1993

# Hydrothermal processing of soy protein products

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Order Number 9414045

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Iowa State University, 1993

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# Hydrothermal processing of soy protein products

by

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfilment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food Science and Human Nutrition
Major: Food Science and Technology

**Approved**

Signature was redacted for privacy.

**In Charge of Major Work**

Signature was redacted for privacy.

**For the Major Department**

Signature was redacted for privacy.

**For the Graduate College**

Iowa State University
Ames, Iowa

1993

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# GENERAL INTRODUCTION

## Literature Review

Soybeans are an abundant source of protein that has long been recognized for its high nutritional value and potential as highly functional food ingredients (1). There has also been increasing evidence that dietary soy protein can lower serum cholesterol levels, which in turn reduce risks of heart disease (2-4). However, only a small portion (3%) of the soy protein supply is used for human consumption, while most is used as feed (5). To increase human consumption of soy protein, there has been enormous effort to understand soy protein structure and functional properties.

### Soy Protein Structure

Soy proteins are composed of four major groups, 2S, 7S, 11S, and 15S globulins. The 7S and 11S globulins, also respectively known as glycinin and ß-conglycinin, are the two major storage proteins of soybeans comprising approximately two-thirds of the total seed protein (6). Glycinin consists of twelve alternately arranged acidic and basic subunits. ß-conglycinin often exists as a dimer, each monomer consists of three

subunits.

Both proteins undergo denaturation when heated. Denaturation connotes any nonproteolytic modification to the native structure of protein, including any process that alters the tertiary or quaternary structures (7). Glycinin and ß-conglycinin have denaturation temperatures of 90 and 75°C, respectively (8). The temperature at which denaturation occurs is a function of protein structure, water concentration, pH, and ionic strength. The denaturation temperatures of both ß-conglycinin and glycinin increase by over 30°C as the water content of the system decreases from 90 to 20% (9). These two proteins have higher thermal stabilities as ionic strength increases (8). ß-conglycinin and glycinin are highly stable to heat when the pH is near their isoelectric points (pH 4.5 and pH 6.5) (9). Thermal denaturation of both ß-conglycinin and glycinin generally follow a three-step process: initial dissociation of the subunits, unfolding of subunits, and subsequent aggregation (10, 11).

A number of tools can be used to study protein conformation changes. Ultracentrifugation and gel chromatography are used to determine whether protein subunits are dissociated (12). Differential scanning calorimetry is often used to decide protein status by detecting thermal transitions (13).

Rocket immunoelectrophoresis is used to measure amounts of biologically native proteins (14).

Functional Properties of Soy proteins

Functional properties, also referred to as functionalities, are the composite of properties and functions that ingredients have in a food system where they are applied. They include solubility, hydration, emulsifying, oil absorbing, foaming, and gelling properties.

Water solubility is probably the most important property of protein in foods. This is not only because soy ingredients must form stable dispersion when incorporated into beverages, but also because other functionalities, such as gelling, emulsifying, and foaming, are closely associated with solubility (1). Protein dispersibility index (PDI) and nitrogen solubility index (NSI) are routinely used to evaluate protein solubility. Solubility is affected by both processing methods and conditions of its determination. During processing, proteins are often denatured and insolubilized by heat and exposure to pH extremes. Ionic strength, pH, and temperature are the three major parameters that affect protein solubility during laboratory determination. Very significant interaction exists between pH and ionic

strength (15).

Hydration properties are extremely important in baked goods, meat products, and cheeses. Water binding, water absorption, water holding, and water hydration capacity are terms that are used interchangeably (16). Hydration can be affected by such factors as amino acid composition, protein conformation, surface topography, surface charge and polarity, ionic strength, ionic species, pH, and temperature (7).

Emulsifying properties of soy proteins are important when used in coffee whiteners, mayonnaise, and comminuted meats. Although a positive correlation exists between emulsifying capacity and solubility, it is possible to use denatured proteins as emulsifying agents, if sufficient mechanical energy (shear) is applied to the emulsion system (7).

In cakes, whipped toppings, and frozen desserts, foaming capacity is critical to product quality. For good foam formation, the protein should be soluble and form protein films to trap gas as fine bubbles. Lipid is often detrimental to foaming ability (17), defatted soy flour and soy isolate have better foaming properties than full-fat or refatted flours.

In tofu manufacture and application of soy protein in meat systems, gelling is a very significant property. Gelling refers to the transformation of

a sol into a gel-like structure by heat or chemical agents (i.e., calcium salts). The transformation may involve initial dissociation of subunits and unfolding of the subunits (7). When the gel is formed by coagulants, the degree of heat treatment affects gel formation and gel quality (18).

Even with the wide range of possible applications, the amount of soy protein used for human consumption remains very small. Over 96% of the available soy protein supply is used for feeds and only 4% is used for food and industrial products (5). Flatulence caused by oligosaccharides, undesirable flavors formed from lipid oxidation, and low protein digestibility due to trypsin inhibitors are among the major obstacles for increasing applications of soy protein in human food. During processing of soy protein products, procedures are taken to minimize these obstacles. Aqueous alcohol is used for washing out oligosaccharides to eliminate flatulence and improve flavor. The washing process, however, seriously reduces the solubility of soy protein, which in turn leads to other poor functional properties. This is also true for heat treatments used to inactivate trypsin inhibitors in improving protein digestibility.

Hydrothermal Cooking System

A steam-infusion cooking process, known as Hydrothermal Cooking (HTC), was developed to process soymilk from ground full-fat soy flour. The process increased recoveries of solids and protein in the final soymilk from 60 and 70% for traditional soymilk to 87 and 90%, respectively (19). It has even been reported that all but hulls of soybeans could be recovered as a stable soymilk (20). High recoveries of solids and protein by the HTC process are very significant features, as the insoluble fraction removed from traditional soymilk is composed of high quality protein (21). Cooking at 154°C for 30 sec gave maximum recovery (19). Besides high recovery, the process also inactivated 90% of the trypsin inhibitor content, which improved the nutritional quality of protein in soymilk. More importantly, the process also produced soymilk with less off-flavor because significant steam stripping occurred during the flashing of steam. The blander flavor was also attributed to the very short time for lipoxygenase-catalyzed lipid oxidation to occur.

The slurring temperature and time are critically important to off-flavor developments and solids and protein recoveries in the HTC process (22). When employing rapid hydration techniques, maximum recovery was

obtained by cold slurring (25°C) and cooking at 154°C for 22 sec. Hot slurring significantly reduced the TBA value and hexanal content of soymilk processed by HTC, which are two major indices for oxidation and off-flavor, but yields were reduced. Compared with traditional and other recent processes, HTC gave much higher recovery, blander flavor, better or equivalent nutritional quality, and higher dispersion stability. The viscosity of HTC soymilk was much higher than that of traditional soymilk, which is desirable in many dairy products.

There is no knowledge about whether HTC can be used as a heat treatment to preserve and improve functional properties of soy protein products, such us soy flour, protein concentrate, and protein isolate. The general objective of the present study was to find new applications for HTC in the soy protein industry. Specific objectives include: (1) to investigate effects of HTC processing on functional properties of soy protein products; (2) to study effects of HTC processing on coagulating properties of HTC-processed soymilk; (3) and to understand structure changes that soy proteins undergo during HTC.

## An Explanation of the Dissertation Organization

This dissertation consists of three papers, which were written in the format required by the Journal of the American Oil Chemists' Society (JAOCS) and will be submitted to JAOCS to be published. There is a General Summary following the three papers. The references cited in the General Introduction follow the General Summary.

# PAPER I

# FUNCTIONAL PROPERTIES OF HYDROTHERMALLY PROCESSED SOY PROTEIN PRODUCTS

# Functional Properties of Hydrothermally Processed Soy Protein Products

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## ABSTRACT

The effects of the Hydrothermal Cooking (HTC) process on the functional properties of defatted soy flour, soy protein concentrate (aqueous alcohol-washed) and soy protein isolate were studied. Samples were processed at 154°C for 11, 19, 30, and 42 sec and spray dried. Bulk density, nitrogen solubility index (NSI), protein solubility profile, foaming capacity and stability, emulsifying capacity, oil absorption, and water hydration properties were studied. HTC processing increased the NSI of soy protein concentrates by approximately four times (from 15 to 56%). HTC changed the solubility profile of soy protein concentrate from a flat profile to one typical of undenatured soy protein. HTC improved foaming and emulsifying properties. For soy protein isolates, HTC also improved NSI, and foaming and emulsifying properties, although the improvements were less dramatic than with soy protein concentrate. NSI and emulsifying properties of soy flour were improved at some processing conditions. The foaming properties of soy flour were not improved by HTC. Dramatically increased protein solubility of soy protein concentrate and slightly improved protein solubility of soy flour and soy protein isolate while inactivating trypsin inhibitors have considerable practical significance.

# INTRODUCTION

Soybeans are a very abundant source of protein that has long been recognized for its high nutritional value and potential as highly functional food ingredients (1). Flatulence caused by oligosaccharides, undesirable flavors formed by lipoxygenase-catalyzed lipid oxidation, and low protein digestibility due to trypsin inhibitors are among major obstacles for increasing acceptance of soy protein ingredients in human food. During processing of soy protein products, procedures are used to minimize these problems. Procedures, such as heat treatment to inactivate lipoxygenase and trypsin inhibitors and aqueous alcohol washing to remove oligosaccharides, usually denature and insolubilize protein and making the protein ingredients less functional in foods (2) and industrial applications (i.e., paper coatings and adhesives).

Soy flour, soy protein concentrate, and soy protein isolate are three major soy protein products. They have different protein contents and are used in different applications. Soy flour, the least refined form among the three, has a protein content of about 55% (db). Different degrees of heat treatment during desolventization/toasting/cooking produce soy flour with various functional properties (3). Flash desolventizing produces soy flour

with minimum protein denaturation and high protein solubility. However, soy flour contains oligosaccharides. It also has high levels of trypsin inhibitor activity, unless it is heat treated. Nitrogen solubility index of soy flour is as low as 20% when treated with moist heat to inactivate trypsin inhibitors (1). Soy protein concentrates contain more than 65% protein because soluble carbohydrates are removed by washing with either acid or aqueous alcohol. Aqueous alcohol washing, the most popular method, reduces protein solubility below 10%, but produces very bland flavor, less flavor than either soy flour or soy protein isolate (1). Soy protein isolate is the most highly refined soy protein product with a protein content of more than 90%. Soy protein isolate is made by alkali extraction of protein to remove insoluble fiber and subsequent acid precipitation to remove soluble sugars. Without any heat treatment, high levels of trypsin inhibitors remain in soy protein isolate.

The Hydrothermal Cooking (HTC) process, a steam infusion process, was developed to process soymilk (4). At optimum conditions, the process increased recoveries of solids and protein from 60 and 70% of the traditional oriental process to 87 and 90%, respectively (5, 6). The process also achieved over 90% reduction in trypsin inhibitor activity (7). Later it was

even reported that all but hulls of soybeans could be recovered as a stable soymilk (8). Soymilk with significantly blander flavor can be made by minimizing cold water contact time for lipoxygenase to be active (9). It was also reported that the functional properties (protein solubility, water absorption, oil absorption, whippability, foam stability, and emulsifying properties) of spray-dried soymilk made from whole or dehulled soybeans using HTC were superior to those of spray-dried soymilk prepared by the traditional method (8).

Thus far, HTC has not been applied to processing of soy flour, soy protein concentrates, or soy protein isolate. HTC is a unique form of heat treatment exposing the product to very high temperatures (120-155°C) for short periods (1-240 sec). Based on previous observations with soymilk, we hypothesize that we can maintain or improve functional properties of soy protein products by employing HTC. Additionally, the flashing of HTC steam should strip volatiles responsible for beany flavors and odors in soy protein ingredients. The objective of the present study was to investigate effects of HTC processing on functional properties of soy flour, aqueous-alcohol-washed soy protein concentrate, and soy protein isolate.

# MATERIALS AND METHODS

## Materials

NutriSoy 7B Flakes (59.8% (mfb) protein and 71.3 NSI) and NutriSoy protein Concentrates (65.4% (mfb) protein and 8.1% NSI) were purchased from the Archier-Daniels-Midland Company (Decatur, IL). NutriSoy 7B Flakes was prepared by dehulling soybeans, hexane defatting, and flash desolventizing. NutriSoy protein concentrates involved the same procedure followed by aqueous alcohol washing. Both were ground using a Fitzmill (The W. J. Fitzpatrick Co., Chicago, IL) equipped with 100-mesh screen.

Soy protein isolate was prepared in the laboratory from ground NutriSoy 7B Flakes using modified procedures described by Circle and Smith (1). We chose to prepare our own isolate so that it would not be exposed to two spray drying steps. Defatted soy flour was slurred in 50°C distilled water at 15% solids instead of 6.7% solids (called for by Circle and Smith) to minimize centrifuging. The slurry was adjusted to pH 8 using 1 N NaOH and stirred constantly using a laboratory stirrer for 1 hr. Cheesecloth filtering followed by centrifuging at 1000 xG was used to remove insoluble residues. The extract was adjusted with 1 N HCl to pH 4.5, the isoelectric

point for soy protein, and the protein curd was allowed to settle for 2 hr. The clear whey was decanted and an equal amount of fresh distilled water was added back and reslurried, and the washed curd was allowed to settle for 2 hr before the clear whey was decanted again. The pH of the washed slurry was adjusted to 7.0 with 1 N NaOH. The protein content of soy protein isolate slurries were 90.6% (mfb).

