cDE	120588 cA	113325 aB	<.0001
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0027	<.0001	<.0001	<.0001	<.0001	

[z]Values are means of three replications; mean values with different upper cases in the same row and different lower cases in the same column are significantly different (P < 0.05).

[y]SM = soy meal; SPC = soy protein concentrate; SPI = soy protein isolate.

[x]Heating times (min).