## Hydrothermal Cooking System and Processing Conditions

Figure 1 shows a schematic drawing of the HTC process. All soy protein products were fed into the HTC system at 5% solids. Slurries of soy flour and soy protein concentrates, but not soy protein isolate, were ground using a Vibroreactor (Model JM14/E/3, Cherry-Burrell Co., Cedar Rapids, IA) to reduce particle size. A variable speed Moyno pump (2MI type SSQ, Robin and Myers, Inc., Springfield, OH) was used to pump the slurry into a hydroheater (size 300 type B, Hydrothermal Co., Milwaukee, WI) where it was infused with 90 psi (6.5 $kg/cm^2$) steam. The slurry flowed through a stainless-steel holding tube (2.54 cm ID and 2.66 cm OD) insulated with fiberglass, passed through a back pressure valve, and discharged into the flashing chamber. Cooking temperatures and pressures were monitored by

thermal couples and pressure gauges, respectively, installed at both the beginning and the ending of the holding tube. The cooking temperature was controlled by adjusting the back pressure valve. The slurry exiting the flash chamber was immediately cooled by pumping through a cooling coil immersed in an ice water bath. The final temperature was approximately 35°C.

All samples were cooked at one cooking temperature, 154°C, the temperature identified as being optimum for soymilk (4). Four different holding tube lengths of 8.3, 14.5, 30.7, and 38.8 ft (2.5, 4.4, 9.3, and 11.8 m) were used to obtain the four different cooking times of 11, 19, 30, and 42 sec, respectively. The cooking times were regularly checked using a food-grade dye.

A 20-T pilot plant tower spray dryer (Food Processing Center, University of Nebraska-Lincoln, Lincoln, NE) was used to spray dry the slurries. Samples were pumped through a heating coil to obtain 60°C to increase drying efficiency before spraying to the drying chamber. An external mixing nozzle (3.2 mm D) was used. Air inlet and outlet temperatures were 168 and 76°C, respectively.

## Determination of Functional Properties

### Nitrogen Solubility Index

AOCS official method Ba 11-65 (11) was used to determine NSI. Nitrogen contents of both the original samples and the soluble fractions were determined using the macro-kjeldahl method.

### Solubility Profile

Protein solubility profiles were determined by using the method of Hamada and Marshall (12).

### Foaming Properties

Foaming capacity and foam stability were determined by using modified methods of Lin and Humber (10). A slurry of 200 ml containing 3% protein (or solids) was stirred for 10 min. The slurry was further dispersed by using a food mixer (KitchenAid, St. Joseph, MI) at low speed for 1 min. Heavy beating at top speed for 5 min was used to generate foam. The foam was transferred to a 2000-ml graduated cylinder to measure total volume (foam plus liquid) and foam volume. Total (foam plus liquid) and foam

volumes were also recorded at 1, 10, 30, 60, and 120-min intervals. Foaming capacity was calculated as the total volume after beating as a percentage of the original slurry volume. Foam stability was calculated as the percentage of the original foam volume remaining after 120 min of standing. Determinations based on the same protein level and the same solids content level were performed.

Emulsifying Properties

Emulsifying capacity was determined by modifying the method of Hung (5). A 50-ml slurry of 1.0% solids (or protein) was prepared in a 600-ml beaker using distilled water and the slurry was stirred for 10 min. A Braun hand-held mixer (Braun Inc., Lynnfield, MA) was used to homogenize the samples. The slurry was mixed for 1 min at 5000 rpm before the mixer was turned up to 10,000 rpm and corn oil was added. The amount of oil required to take the emulsion to the breaking point, which was recognized by a profound drop in emulsion viscosity, was used as a measure of emulsifying capacity. Determinations were made at both the same levels of solids and protein.

Oil Absorption Capacity

The Lin and Hubert method (13) was used for measuring oil absorption capacity, except corn oil was used.

Hydration Properties

One-gram samples of powder were placed in centrifuge tubes and the tubes were kept in a 100%-RH moisture chamber. The centrifuge tubes were weighed during and after 1-wk storage to determine the amount of moisture absorbed. Hydration values were calculated as the amounts of water absorbed per gram of sample over a 1-wk period.

## Determination of Physical Properties

Compositions

Moisture contents of spray-dried samples were determined by using AOAC method 14.003 (10). Nitrogen content was determined by using a Kjeltec system (Tecator, Inc., Hogana, Sweden). The nitrogen-protein conversion factor N X 6.25 was used.

Bulk Density

The method of Wang and Kinsella (14) was modified to determine bulk density. Samples were gently packed in 50-ml plastic centrifuge tubes by tapping on the bench 10 times from a height of 5 cm. Extra sample remaining on top of the centrifuge tube was removed by scrupling a ruler across the top of the tube. The centrifuge tube was tared and its volume was determined by measuring the amount of distilled water required to fill it.

Colors

Colors of protein powders were measured by using a Hunter colorimeter (Hunter Associates Laboratory, Reston, VA). The Lab unit system was used, where 'L' measured lightness, 'a' measured red (+) and green (-), and 'b' measured yellow (+) and blue (-). Tile LS-12414 was used for standardization.

## Experimental Design and Statistical Analysis

Each treatment was replicated three times in a complete random design. The results were analyzed using the General Linear Model of the Statistical Analysis System. T-tests were performed to compare means.

# RESULTS AND DISCUSSION

## Functional Properties

### Nitrogen Solubility Index

Water solubility is probably the most important property of protein in foods. This is not only because soy ingredients must form stable dispersion when incorporated into beverages, but also because other functionalities, such as gelling, emulsifying, and foaming, are closely associated with solubility (2). NSI is routinely used to evaluate protein solubility.

HTC processing significantly improved the NSI of soy protein concentrate (Table 1). NSI increased steadily to a maximum value at 30 sec. Most notably, HTC processing increased the NSI of untreated soy protein concentrate from 15% in the HTC unprocessed soy protein concentrate to 56% for the soy protein concentrate HTC processed for 30 sec, nearly a three-fold increase.

NSI's of both soy flour and soy protein isolates were also improved by HTC processing, although the effects were not as significant as those for concentrates. Soy flour processed for 11 sec had the highest NSI; NSI

dropped when the processing time increased to 19 sec; and then it started to improve again with increasing processing time. Soy protein isolates trended toward a similar pattern, however, the peak NSI occurred at 19 sec of cooking.

It is not readily apparent why HTC improved soy protein solubility, but we speculate that more than one mechanism is involved. HTC processing perhaps could disrupt large particles or any previously aggregated proteins due to earlier heat or other treatments. HTC could also prevent further formation of large aggregates by high-shear mixing during cooking.

Protein Solubility Profile

The protein solubilities of soy flour in the low acid range were significantly improved by HTC, especially at longer processing times (Fig. 2). It was also noted that the isoelectric points were shifted from pH 4.5 to pH 5.0 when soy flour was processed for 19 sec or longer.

The protein solubility profiles of unprocessed soy protein concentrates or those processed only for 11 sec were not very responsive to changes of pH, solubilities were low over the range pH 2 to pH 9 (Fig. 3). Soy protein concentrates processed longer than 19 sec exhibited protein solubility

profiles more typical of undenatured soy protein. The solubility of soy protein concentrate processed for 19 sec was lower in the acid range and higher in the alkaline range than observed in soy protein concentrate processed for 35 and 42 sec. The isoelectric point also shifted from pH 4.5 to pH 5.0 with soy protein concentrates processed for 35 and 42 sec. HTC restored solubility properties of soy protein concentrate to nearly that of native soy protein.

All soy protein isolates showed the typical solubility profile of soy proteins (Fig. 4). However, longer HTC processing time led to higher protein solubilities at all pH's. The curves uniformly shifted to higher levels as cooking time increased.

The reason for the shift in the isoelectric point is not understood. In some samples we noted ammonia-like odors that might be attributed to deamidation. If deamidation had occured, the isoelectric pH should have shifted to the acid side rather than the alkaline side as was observed. Decarboxylation would be expected to shift the isoelectric point to the alkaline side.

Foaming Properties

Effects of HTC processing on foaming capacity (equivalent protein levels) of different soy protein products are shown in Table 2. HTC processing decreased the foaming capacity of soy flour. There were no significant differences among HTC-processed soy flour samples processed for different times.

HTC processing significantly improved foaming capacities of soy protein concentrates. Generally, the longer soy protein concentrate was processed by HTC, the greater the foaming capacity. HTC processing increased the foaming capacity of soy protein concentrate almost three times when cooked for 42 sec.

Improvement was also observed in HTC-processed soy protein isolate; however, the effects were not as significant as those for soy protein concentrate. It was also noted that, whether processed by HTC or not, soy protein isolate samples had the highest foaming capacity followed in order by soy protein concentrate and soy flour. This is partially due to differences in composition and protein solubility. Residual lipids and fiber of soy flour and residual fiber of concentrate probably reduce foaming capacity.

Foaming capacities were also determined based on solids level (Table 3A). The general trends were the same as those observed in experiments in which the same protein level was used.

Foam stabilities determined on the same protein level are shown in Table 3. Foam stabilities of soy flour were reduced when processed for 11 sec, but were improved to nearly the same value of unprocessed soy flour as cooking time increased. However, those of soy protein concentrate were significantly improved by HTC processing. Cooking time had no significant effect. The foam stabilities of soy protein isolate decreased when processed for 19 sec or longer.

Emulsifying Capacity

Table 4 shows emulsifying capacities (equivalent protein levels) of soy protein products processed at different conditions. Emulsifying capacities of soy flour were initially reduced (samples processed for 11 or 19 sec), but emulsifying capacity was restored at longer cooking times. At 42 sec of cooking the emulsifying capacity of HTC-processed soy flour was significantly better than that of unprocessed soy flour. Emulsifying capacities of soy protein concentrate were dramatically improved. The

emulsifying capacity increased as HTC processing time increased. The emulsifying capacity of soy protein isolates also were significantly improved by HTC processing. There were no significant differences among soy protein isolates cooked for different times.

Overall, soy protein isolates had the highest emulsifying capacities among the three products. Unlike foaming capacity, soy flours generally had better emulsifying properties than soy protein concentrates.

Emulsifying capacities determined on the same solids level were basically the same as was described for those performed on an equivalent protein level (Table 5A), except that soy protein isolates had the highest emulsifying capacities, followed by flour and concentrate. This was probably due to differences in protein content.

Oil Absorption

HTC processing had no significant effect on oil absorption capacities of soy flour and soy protein concentrates (Table 5). However, soy protein isolates had significant higher oil absorption capacities when HTC processed. The improved oil absorption of soy protein isolates should be useful in meat systems where substantial amounts of soy protein isolate are used to reduce

cooking losses.

Hydration Capacity

Hydration capacities of soy flour were reduced by the HTC processing (Table 6). There were no significant differences among HTC-processed soy flour or soy protein concentrate. However, HTC processing significantly improved the hydration capacities of soy protein isolates.

It should also be noted that attempts were made to measure the hydration properties by the AACC method (No. 8804) (14). Samples in the present study hydrated unevenly when water was added and results were not highly reproducible.

## Physical Properties

Dry Characteristics

Soy flours, soy protein concentrates and soy protein isolates had moisture content ranges of 1.60-2.23%, 0.56-2.60%, and 1.37-2.40%, respectively (Table A1). There was a general trend of samples without HTC processing having higher moisture contents than HTC-processed samples.

As cooking time increased, moisture content after spray drying decreased. Thus, HTC processing seemed to facilitate drying.

Bulk Density

Soy flour became denser at longer cooking times (Table 7). Both HTC-processed soy protein concentrate and HTC-processed soy protein isolate, however, were less dense than unprocessed samples. The HTC-processed soy protein isolate had much lower bulk density (0.17-0.19 g/cc), compared with uncooked isolate (0.47 g/cc). Changes in bulk densities were probably the result of changes in particle size and particle shape, but the precise mechanism by which HTC altered these physical properties was not clear.

Colors

Spray-dried soy protein products had 'L' values ranging 90-94 (Table 8). At very short cooking times (11 and 19 sec), the colors of HTC-processed soy flour and soy protein isolate were lighter than unprocessed controls. This has also been observed in HTC-processed soymilk (4). As cooking time increased, all samples became slightly darker due to generation of Maillard reaction products; however, the extent of darkening was not

great. The redness ('a' values) of samples ranged from -1.80 to 0.27. For all the samples, 'a' values increased with increasing cooking time, going from negative values to positive values (i.e., the color changed from green color to red). The change from green to red was probably the result of the destruction of natural green color (chlorophyll) and the generation of Maillard colors. The yellowness ('b' values) of samples ranged from 8.8 to 14.1. As cooking time increased, there was generally an initial decrease of yellowness and then a gradual increase. This was likely caused by the destruction of natural yellow pigments and subsequent generation of Maillard reaction products.

Although these changes are generally undesirable, the extent of color development is regarded as being of little practical significance, especially in foods. Only in the most demanding applications, such as paper coatings, would these small changes be regarded as being important.

# CONCLUSIONS

HTC processing proved to be very effective in improving functional properties of soy protein products. HTC processing markedly increased the solubility, foaming capacity, and emulsifying capacity of aqueous-alcohol-washed soy protein concentrates. Although aqueous-alcohol-washed soy protein concentrate is often preferred in foods because of its bland flavor and low tendency to produce flatulence, it lacks desirable functional properties. HTC processing restored functional properties to nearly those of native soy protein. HTC also improved functional properties of soy flour and soy protein isolate; but the effects of HTC processing were not as dramatic with these products as with soy protein concentrate. However, protein solubilities of soy flour and soy protein isolate are maintained, HTC can be used to inactivating trypsin inhibitors. Because it is now possible to combine the attributes of aqueous-alcohol-washed soy protein concentrate (lower flavor, reduced flatulence, and low cost) with the functional properties of soy protein isolate, HTC has considerable commercial potential to increase soy protein utilization in foods and industrial products.

## REFERENCES

1. Smith, A.K, and S.J. Circle. in *Soybeans: Chemistry and Technology*, edited by A.K. Smith and S.J. Circle, AVI Publishing Co., Westport, CN, p. 339, 1976.

2. Kinsella, J.E., S. Damodaran, and B. German. in *New protein foods, Vol 5, Seed storage proteins*, edited by A. M. Altschul and H. L. Milcke, Academic Press, New York, NY, p. 107, 1985.

3. Wolf, W.J., and J.C. Cowan. in *Soybeans as Food Sources*, edited by W.J. Wolf, CRC Press, Miami, FL, 1976.

4. Johnson, L.A., C.W. Deyoe, and W.J. Hoover. U.S. Patent 4,409,256. (1981)

5. Johnson, L.A., C.W. Deyoe, and W.J. Hoover. *J. Food Sci. 46*:239 (1981).

6. Johnson, L.A. *Processing Aqueous Extracts of Soybeans by Rapid-Hydration Hydrothermal Cooking*, Ph.D. Thesis. Kansas State University, Manhattan, KS, 1978.

7. Johnson, L.A., C.W. Deyoe, W.J. Hoover, and J.R. Schwenke. *Cereal Chem 57*:376 (1980).

8. Hung, J.S. *Studies on Processing, Functional Characteristics and Nutritional Quality of Hydrothermal Extracts of Soybeans*, Ph.D. Dissertation. Kansas State University, Manhattan, KS, 1984.

9. Kim, C.J. *Physico-Chemical, Nutritional, and Flavor Properties of Soybean Extracts Processed by Rapid-Hydration Hydrothermal Cooking*, Ph.D. Dissertation. Iowa State University, Ames, IA, 1988.

10. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th edn, edited by Kenneth Helrich, the association, Arlington, VA, 1990.

11. *Official and Tentative Methods of the American Oil Chemists' Society*, 3rd edn., edited by D. Firestone, the association, Champaign, IL, 1991.

12. Hamada, J.S., and W.E. Marshall *J. Food Sci. 54*:598, (1989).

13. Lin, M.J.Y., E.S. Humber, and F.W. Sosulsiki. *J. Food Sci. 39*:368 (1974).

14. Wang, J.C., and J.E. Kinsella. *J. Food Sci. 41*:286 (1976).

15. *Approved Methods of the American Association of Cereal Chemistry*, the association, St. Paul, MN.

Table 1.

Effects of Hydrothermal Cooking on Nitrogen Solubility Indices (%) of Soy Protein Products[1]

| Product | Cooking Time (sec.) | | | | |
|---|---|---|---|---|---|
| | 0[2] | 11 | 19 | 30 | 42 |
| Flour | $64.5^{bcdef}$ | $79.4^{a}$ | $59.9^{def}$ | $67.7^{abcde}$ | $72.6^{abcd}$ |
| Concentrate | $14.8^{h}$ | $28.5^{g}$ | $54.2^{f}$ | $56.4^{ef}$ | $55.3^{ef}$ |
| Isolate | $63.6^{cdef}$ | $71.9^{abcd}$ | $77.3^{ab}$ | $73.0^{abcd}$ | $75.6^{abc}$ |

[1] Means with common superscripts are not significantly different. Least significant difference ($p \leq 0.05$) was 13.2%.

[2] Controls, no HTC processing.

Table 2.

Effects of Hydrothermal Cooking on Foaming Capacities (%) of Soy Protein Products[1]

| Product | Cooking Time (sec.) | | | | |
|---|---|---|---|---|---|
| | 0[2] | 11 | 19 | 30 | 42 |
| Flour | $490^{f}$ | $287^{g}$ | $304^{g}$ | $302^{g}$ | $325^{g}$ |
| Concentrate | $193^{g}$ | $551^{ef}$ | $517^{ef}$ | $653^{ef}$ | $736^{d}$ |
| Isolate | $925^{c}$ | $1060^{bc}$ | $1260^{a}$ | $1170^{ab}$ | $1200^{ab}$ |

[1] Foaming tests were performed at the same level of protein. Means with common superscripts are not significantly different. Least significant difference ($p \leq 0.05$) was 158%.

[2] Controls, no HTC processing.

Table 3.

Effects of Hydrothermal Cooking on Foam Stabilities (%) of Soy Protein Products[1]

| Product | Cooking Time (sec.) | | | | |
|---|---|---|---|---|---|
| | 0[2] | 11 | 19 | 30 | 42 |
| Flour | $82.4^{ab}$ | $28.2^{f}$ | $60.7^{de}$ | $66.0^{cde}$ | $73.1^{bcd}$ |
| Concentrate | $8.7^{g}$ | $88.3^{ab}$ | $87.8^{ab}$ | $89.2^{a}$ | $90.2^{a}$ |
| Isolate | $87.8^{ab}$ | $92.0^{a}$ | $57.2^{e}$ | $79.0^{abc}$ | $66.4^{cde}$ |

[1] Foaming tests were performed at the same level of protein. Means with common superscripts are not significantly different. Least significant difference ($p \leq 0.05$) was 15.2%.

[2] Controls, no HTC processing.

Table 4.

Effects of Hydrothermal Cooking on Emulsifying Capacities (ml oil/g protein) of Soy Protein Products[1]

| Products | Cooking Time (sec.) | | | | |
|---|---|---|---|---|---|
| | 0[2] | 11 | 19 | 30 | 42 |
| Flour | $820^{de}$ | $487^{h}$ | $570^{g}$ | $847^{cde}$ | $920^{ab}$ |
| Concentrate | $170^{i}$ | $437^{h}$ | $723^{f}$ | $787^{ef}$ | $750^{f}$ |
| Isolate | $790^{ef}$ | $927^{ab}$ | $957^{a}$ | $870^{cde}$ | $907^{abc}$ |

[1] Emulsifying tests were performed at the same level of protein. Means with common superscripts are not significantly different. Least significant difference ($p \leq 0.05$) was 68.3 ml oil/g protein.

[2] Controls, no HTC processing.

Table 5.

Effects of Hydrothermal Cooking on Oil Absorption Capacities (g oil/100 g sample) of Soy Products[1]

| Product | Cooking Time (sec.) | | | | |
|---|---|---|---|---|---|
| | 0[2] | 11 | 19 | 30 | 42 |
| Flour | 262$^{d}$ | 238$^{d}$ | 236$^{d}$ | 225$^{d}$ | 225$^{d}$ |
| Concentrate | 283$^{cd}$ | 240$^{d}$ | 264$^{d}$ | 264$^{d}$ | 268$^{d}$ |
| Isolate | 204$^{d}$ | 511$^{a}$ | 392$^{bc}$ | 386$^{bc}$ | 408$^{ab}$ |

[1] Means with common superscripts are not significantly different. Least significant difference ($p \leq 0.05$) was 110.0.

[2] Controls, no HTC processing.

Table 6.

Effects of Hydrothermal Cooking on Hydration of Soy Protein Products (moisture content after 1 wk)[1]

| Products | Cooking Time (sec.) | | | | |
|---|---|---|---|---|---|
| | 0[2] | 11 | 19 | 30 | 42 |
| Flour | $9.6^{de}$ | $9.2^{f}$ | $9.2^{f}$ | $9.3^{ef}$ | $9.4^{ef}$ |
| Concentrate | $10.0^{bc}$ | $9.7^{cd}$ | $9.8^{bcd}$ | $9.6^{de}$ | $9.7^{c}$ |
| Isolate | $10.0^{b}$ | $10.8^{a}$ | $10.7^{a}$ | $10.8^{a}$ | $10.7^{a}$ |

[1] Means with common superscripts are not significantly different. Least significant difference ($p \leq 0.05$) was 0.3%.

[2] Controls, no HTC processing.

Table 7.

Effects of Hydrothermal Cooking on Bulk Densities (g/cc) of Soy Protein Products[1]

| Product | Cooking Time (sec.) | | | | |
|---|---|---|---|---|---|
| | 0[2] | 11 | 19 | 30 | 42 |
| Flour | $0.33^{de}$ | $0.35^{cd}$ | $0.33^{de}$ | $0.38^{b}$ | $0.38^{bc}$ |
| Concentrate | $0.35^{bcd}$ | $0.36^{bc}$ | $0.32^{ef}$ | $0.31^{ef}$ | $0.29^{f}$ |
| Isolate | $0.47^{a}$ | $0.19^{g}$ | $0.18^{g}$ | $0.19^{g}$ | $0.17^{g}$ |

[1] Means with common superscripts are not significantly different. Least significant difference ($p \leq 0.05$) was 0.03 g/cc.

[2] Controls, no HTC processing.

Table 8.

Effects of Hydrothermal Cooking on Colors of Soy Protein Products[1]

| Product | Cooking Time (sec) | Hunter Color Values | | |
|---|---|---|---|---|
| | | L | a | b |
| Flour | 0[2] | $92.2^{bcdf}$ | $-0.85^{ef}$ | $10.3^{e}$ |
| | 11 | $93.1^{ab}$ | $-0.62^{cd}$ | $11.0^{c}$ |
| | 19 | $93.0^{abc}$ | $-0.43^{c}$ | $10.8^{cd}$ |
| | 30 | $90.8^{ghi}$ | $0.15^{b}$ | $11.7^{b}$ |
| | 42 | $90.5^{hi}$ | $0.38^{a}$ | $12.0^{b}$ |
| Concentrate | 0 | $93.7^{a}$ | $-1.80^{h}$ | $11.7^{b}$ |
| | 11 | $91.9^{cdef}$ | $0.26^{ab}$ | $8.75^{h}$ |
| | 19 | $91.7^{defg}$ | $0.22^{ab}$ | $9.01^{gh}$ |
| | 30 | $90.8^{fghi}$ | $0.27^{ab}$ | $9.35^{fg}$ |
| | 42 | $89.9^{i}$ | $0.21^{ab}$ | $9.56^{f}$ |
| Isolate | 0 | $92.7^{abcd}$ | $-2.76^{i}$ | $14.1^{a}$ |
| | 11 | $93.2^{ab}$ | $-1.55^{g}$ | $10.4^{de}$ |
| | 19 | $91.6^{efgh}$ | $-1.39^{g}$ | $10.3^{e}$ |
| | 30 | $91.8^{degf}$ | $-0.96^{f}$ | $10.7^{cde}$ |
| | 42 | $91.0^{fghi}$ | $-0.69^{ed}$ | $10.8^{cd}$ |

[1] Means with common superscripts are not significantly different. Least significant differences ($p \leq 0.05$) for 'L', 'a', and 'b' were 1.13, 0.23, and 0.50, respectively.

[2] Controls, no HTC processing.

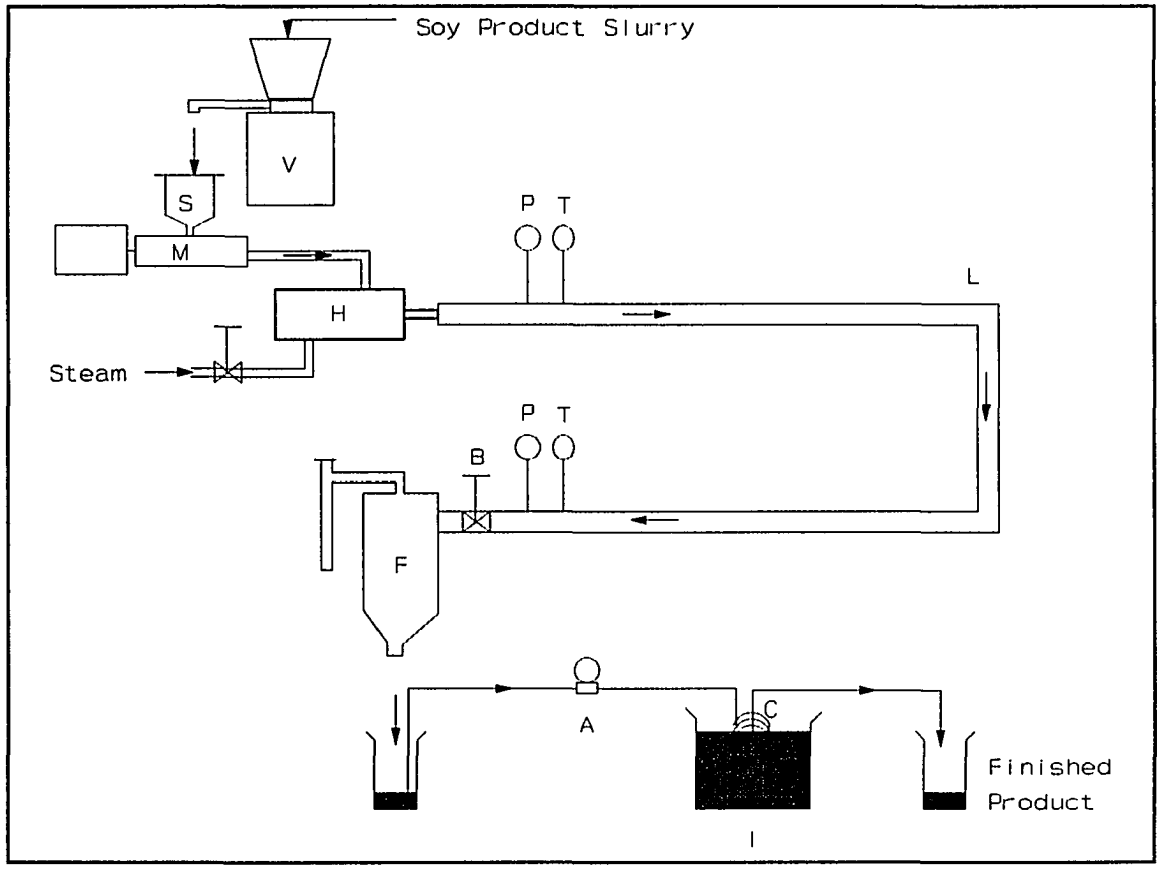


Figure 1. Hydrothermal cooking system (V, Vibroreactor; S, surge tank; M, Moyno pump; H, hydroheater; L, holding tube; P, pressure gauges; T, thermal-couples; B, back pressure valve; A, air-driven pump; F, flash chamber; C, cooling coil; and I, ice water bath.)

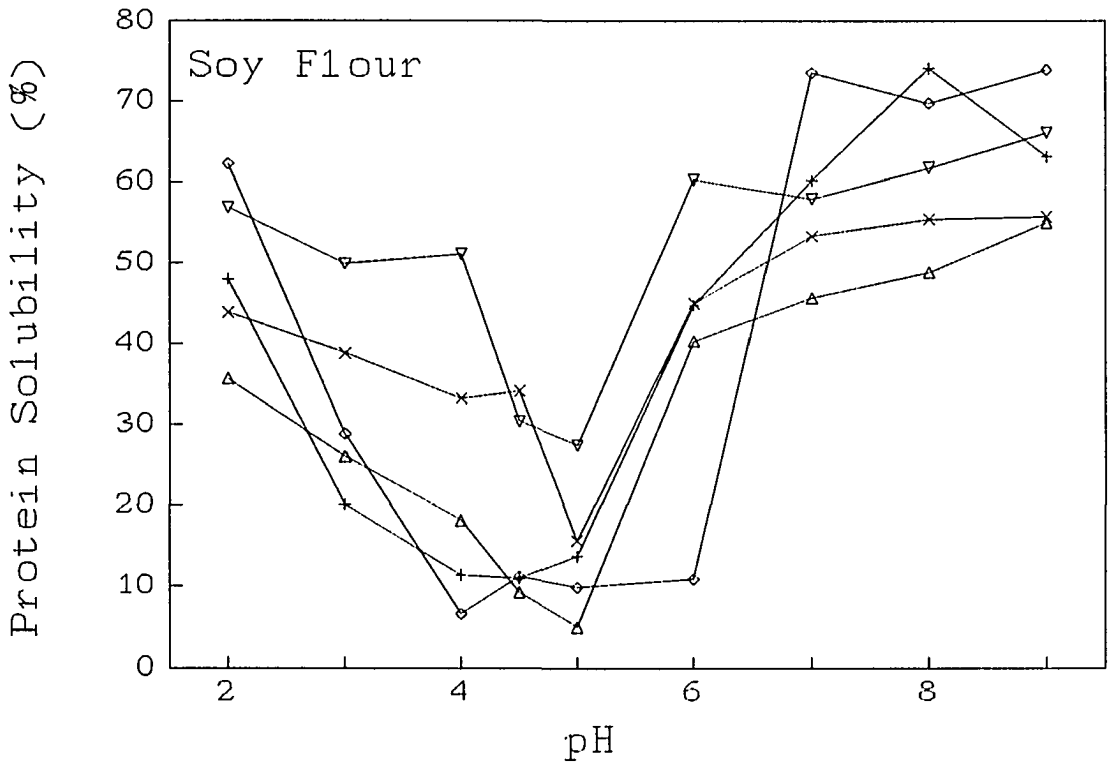


Figure 2. Effects of HTC processing on the protein solubility profiles of soy flour (+, 0 sec; ◇, 11 sec; ▵, 19 sec; ×, 30 sec; and ▽, 42 sec). Least significant difference ($p \leq 0.05$) was 8.26%.

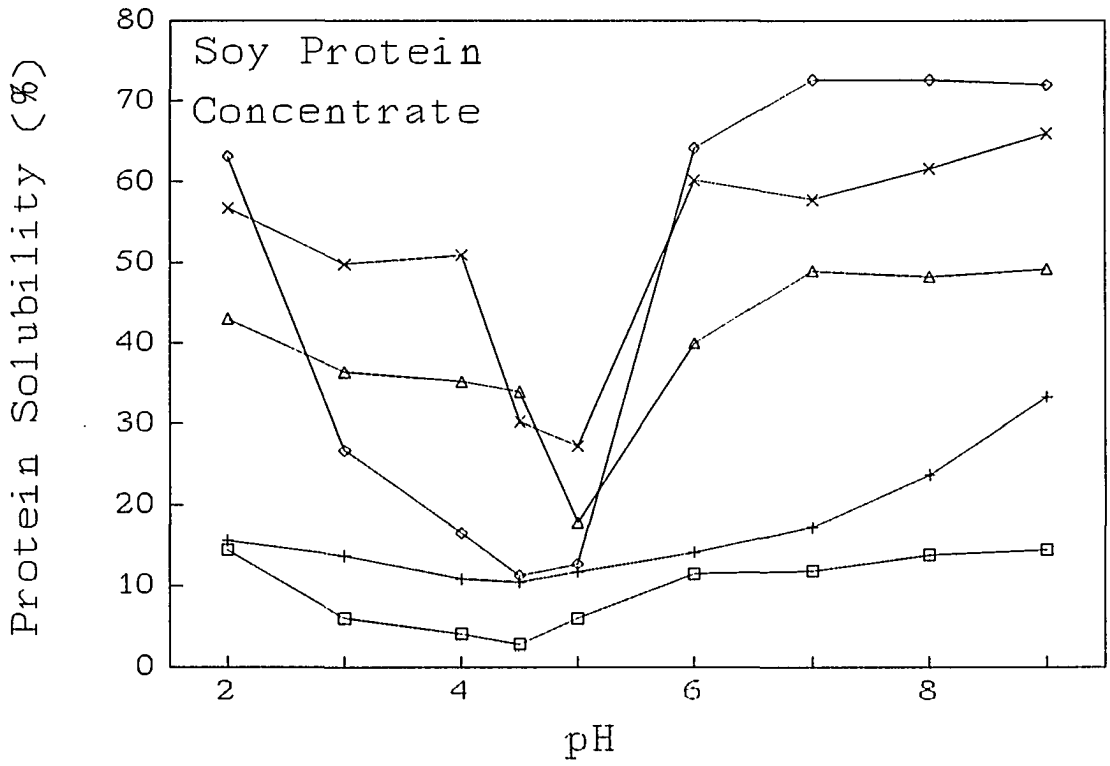


Figure 3. Effects of HTC processing on the protein solubility profiles of soy protein concentrate (□, 0 sec; +, 11 sec; ◇, 19 sec; ▵, 30 sec; and ×, 42 sec). Least significant difference ($p \leq 0.05$) was 5.45%.

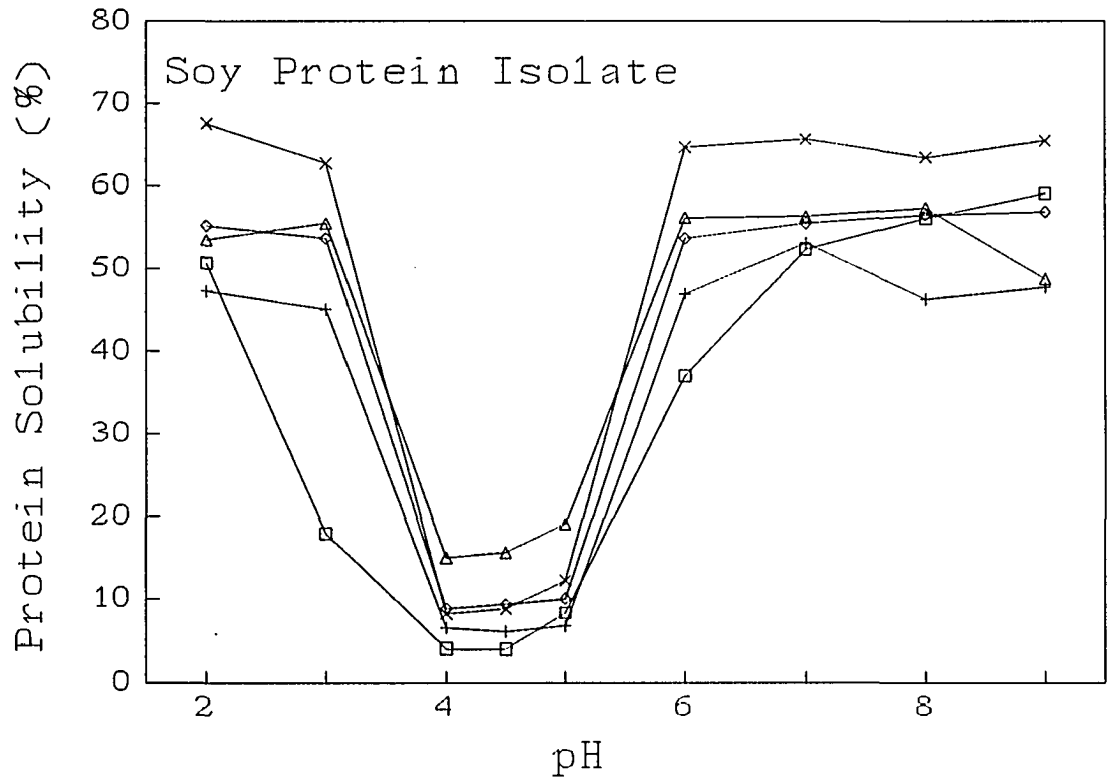


**Figure 4.** Effects of HTC processing on the protein solubility profile of soy protein isolate (□, 0 sec; +, 11 sec; ◇, 19 sec; ▵, 30 sec; and ×, 42 sec). Least significant difference ($p \leq 0.05$) was 4.60%.

# PAPER II

# COAGULATION PROPERTIES OF HYDROTHERMALLY PROCESSED SOYMILK

# Coagulation Properties of Hydrothermally Processed Soymilk

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## ABSTRACT

The effects of Hydrothermal Cooking (HTC), a steam-injection process, on calcium salt coagulation properties of soymilk were studied. Full-fat soymilk were processed at five different processing conditions (traditional kettle cooking at 100°C for 5 min, HTC at 100°C for 20 sec, HTC at 134°C for 26 sec, HTC at 154°C for 31 sec, and HTC at 162°C for 35 sec) and soymilk was coagulated at four $CaCl_2$ concentrations (0.05, 0.10, 0.20 and 0.30%). Tofu yield and recoveries of solids and protein in the coagulated curd followed similar trends with increasing $CaCl_2$ concentration, namely, an initial increase rising to a peak followed by a decrease. HTC-processed soymilks, especially those processed at high temperature (162°C), gave lower tofu yields and lower solids and protein recoveries in tofu. HTC-processed soymilks, especially those processed at high temperature (132, 154 and 162°C), resulted in tofu with inferior texture characteristics, namely, very soft, fragile, and adhesive. Both the mass balance and tofu texture data indicated that HTC-processed soymilk was not suitable for tofu manufacturing. However, the characteristic of high calcium salt tolerance of HTC-processed soymilk might be utilized to improve dispersion stability of calcium-fortified soy-based dairy analogs.

# INTRODUCTION

Tofu, also known as soybean curd, has been widely consumed in the Orient for many centuries. It provides an important source of protein and calcium in Asian diets. Soybean curd is prepared by coagulating soymilk, an aqueous extract of soybeans, using coagulating agents and subsequently molding the coagulum (1). Coagulation properties of soymilk are critical to achieving high yields of tofu and desired texture. Calcium sulfate is the most commonly used coagulating agent, but others include calcium chloride, calcium lactate, calcium acetate, calcium carbonate, calcium phosphate, calcium gluconate, and glucono-$\delta$-lactone (1).

Soymilk preparation is the first step of tofu manufacturing. Traditionally, soybeans are soaked overnight, ground with a small amount of water, kettle cooked at 100°C for 3-10 min and filtered to remove insolubles (2). The resulting soymilk is normally adjusted to 5-6% solids content before coagulating. Traditionally prepared soymilk has off-flavors (painty and beany) due to lipid oxidation catalyzed by lipoxygenase during soaking and grinding. In Western societies, this unacceptable flavor is the major obstacle for widespread acceptance of almost all soy food products, especially soymilk and tofu.

A steam-infusion cooking process, known as Hydrothermal Cooking (HTC), was developed to continuously process soymilk from ground full-fat soy flour (3, 4, 5). HTC-processed soymilk had less off-flavors because of much shorter time for lipoxygenase activity and the steam stripping occurring during the flashing of steam. The process also increased recoveries of solids and protein in the soymilk from 60 and 70% for traditional soymilk to 87 and 90%, respectively (6). It is not known whether HTC-processed soymilk can be used for tofu manufacturing, despite its superior flavor characteristics and yield. However, it is known that the degree of heat treatment of soymilk affects gel formation and gel quality (7).

Another significance of coagulation properties of soymilk is the sensitivity of soy protein to calcium fortification of soy-based dairy analogs. Compared with bovine milk, soymilk has much lower calcium content (3 mM for soymilk versus 30 mM for bovine milk) (8). Calcium fortification efforts have largely been unsuccessful due to the tendency for calcium to coagulate the proteins forming precipitates and causing gelation of the soymilk during storage. Weingartner et al (8) were able to fortify soymilk (6% solids) to a comparable calcium level of bovine milk using mixtures of calcium citrate and tricalcium phosphate. Addition of these calcium salts did

not adversely affect protein stability of the beverages. Zemel et al (9) also patented a method for fortifying soymilk with a calcium source in which an alkali metal polyphosphate salt was added to suppress aggregation between soymilk constituents and the added calcium ions. All these methods require addition of special calcium salts. It is not known whether HTC-processed soymilk has lower calcium sensitivity, which would enable use of more common and less expensive forms of calcium.

The objectives of the present study were to investigate the effects of HTC processing conditions on coagulation properties of soymilk and to explore the possibilities of utilizing the HTC process for tofu manufacturing and/or calcium fortification of soymilk.

# MATERIALS AND METHODS

## Soymilk Processing

Soymilk was prepared using full-fat soy flour, which was obtained by grinding Vinton 81 soybeans (West Central Cooperative, Jefferson, IA). The same processing methods used in our previous study on effects of HTC processing on functionalities of soy protein products (10) were followed. The ground soybean slurry was processed at 8% solids in the present study. Four different HTC processing temperatures (100, 130, 154, and 162°C) were used. Only one holding tube length (9.3 m) was used, which resulted four different cooking times (20, 26, 30, and 35 sec) for the four temperatures, respectively. Traditional soymilk prepared by kettle boiling for 5 min was used as a control. The cooked slurry was centrifuged at 1000 xG for 10 min and the supernatant was decanted and collected as soymilk. Solids and protein contents were determined using the same procedures as were used before (10).

## Tofu Coagulation

Coagulation was carried out by modifying the tofu making procedure used by Saio et al (11). Calcium chloride, instead of calcium sulfate, was used as coagulating agent, because it has high water solubility leading to more reproducible results (1). Soymilk samples were adjusted to 5.0% solids. Samples (39 ml) were placed in 50-ml centrifuge tubes and incubated in a 70°C waterbath for 30 min. Then 1 ml of various calcium chloride solutions of different concentrations (0.02, 0.04, 0.08, and 0.12 g/ml) were added to the warm soymilk samples. The samples were promptly mixed for 10 sec using a vibrating mixer (Barnstead/Thermolyne, Dubuque, IA). The final calcium chloride concentrations in the soymilk samples were 0.05, 0.10, 0.20, and 0.30%. Then the samples were placed in the waterbath for 30 min. The samples were cooled by placing them in a cold waterbath for 20 min before centrifuging at 1000 xG for 10 min. The resulting curds at the bottom of the tubes were tofu. The test-tube tofu procedure requires less sample and gives reproducible mass-balance information.

## Tofu Evaluation

The amounts of supernatant and tofu were measured gravimetrically. The tofu yield was calculated as the percentage of wet precipitate weight after centrifuging divided by the starting soymilk weight (as-is basis). Solids and protein contents of the supernatant were analytically determined and solids and protein contents of the tofu fractions were calculated by differences in the mass balances.

Texture profiles of curds were evaluated using a Voland Stevens Texture Analyzer (Voland Corporation, Hawthorne, NY). Curds were carefully cut using a razor blade into blocks with a 2 cm x 2 cm base and a height of 1.5 cm. Care was taken to ensure that the texture analyzer probe pressed and penetrated the center of the block. The TA58 probe with a 5-mm travel distance and a speed of 0.5 mm/sec in the cycle mode were chosen as operation parameters. Texture profiles were recorded with a chart recorder. The chart speed and sensitivity were set to 10 cm/min and 2 V, respectively. The analyzer was calibrated so that the full-scale force was 100 g. Hardness, fracturability, and adhesiveness were read from the profile according to definitions by Saio et al (11).

## Experimental Design

Complete random factorial design (two factors, five levels of processing conditions, and four levels of calcium concentrations) was used. The results were analyzed by using the SAS system and means were compared by using the T-test.

# RESULTS AND DISCUSSIONS

## Tofu Mass Balances

### Tofu Yield

The effects of calcium chloride concentration and HTC-processing conditions on tofu yields were very dramatic (Table 1). When 0.05% of calcium chloride was added to HTC soymilk processed at 132, 154, and 162°C, there was little coagulation of tofu (1.2-2.3%). There were significantly greater amounts of tofu coagulation (37.8%) in traditional soymilk, although the precipitates were in a semi-liquid form. When the calcium chloride concentration was increased to 0.1%, tofu yields from all soymilks increased significantly. Traditionally prepared soymilk had the highest yield (34.4%), followed by HTC soymilk processed at 100, 132, and 152°C (31.2-32.4%). The HTC soymilk processed at 162°C had the lowest tofu yield (25.8%). When the calcium chloride concentration was increased to 0.2% and more, tofu yields for all soymilk trended downward but most of the differences were not statistically significant. However, traditionally prepared soymilk declined to a greater extent than HTC processed soymilk

so that HTC soymilk processed at 100, 132, and 154°C had higher tofu yields than traditionally prepared soymilk when 0.2 or 0.3% calcium chloride was added. Overall, HTC-processed soymilk had lower tofu yields than traditionally prepared soymilk, especially when processed at a higher temperature (162°C).

Solids Recovery in Tofu

Solids (dry matter basis) recovery in tofu is a better indicator of than the tofu yield based on an "as-is" wet basis. The effects of HTC processing conditions and calcium chloride concentrations on solids recoveries followed similar trends as wet tofu yields (Table 2).

Higher concentrations of calcium chloride generally lead to higher solids recoveries. However, solids recoveries started to decline when the calcium chloride concentration exceeded critical levels. Traditionally prepared soymilk reached maximum solids recovery at 0.1% calcium chloride concentration. This compares favorably with an earlier study (1), where 0.09% calcium chloride was considered optimum. However, HTC soymilk processed at 162°C had its highest solids recovery at 0.2% calcium chloride. HTC soymilk processed at lower temperatures (100, 132, and

154°C) exhibited maximum solids recoveries at either 1% or 2% calcium chloride.

When 0.05% calcium chloride was added, solids recoveries for HTC soymilk processed at 132, 154, and 162°C were very low (4-7%) compared with traditionally-processed soymilk which gave the highest solids recovery (53%). At higher calcium chloride concentrations, HTC soymilk processed at lower temperatures (100, 132, and 154°C) had higher solids recoveries followed by traditional soymilk, and followed HTC soymilk processed at 162°C. The higher the HTC processing temperature (and time), the lower the solids recovery in tofu.

Protein Recovery in Tofu

Protein recovery was calculated as the percentage of protein in the original soymilk that recovered in tofu fraction (Table 3). The effects of HTC processing condition and calcium chloride concentration followed a similar trends those of tofu yield and solids recovery.

The higher the calcium chloride concentration, the more protein recovered in tofu. Traditionally prepared soymilk reached the highest protein recovery at 0.10% calcium chloride; higher concentration led to

lower protein recovery in tofu. HTC soymilk processed at 100°C merely rose from its low value at 0.05% calcium chloride to a plateau at the other three concentrations. HTC soymilks processed at 132, 154, and 162°C reached their maximum protein recoveries at 0.20% calcium chloride.

When 0.05% calcium chloride was added to HTC-processed soymilk, especially soymilk processed at higher temperatures (132, 154, and 165°C), very little protein was recovered in the precipitated fraction and the majority of protein remained in supernatant. However, traditionally prepared soymilk had about 42% of the protein recovered in the precipitated fraction (tofu). When the calcium chloride concentration increased to 0.1%, traditionally prepared soymilk and HTC soymilk processed at 100°C had the highest protein recoveries in tofu. HTC soymilk processed at 162°C had the lowest protein recovery, where more than 40% of the protein remained in the supernatant. When the calcium chloride concentration was increased to 0.2 and 0.3%, HTC soymilk processed at lower temperatures (100, 134, and 154°C) gave about the same protein recovery as traditionally prepared soymilk. However, HTC soymilk processed at 162°C still had significantly lower protein recovery in tofu than the rest.

Mass Balances

Tofu yield, solids recovery, and protein recovery for soymilk processed differently all followed a common trend with increasing calcium chloride concentration, initial increases to maximum values followed by declines. This can be explained as salt-in effects, excess salts can make protein more soluble (12). The manner in which the soymilk was processed affected the amounts of calcium chloride required for maximum values. Traditionally processed soymilk reached maximum values at lower calcium chloride concentrations than HTC-processed soymilk, and the higher the processing temperature, the higher the calcium chloride concentration required to maximize tofu yields and recoveries of solids and protein. The mechanism for this peak delay or high tolerance of HTC-processed soymilk to calcium salts is not known.

The high tolerance of HTC-processed soymilk to low calcium chloride concentration may prove to be useful in calcium fortification of soymilk. Soymilk with 6% solids naturally contains approximately 3 mM calcium, whereas bovine milk contains 25 mM calcium. Our data indicates that the calcium content of soymilk could be increased to at least 7 mM, which more than doubles its natural content, without adversely affecting stability. This is

not possible with traditional soymilk.

## Tofu Texture Profiles

Effects of HTC processing conditions and calcium chloride concentration on tofu texture profiles (including hardness, fracturability, and adhesiveness) were also studied to predict the suitability of HTC-processed soymilk for tofu manufacturing. When 0.05% of calcium chloride was added, too little tofu resulted to perform texture analyses. Therefore, texture data for this calcium chloride concentration could not be obtained.

### Tofu Hardness

Hardness was defined as the force encountered by the analyzer probe when it traveled to the maximum distance when penetrating the curd. There was a very apparent and consistent trend for tofu hardness by calcium chloride concentration (Table 4). For all soymilks, tofu hardness values initially increased to maximum and then declined with increasing calcium concentration, 0.2% calcium chloride gave the hardest tofu.

The effects of HTC processing were also extremely significant. HTC processing greatly reduced the hardness of tofu and the higher the processing

temperature, the softer the resulting tofu.

Tofu Fracturability

Fracturability was defined as the force encountered by the probe when it first punctured the sample surface. The higher the facturability value, the less fragile the texture. The effects of HTC processing condition and calcium chloride concentration followed the same trend as for hardness. HTC-processed soymilks, especially those processed at higher temperatures, resulted in very fragile tofu compared with traditionally prepared soymilk.

Tofu Adhesiveness

Adhesiveness was defined as the negative force encountered by the probe when it withdraws from the penetrated curd. It was measured as negative peak area ($mm^2$) from the texture profile. Tofu adhesiveness increased with increasing calcium chloride concentration. HTC-processed soymilk resulted tofu that was more adhesive, and the higher the processing temperature, the more adhesive the tofu.

HTC-processed soymilk, especially those processed at high temperatures (132, 154, and 162°C), gave tofu with inferior texture characteristics, namely very soft, fragile, and adhesive textures.

# CONCLUSIONS

Yields, solids recoveries, and protein recoveries for tofu from soymilk processed under different conditions followed a common pattern with increasing calcium chloride concentration, namely, initial increases to maximum values followed by decreases. HTC-processed soymilks, especially those processed at high temperature (162°C), gave lower tofu yields and lower solids and protein recoveries in tofu. HTC-processed soymilks, especially those processed at high temperature (132, 154, and 162°C), resulted in tofu with inferior texture characteristics, being very soft, fragile, and adhesive. Both the mass balance data and tofu texture data revealed that HTC-processed soymilk was not suitable for tofu manufacturing. However, the high calcium salt tolerance of HTC-processed soymilk might prove beneficial in calcium fortification of soy-based dairy analogs.

## REFERENCES


1. Lu, J.Y., E. Carter, and R.A. Chung. *J. Food Sci. 45*:32 (1980).
2. Fukushima, D. *J. Am. Oil Chem. 58*:346 (1981).
3. Johnson, L.A., C.W. Deyoe, and W.J. Hoover. U.S. Patent 4,409,256. (1981).
4. Hung, J.S. *Studies on Processing, Functional Characteristics and Nutritional Quality of Hydrothermal Extracts of Soybeans*, Ph.D. Dissertation. Kansas State University, Manhattan, KS, 1984.
5. Kim, C.J. *Physico-Chemical, Nutritional, and Flavor Properties of Soybean Extracts Processed by Rapid-Hydration Hydrothermal Cooking*, Ph.D. Dissertation. Iowa State University, Ames, IA, 1988.
6. Johnson, L.A., C.W. Deyoe, and W.J. Hoover. *J. Food Sci. 46*:239 (1981).
7. Hashizume, K., and T. Watanabe. *Agr. Biol. Chem. 43*:683-690 (1979).
8. Weingartner, K.E., A.I. Nelson, and J.W. Erdman Jr. *J. Food Sci. 48*:256 (1983).
9. Zemel, M.B. U.S. Patent 4,906,482. (1990)

10. Wang, C., and L.A. Johnson. *J. Am. Oil Chem.* In Press.

11. Saio, K., M. Kamiya, and T. Watanabe. *Agr. Biol. Chem. 33*:1301 (1969).

12. Kinsella, J.E., S. Damodaran, and B. German. In *New protein foods, Vol 5, Seed storage proteins*, edited by A. M. Altschul and H. L. Milcke, Academic Press, New York, NY, p. 107, 1985.

Table 1.

Effects of HTC Processing on Tofu Yield (%)[1]

| Processing Conditions | | $CaCl_2$ Concentration (%) | | | |
|---|---|---|---|---|---|
| Temperature (°C) | Time | 0.05 | 0.10 | 0.20 | 0.30 |
| HTC | | | | | |
| 100 | 20 sec | $9.3^{f}$ | $31.2^{cd}$ | $29.7^{d}$ | $30.6^{cd}$ |
| 132 | 26 sec | $1.2^{g}$ | $32.1^{c}$ | $29.8^{d}$ | $31.6^{cd}$ |
| 154 | 31 sec | $2.3^{g}$ | $32.4^{bc}$ | $29.8^{d}$ | $31.1^{cd}$ |
| 162 | 35 sec | $1.9^{g}$ | $25.8^{e}$ | $25.3^{e}$ | $25.3^{e}$ |
| Traditional | | | | | |
| 100 | 5 min | $37.8^{a}$ | $34.4^{b}$ | $27.1^{e}$ | $27.2^{e}$ |

[1] Tofu yield was calculated as wet tofu weight percentage of soymilk (as-is basis). Means with common superscripts are not significantly different at $p \leq 0.05$. Least significant difference ($p \leq 0.05$) was 2.1%.

Table 2.

Effects of HTC Processing on Solids Recovery in Tofu (%)[1]

| Processing Conditions | | $CaCl_2$ Concentration (%) | | | |
|---|---|---|---|---|---|
| Temperature (°C) | Time | 0.05 | 0.10 | 0.20 | 0.30 |
| HTC | | | | | |
| 100 | 20 sec | $21.1^{j}$ | $80.3^{a}$ | $79.3^{abc}$ | $77.7^{cdef}$ |
| 132 | 26 sec | $4.4^{l}$ | $80.1^{ab}$ | $79.1^{abcd}$ | $78.1^{bcde}$ |
| 154 | 31 sec | $6.2^{lk}$ | $77.4^{cdef}$ | $76.9^{ef}$ | $76.1^{f}$ |
| 162 | 35 sec | $7.4^{k}$ | $65.5^{h}$ | $67.6^{h}$ | $66.5^{h}$ |
| Traditional | | | | | |
| 100 | 5 min | $53.1^{i}$ | $77.1^{def}$ | $73.9^{g}$ | $73.2^{g}$ |

[1] Means with common superscripts are not significantly different at $p \leq 0.05$. Least significant difference ($p \leq 0.05$) was 2.0%.

Table 3.

Effects of HTC Processing on Protein Recovery (%) in Tofu[1]

| Processing Conditions | | $CaCl_2$ Concentration (%) | | | |
|---|---|---|---|---|---|
| Temperature (°C) | Time | 0.05 | 0.10 | 0.20 | 0.30 |
| HTC | | | | | |
| 100 | 20 sec | $8.2^{g}$ | $77.9^{a}$ | $77.4^{a}$ | $77.1^{a}$ |
| 132 | 26 sec | $0.3^{i}$ | $73.6^{b}$ | $76.4^{a}$ | $77.3^{a}$ |
| 154 | 31 sec | $3.2^{h}$ | $70.9^{c}$ | $77.4^{a}$ | $76.6^{a}$ |
| 162 | 35 sec | $4.8^{h}$ | $58.0^{e}$ | $66.2^{d}$ | $66.9^{d}$ |
| Traditional | | | | | |
| 100 | 5 min | $41.7^{f}$ | $78.8^{a}$ | $76.1^{a}$ | $76.6^{a}$ |

[1] Means with common superscripts are not significantly different at $p \leq 0.05$. Least significant difference ($p \leq 0.05$) was 2.1%.

Table 4.

Effects of HTC Processing on Hardness (g) of Tofu[1]

| Processing Conditions | | $CaCl_2$ Concentration (%) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 0.10 | 0.20 | 0.30 |
| HTC | | | | |
| 100 | 20 sec | $10.3^{e}$ | $16.2^{c}$ | $13.9^{d}$ |
| 132 | 26 sec | $4.4^{hi}$ | $9.7^{ef}$ | $7.9^{fg}$ |
| 154 | 31 sec | $2.7^{ji}$ | $7.2^{g}$ | $5.9^{gh}$ |
| 162 | 35 sec | $2.2^{j}$ | $4.9^{h}$ | $5.0^{h}$ |
| Traditional | | | | |
| 100 | 5 min | $24.6^{b}$ | $30.0^{a}$ | $18.2^{c}$ |

[1] Means with common superscripts are not significantly different at $p \leq 0.05$. Least significant difference ($p \leq 0.05$) was 2.1 g.

Table 5.

Effects of HTC Processing on Fracturability (g) of Tofu[1]

| Processing Conditions | | $CaCl_2$ Concentration (%) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 0.10 | 0.20 | 0.30 |
| HTC | | | | |
| 100 | 20 sec | $11.5^{e}$ | $18.7^{c}$ | $16.3^{d}$ |
| 132 | 26 sec | $4.6^{hi}$ | $12.6^{e}$ | $9.3^{f}$ |
| 154 | 31 sec | $2.3^{kj}$ | $7.2^{g}$ | $5.2^{h}$ |
| 162 | 35 sec | $1.0^{kl}$ | $4.5^{hi}$ | $2.8^{ij}$ |
| Traditional | | | | |
| 100 | 5 min | $22.1^{b}$ | $30.0^{a}$ | $20.2^{c}$ |

[1] Means with common superscripts are not significantly different at $p \leq 0.05$. Least significant difference ($p \leq 0.05$) was 1.7 g.

Table 6.

Effects of HTC Processing on Adhesiveness (mm) of Tofu[1]

| Processing Conditions | | $CaCl_2$ Concentration (%) | | |
|---|---|---|---|---|
| Temperature (°C) | Time | 0.10 | 0.20 | 0.30 |
| HTC | | | | |
| 100 | 20 sec | $21.4^{ji}$ | $30.5^{ghi}$ | $48.8^{def}$ |
| 132 | 26 sec | $37.6^{fgh}$ | $40.7^{efg}$ | $54.0^{cde}$ |
| 154 | 31 sec | $52.9^{de}$ | $48.9^{def}$ | $57.0^{cd}$ |
| 162 | 35 sec | $68.2^{cdc}$ | $94.7^{b}$ | $120.1^{a}$ |
| Traditional | | | | |
| 100 | 5 min | $15.3^{j}$ | $24.4^{hij}$ | $33.6^{ghi}$ |

[1] Means with common superscripts are not significantly different at $p \leq 0.05$. Least significant difference ($p \leq 0.05$) was 14.8 $mm^2$.

# PAPER III

# ULTRASTRUCTURE CHANGES OF SOY PROTEIN PRODUCTS CAUSED BY HYDROTHERMAL PROCESSING

# Ultrastructure Changes of Soy Protein Products Caused by Hydrothermal Processing

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## ABSTRACT

Light microscopy, transmission electron microscopy, gel filtration chromatography, immuno rocket electrophoresis, and differential scanning calorimetry (DSC) were used to investigate ultrastructure changes of soy protein during a high-temperature, short-time heat treatment known as hydrothermal cooking (HTC) processing or steam-infusion cooking. Light microscopy and particle size analysis showed that HTC processing disrupted large particles from aqueous-alcohol-washed soy protein concentrates, which contributed to increased protein solubility and dispersibility of soy protein concentrate. HTC also reduced the particle size of soy flour particles by both disrupting and preventing formation of large protein aggregates during heating, which increased protein solubility. Transmission electron microscopy indicated that HTC processing transformed the proteins into small protein aggregates that were water soluble. Gel filtration chromatography showed proteins formed aggregates during or after HTC processing. Rocket immunoelectrophoresis indicated the proteins (glycinin and ß-conglycinin) were no longer in their biologically active form. DSC confirmed that the soy proteins were no longer in native conformations after HTC processing.

## INTRODUCTION

A steam-infusion cooking process, known as hydrothermal cooking (HTC), was developed to continuously process soymilk from ground soy flour (1, 2, 3). The process significantly increased recoveries of solids and protein in the final soymilk from 60 and 70% for traditional soymilk to 87 and 90%, respectively (4). HTC also improved solubilities and other functional properties of soy protein products, especially aqueous-alcohol-washed soy protein concentrate (5). However, there is little understanding of the mechanisms accounting for these effects. In order to understand mechanisms for the effects of HTC processing and to further improve functional properties of soy protein products, it is important to understand ultrastructure changes of soy protein products, especially changes to soy proteins.

Soy proteins are composed of four major groups, 2S, 7S, 11S and 15S globulins. The 7S and 11S globulins, also respectively known as glycinin and ß-conglycinin, are the two major storage proteins of soybeans comprising approximately two-thirds of the total seed protein (6). Glycinin consists of alternately arranged acidic and basic subunits, which normally exist as two hexamers. ß-conglycinin often exists as a dimer, each monomer

consists of three subunits.

Both proteins undergo denaturation when heated. Denaturation connotes any nonproteolytic modification to the native structure of protein, including any process that alters the tertiary or quaternary structures (7). Glycinin and ß-conglycinin have denaturation temperatures of 90 and 75°C, respectively (8). The temperature at which denaturation occurs is a function of protein structure, water concentration, pH, and ionic strength. The denaturation temperatures of both ß-conglycinin and glycinin increase by over 30°C as the water content of the system decreases from 90 to 20% (9). These two proteins have higher thermal stabilities as ionic strength increases (8). ß-conglycinin and glycinin are highly stable to heat when the pH is near their isoelectric points (pH 4.5 and pH 6.5, respectively) (9). Thermal denaturation of both ß-conglycinin and glycinin generally follows a three-step process: initial dissociation of the subunits, unfolding of subunits, and subsequent aggregation (10, 11). Usually the end result is insoluble protein aggregates with poor functional properties.

When the protein concentration is high, gelation also occurs after denaturation by heat. The minimum protein concentration required for heat gelation depends on heating temperature and heating time (12). Heat-

induced protein gels can be divided into two types: gels formed by random aggregation and gels formed by association of molecules into strands in a more ordered manner (13). The gelation mechanisms for pure ß-conglycinin and glycinin solutions are believed to be different, ß-conglycinin globulin forms gel by random aggregation and the glycinin forms a gel in a more ordered manner (13, 14).

The mechanism for gelation of a mixture of ß-conglycinin and glycinin is different from those of pure individual globulin solutions (15). The gelation mechanism of any given soy protein product is even more complex due to interactions of protein with non-protein materials.

The objective of the present study was to investigate ultrastructure changes of soy protein products, especially protein structural changes, during HTC processing. This may help elucidate the mechanism for the effects of HTC processing on functional properties of soy protein products and lead to economical methods to improve functional properties.

# MATERIALS AND METHODS

## HTC Processing

The same processing procedures (HTC at 154°C for 31 sec) used in our previous study on functional properties of HTC-processed soy protein products (5) were used to prepare liquid dispersions for particle size analysis, light microscopy, and transmission electron microscopy (TEM). Liquid dispersions without heat treatment and those processed by traditional heating (kettle heating at 100°C for 5 min) were used as controls.

In gel filtration chromatography, the freshly prepared samples were processed under the same conditions (HTC at 100°C for 20 sec, HTC at 134°C for 26 sec, HTC at 154°C for 31 sec, and HTC at 162°C for 35 sec) as were used in our previous coagulation study (16).

## Particle Size Distribution

Nylon filter cloths with mesh openings of 30 and 100 $\mu$m were used to study particle size distribution. A 20-ml liquid dispersion was filtered through 100-$\mu$m filter cloth using vacuum and 60 ml of distilled water was used to spray-wash the retained material. Then the filtrate was filtered

through a 30-$\mu$m filter cloth. Percentages of solids in three fractions (smaller than 30 $\mu$m, between 30 and 100 $\mu$m, and larger than 100 $\mu$m) were used to characterize particle size distributions.

## Light Microscopy

Freshly prepared liquid dispersions were examined using a Nikon light microscope (Nippan Kogaku K. K., Tokyo, Japan). Micrographs were obtained by using an attached 35-mm camera.

## Transmission Electron Microscopy

Sample dispersions were centrifuged (1000 xG) to avoid interference from large particles. A small drop of a freshly prepared solution was applied to a carbon-coated electron microscope grid, stained with potassium phosphotungstate (14) and then examined under a JOEL 1200ES STEM electron microscope (JOEL USA, Peabody, MA).

## Gel Filtration

Spray-dried soy flours and soy protein concentrates from our previous functional property study (5) and freshly processed liquid soy flour samples

were subjected to gel filtration chromatography.

The method used by Iwabuchi et al (17) was slightly modified. Sepharose CL-6B Gel was packed in a 2.5 X 110-cm column. The void volume was determined by eluting blue dextrin. Samples were slurried in the elution buffer, 3.2 mM potassium phosphate (pH 7.6, I=0.01) and stirred for 30 min. The slurry was centrifuged at 2000 xG for 5 min, and the protein content of the clear supernatant was determined by using the Biuret method (17). The protein solution was diluted to 5 mg/ml and 5 ml of the solution was loaded onto the gel.

Fractions (5 ml) were collected using a fraction collector. The flow rate was 0.5 ml/min. The protein content of each faction was determined by using the Biuret method. Absorbance at 540 nm after the reaction with biuret solution was plotted against the ratio of elution volume to void volume (V/Vo).

## Rocket Immunoelectrophoresis

Samples from our previous functional property study (5) were subjected to rocket immunoelectrophoresis to determined the amounts of native ß-conglycinin and glycinin. The test was carried out according to the

method of Murphy and Resurreccion (19).

## DSC Analysis

DSC analysis was carried out using the method described by Anderson (20). Sample slurries with 12% solids were prepared by using vacuum degassing so that the slurries were more uniform and the dry sample had maximum contact with water.

A Perkin Elmer DSC7 system (Perkin Elmer Corp., Norwalk, CT) equipped with a personal computer was used for thermal analysis. Stainless-steel capsules were used to hold about 65 mg of slurry. All samples were scanned from 25 to 130°C. Thermograms were normalized, so that all curves were based on 1 mg of protein.

## Degree of Hydrolysis

The method of Adler-Nissen (21) was modified to determined degrees of hydrolysis. No sodium dodecyl sulfate (SDS) was used to disperse the sample because SDS caused the sample to become turbid when added to the buffer (0.2125M sodium phosphate buffer, pH 8.2).

# RESULTS

## Particle Size Analysis

There were marked differences between uncooked soy protein concentrate and uncooked soy flour (Table 1). The uncooked soy protein concentrate consisted of much larger particles than soy flour (i.e. soy concentrate had 8.8% of solids in particles less than 30 $\mu$m versus 74.7% for soy flour). Presumably, the aqueous alcohol used to extract oligosaccharides and the heat used in desolventizing soy protein concentrate denatured the protein and caused it to aggregate into large particles.

HTC processing significantly altered particle size distributions of soy protein concentrate and soy flour. Over 72% of the solids in the uncooked soy protein concentrate slurry were in particles larger than 100 $\mu$m. When the slurry was traditionally cooked (100°C for 5 min), there was a slight reduction in particle size. However, HTC processing (154°C for 30 sec) dramatically reduced particle size; only 3.7% of the solids remained as particles larger than 100 $\mu$m. The large particles were apparently disrupted into smaller particles.

Unlike soy protein concentrate, uncooked soy flour had very little solids (12%) in particles larger than 100 μm. Traditional cooking significantly increased the proportion of particles larger than 100 μm. The increase was attributed to protein precipitation and/or aggregation. Traditionally cooked soy flour had the lowest percentage of solids in particles of intermediate size (30-100 μm). The increase in large particles came largely at the expense of intermediate-size particles and the proportion of small particles remained unchanged. HTC dramatically reduced particles larger than 100 μm, but the proportion of intermediate-size particles remained unchanged. Almost 87% of the total solids were in particles smaller than 30 μm. Reduced particle size of HTC-processed proteins may be due to the high temperature and shear. Also, the high shear mixing action of HTC processing probably prevented soy protein from forming large aggregates.

## Light Microscopy

Light micrographs showed the effects of HTC on the ultrastructures of aqueous-alcohol-washed soy protein concentrate and full-fat soy flour dispersions (Fig. 1). HTC disrupted large particles in soy protein

concentrate into smaller particles and dispersible proteins (the latter not being visible under a light microscope). No differences were observed between slurries without heat treatment and with traditional heat treatment.

Compared with the HTC-processed dispersion of full-fat soy flour, dispersions of full-fat flour with no heat and with traditional heat had fat droplets fewer in number and larger in size (10 $\mu$m for no heating and traditional heating versus 1 $\mu$m for HTC processing). Thus, the high-shear mixing action during HTC processing also reduced fat droplet size stabilizing the emulsion system. We have constantly observed more lipid separation in traditionally cooked soymilk than in HTC-processed soymilk during centrifugation after cooking.

## Transmission Electron Microscopy (TEM)

Samples were centrifuged at 1000 xG before examining under the elctronic microscope to avoid interference from large particles. Therefore, TEM micrographs showed only dispersible portions of samples.

Unheated soy flour dispersions had small light spherical structures (a), which were native soy proteins (Fig. 2A). Traditionally processed soy flour showed aggregates (a) of irregular shape (Fig. 2B). Dispersions of HTC-

processed soy flour (Fig. 2C) had larger dark aggregates (a) than traditionally-processed soy flour. The high viscosity of HTC-processed dispersions (3) may have kept these large aggregates in the water dispersion after centrifugation. In addition to dark aggregates, there were small irregularly-shaped aggregates in HTC-processed soy flour (b).

Both unheated (Fig. 2D) and traditionally-heated (Fig. 2E) soy protein concentrate dispersions showed small spherical structures (a), which were probably soybean globulins. HTC-processed soy protein concentrate disperion (Fig. 2F) had some large aggregates (a) and a lot of irregularly-shaped aggregates (b). There were also some small spherical structures (c), which were probably low molecular weight (MW) proteins.

## Gel Filtration Chromatography

Gel filtration chromatographic profiles of soy flours, which were HTC processed at 154°C for 11 and 45 sec, were compared with those of ß-conglycinin, glycinin, and raw flour (Fig. 3). The proteins in the HTC-processed soy flours had much larger molecular weights (MW) eluting from the gel near the void volume (V/Vo = 1). These were probably protein aggregates. In the raw flour profile, a shoulder after the main peak was

observed, which was probably dissociates of either ß-conglycinin or glycinin, or 2S proteins. The shoulder was absent in the other profiles. The MW difference between ß-conglycinin and glycinin was not noticeable at the level of resolution used (both had V/Vo ratios near 1.5).

The change in the gel filtration pattern could have been the result of either HTC or spray drying. In order to eliminate the effect of spray drying, gel filtration patterns of freshly-prepared HTC-processed liquid samples were also compared with standards (Fig. 4). However, the results were the same, proteins in all HTC-processed samples were aggregated. This demonstrated that HTC processing alone caused protein aggregation and was not caused by spray drying.

The gel filtration chromatographic profiles of aqueous-alcohol-washed soy protein concentrate are shown in Figure 5. It should be noted that the profiles are for only those of dispersible proteins, and any precipitated fraction could not be loaded onto the column. The proteins in the soluble portion of the unprocessed soy protein concentrate (Fig. 5B) had similar MW's (V/Vo = 1.5) as native ß-conglycinin and glycinin. It also had two fractions of smaller MW's (2.1 and 2.5 V/Vo), which we speculate to be dissociates of ß-conglycinin and glycinin, or 2S globulins. The soy protein

concentrate with only 11 sec of HTC processing at 154°C had much greater proportion of protein with smaller MW at the expense of the native protein portion, which was absent. It is believed that the fraction with smaller MW was a protein that was heat and alcohol stable. HTC-processed soy protein concentrate samples, especially those processed at 154°C for 19, 35, and 42 sec (Fig. 5C-F), consisted of proteins with different MW's (1.0, 1.2, and 1.8 V/Vo). The larger MW fractions were probably protein aggregates. The smaller MW fraction was believed to be the dissociated subunits of ß-conglycinin and glycinin, or 2S globulins.

## Immuno-Rocket Gel Electrophoresis

Immuno-rocket gel electrophoresis was used to qualitatively determine whether ß-conglycinin and glycinin in HTC-processed samples were still in their biologically native structures. On both ß-conglycinin and glycinin antibody gels, none of the HTC-processed samples had rockets typical of their standards, while unprocessed soy flours and soy protein isolates did. HTC completely destroyed the biological activity of the two globulins. On the glycinin antibody gel, unprocessed soy protein concentrate had a very small rocket. However, it was absent on ß-conglycinin globulin antibody

gel. This indicated that glycinin was more stable to aqueous-alcohol-washing than ß-conglycinin globulins.

## DSC Analysis

All HTC-processed samples and the unprocessed soy protein concentrate sample had no thermal transition (Fig. 6). Unprocessed soy flour and soy protein isolate exhibited two thermal transitions. One was the ß-conglycinin transition with an onset temperature around 75°C; the other was the glycinin transition with an onset temperature of 95°C. This was consistent with values previously reported (3, 10). No transition was observed for trypsin inhibitor (60°C), as reported by Anderson (10). The DSC study confirmed that proteins in the HTC-processed samples were completely denatured.

## Degree of Hydrolysis

Soy flours, soy protein concentrates, and soy protein isolates had degrees of hydrolysis (DH) of 1.33-1.55, 1.22-1.45, and 1.39-1.64 L-leucine equ/mg protein, respectively (Table A8). There were no significant

differences among any of the samples. Therefore, there was no indication that protein hydrolysis occurred during HTC processing.

# DISCUSSION

## Hypotheses

Earlier studies (1, 2, 3) showed that HTC produced very stable soymilk emulsions, and that HTC recovered much more solids and protein than those obtained by traditional cooking. Our previous study (5) on functional properties of HTC-processed soy protein products showed that HTC processing significantly increased nitrogen solubility index (NSI) of aqueous-alcohol-washed soy protein concentrate and slightly improved NSI's of soy flour and soy protein isolates. HTC also improved emulsifying capacities of different soy protein products to different degrees.

Prior to the present study, we hypothesized the following mechanism (Fig. 7) to explain these observations. Upon heating, proteins in different soy protein products dissociate into subunits. With prolonged heating, these subunits completely unfold, which in turn form aggregates. With HTC processing, high temperatures for short periods partially unfold subunits. These partially unfolded subunits lead to the improved solubility and emulsifying capacity shown in earlier studies. In the case of full-fat soymilk, a stable emulsion formed due to exposed hydrophobic regions of

partially-unfolded subunits, which functioned much more efficiently as emulsifiers. Protein hydrolysis could have also played a role in improved protein solubility.

## Evaluation of Hypotheses

The present study disapproved many elements of the preceding hypotheses. Firstly, gel filtration data indicated proteins in HTC-processed soy flour and majority of proteins in HTC-processed soy concentrate were in aggregates. Secondly, DSC results did not show any thermal transition, which eliminated the existence of partially-unfolded subunits. Finally, DH determinations showed there were not any DH changes caused by HTC processing, ruling out the possibility of protein hydrolysis as a mechanism for improved protein solubility.

The gel filtration data also showed that there were significant amounts of small MW proteins in HTC-processed soy protein concentrate, although the majority of proteins were in aggregates. The small MW proteins might be partially responsible for the improved solubility and emulsifying capacity.

## New Models

Based on the present study, the following mechanisms for different soy protein products are proposed (Fig. 8).

Soy Protein Concentrate

Large particles of aqueous-alcohol-washed soy protein concentrate were disrupted into much smaller particles, as demonstrated by particle size analysis and light microscopy. There are three ways that the disruption process can increase NSI and improve functional properties of aqueous-alcohol-washed soy protein concentrate. Firstly, ultra-small particles from the disruption process can remain dispersed when NSI is determined. These ultra-small protein particles are able to function in foaming and emulsifying just as native soy proteins do. Secondly, during the disruption process, proteins are also released into solution, some may form dissociates of proteins, which was indicated by gel filtration chromatography. These dissociated protein subunits were soluble and had high foaming and emulsifying capacities. Finally, even with the formation of aggregates from single protein molecules released by disruption, these aggregates were sufficiently small to remain in solution and be functional. The disruption

process was attributed to both high temperature and high shear.

Soy Flour and Soy Protein Isolate

Both HTC processing and traditional cooking can disrupt any large particles in soy flour, which release proteins and increases protein solubility of soy flour. This is proven by the particle size analysis. Secondly, soluble proteins in soy flour and soy protein isolate form aggregates when heated. However, HTC processing formed aggregates which are small enough to stay in solution and function as emulsifying agents. This is attributed to the high shear of HTC preventing formation of large aggregates. Traditional cooking formed much lager aggregates due to lack of shear. Finally, the high viscosity of the HTC-processed slurry might also prevent aggregates from separating from the dispersion.

Full-Fat Soymilk

A similar mechanism is proposed for full-fat soymilk as has been proposed for soy flour and soy protein isolate, except there are some additional elements. Small dispersible aggregates formed during HTC processing still function as emulsifiers and interacted with oil droplets.

Secondly, the high shear of HTC processing disrupted large oil droplets into much smaller droplets, which improved the stability of soymilk emulsions.

# CONCLUSIONS

Light microscopy and particle size analysis showed that HTC processing disrupted large particles from aqueous-alcohol-washed soy protein concentrates, which normally are poorly soluble in water. This led to greater water solubility of proteins in soy protein concentrates. HTC also reduced the particle size of soy flour dispersions by both disruption and preventing formation of large protein aggregates during heating. Transmission electron microscopy indicated that HTC processing transformed proteins into protein aggregates. Gel filtration chromatography showed proteins formed aggregates during or after HTC processing. Rocket immunoelectrophoresis and DSC indicated the proteins (glycinin and ß-conglycinin) were no longer in their native form.

## REFERENCES


1. Johnson, L.A., C.W. Deyoe, and W.J. Hoover. U.S. Patent 4,409,256. (1981).
2. Hung, J.S. *Studies on Processing, Functional Characteristics and Nutritional Quality of Hydrothermal Extracts of Soybeans*, Ph.D. Thesis. Kansas State University, Manhattan, KS, 1984.
3. Kim, C.J. *Physico-Chemical, Nutritional, and Flavor Properties of Soybean Extracts Processed by Rapid-Hydration Hydrothermal Cooking*, Ph.D. Thesis. Iowa State University, Ames, IA, 1988.
4. Johnson, L.A., C.W. Deyoe, and W.J. Hoover. *J. Food Sci. 46*:239 (1981).
5. Wang, C., and L.A. Johnson. *J. Am. Oil Chem.* In Press.
6. Nielsen, N.C. In *New Protein Foods, Vol 5* edited by A.M. Altschul, and H.L. Milcke, Academic Press, New York p. 27, 1985.
7. Kinsella, J.E., S. Damodaran, and B. German. In *New protein foods. Vol 5*, edited by A.M. Altschul, and H.L. Milcke, p. 107, Academic Press, New York, 1985.
8. Hermansson, A.M. *J. Am. Oil Chem. Soc. 56*:595 (1979).

9. Sheard, P.R., A. Follows, D.A. Ledward, and J.R. Mithchell, *J. Food Tech. 51*:65 (1986).

10. Wolf, W.J., and T. Tamura. *Cereal Chem. 46*:331 (1969).

11. Lillford, P.J. In *Plant Proteins,* edited by G. Norton, p. 298. Butterworths, London, 1978.

12. Catsimpoolas, N., and E.W. Meyer. *Cereal Chem. 47*:559 (1970).

13. Hermansson, A.M. *J. Am. Oil Chem. 63*:658 (1986).

14. Nakamura, T., S. Utsumi, and T. Mori. *Agri. Biol. Chem. 50*:1287 (1986).

15. Nakamura, T., S. Utsumi, and T. Mori. *Agri. Biol. Chem. 50*:2429 (1986).

16. Wang, C., and L.A. Johnson. *J. Am. Oil Chem.* In Press.

17. Iwabuchi, S., H. Watanabe, and F. Yamauchi. *J. Agric. Food Chem. 39*:27 (1991).

18. Robyt, J.F., and B.J. White. In *Biochemical Techniques, Theory and Practice* p. 235, Brooks/Cole, Monterey, CA, 1988.

19. Murphy, P.A., and A.P. Resurreccion, J. Agric. *Food Chem. 32*:911 (1984).

20. Anderson, R.L. *J. Am. Oil Chem. Soc.* In Press (1992).

21. Adler-Nissen, J. *J. Agri. Food Chem. 27*:1256 (1979).

Table 1.

Effects of HTC Processing on Particle Size Distributions of Soy Protein Products[1]

| Soy Products | Heating Process | Particle Size Distribution (%) | | |
|---|---|---|---|---|
| | | Smaller[2] than 30 $\mu$m | Between[3] 30 and 100 $\mu$m | Larger[4] than 100 $\mu$m |
| Concentrate | None[5] | 8.8[a] | 18.8[c] | 72.5[f] |
| | Traditional[6] | 25.2[b] | 11.4[b] | 63.4[e] |
| | HTC[7] | 59.5[c] | 36.8[d] | 3.7[b] |
| Defatted Flour | None | 74.7[d] | 12.8[b] | 12.5[c] |
| | Traditional | 72.7[d] | 4.6b[a] | 22.7[d] |
| | HTC | 86.9[e] | 12.3[b] | 0.8[a] |

[1] Values sharing the same superscript in the same column are not significantly different ($p \leq 0.05$).
[2] Least significant difference ($p \leq 0.05$) was 3.2%.
[3] Least significant difference ($p \leq 0.05$) was 2.8%
[4] Least significant difference ($p \leq 0.05$) was 3.5%
[5] No heat treatment.
[6] Slurries were kettle heated at 100°C for 5 min.
[7] Slurries were HTC processed at 154°C for 31 sec.

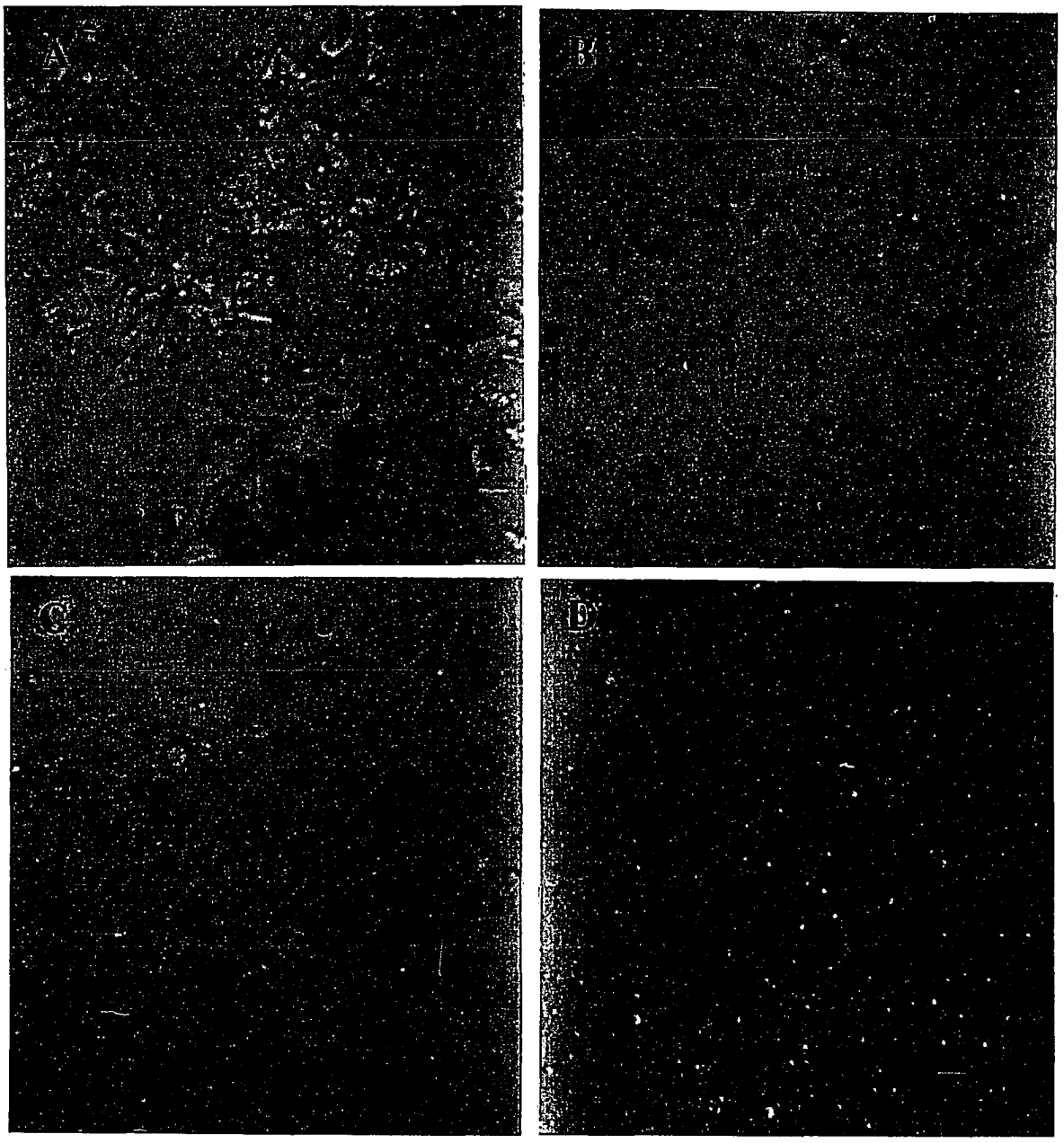


Figure 1. Light micrographs (400X) of soy protein products dispersions. (A, soy protein concentrate dispersion without heat treatment; B, HTC-processed soy protein concentrate dispersion; C, traditionally-processed full-fat soy flour dispersion; and D, HTC-processed full-fat soy flour dispersion. Bar size is 25 $\mu$m.)

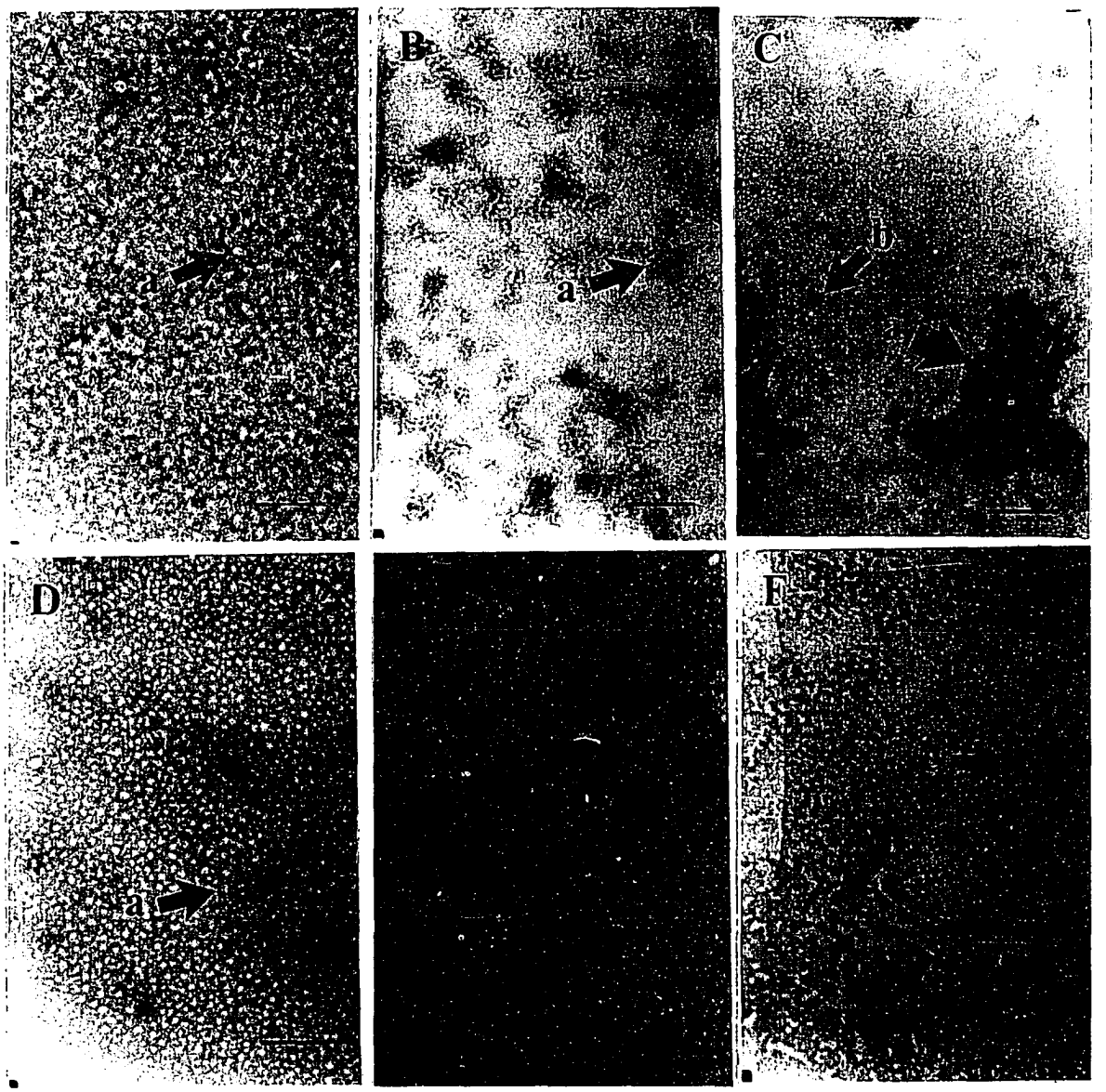


Figure 2. Transmission electron micrographs of soy protein product dispersions. (A, unheated soy flour; B, traditionally-heated soy flour; C, HTC-processed soy flour; D, unheated soy protein concentrate; E, traditionally-heated soy protein concentrate; and F, HTC-processed soy protein concentrate. Bar size is 200 nm).

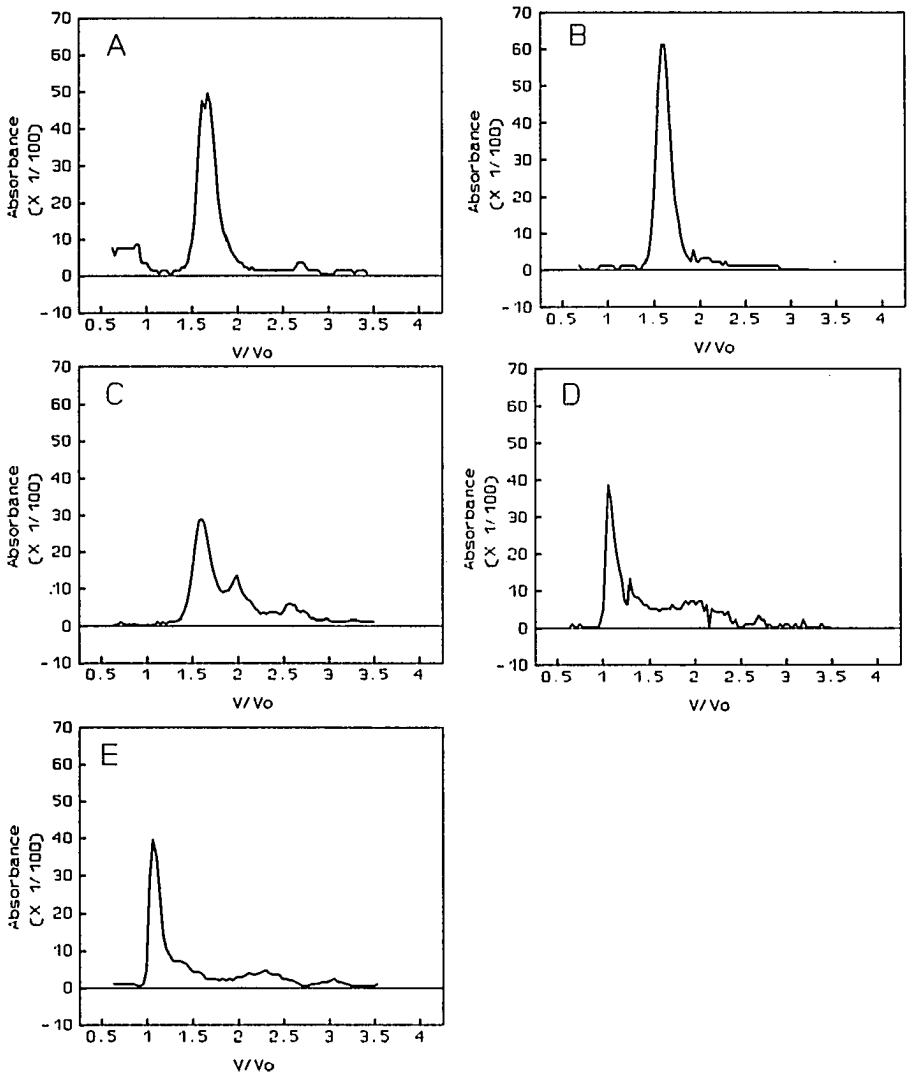


Figure 3. Effects of HTC processing on gel filtration chromatographic patterns of spray-dried soy flour proteins (A, ß-conglycinin standard; B, glycinin standard; C, raw soy flour; D, HTC processed at 154°C for 11 sec; and E, HTC processed at 154°C for 42 sec).

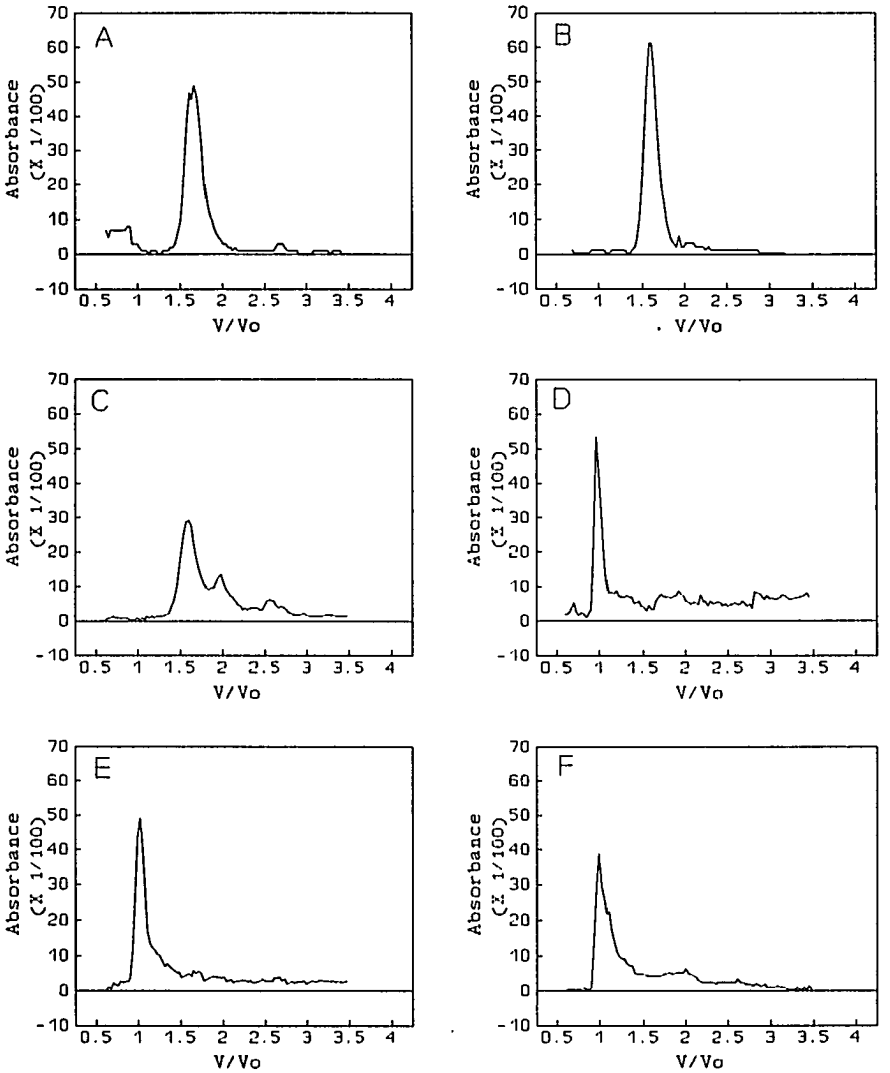


Figure 4. Effects of HTC on gel filtration chromatographic patterns of freshly processed (without spray drying) soy flour proteins (A, ß-conglycinin standard, B, glycinin standard; C, no heat; D, HTC processed at 100°C for 20 sec; E, HTC processed at 132°C for 26 sec; and F. HTC processed at 154°C for 31 sec).

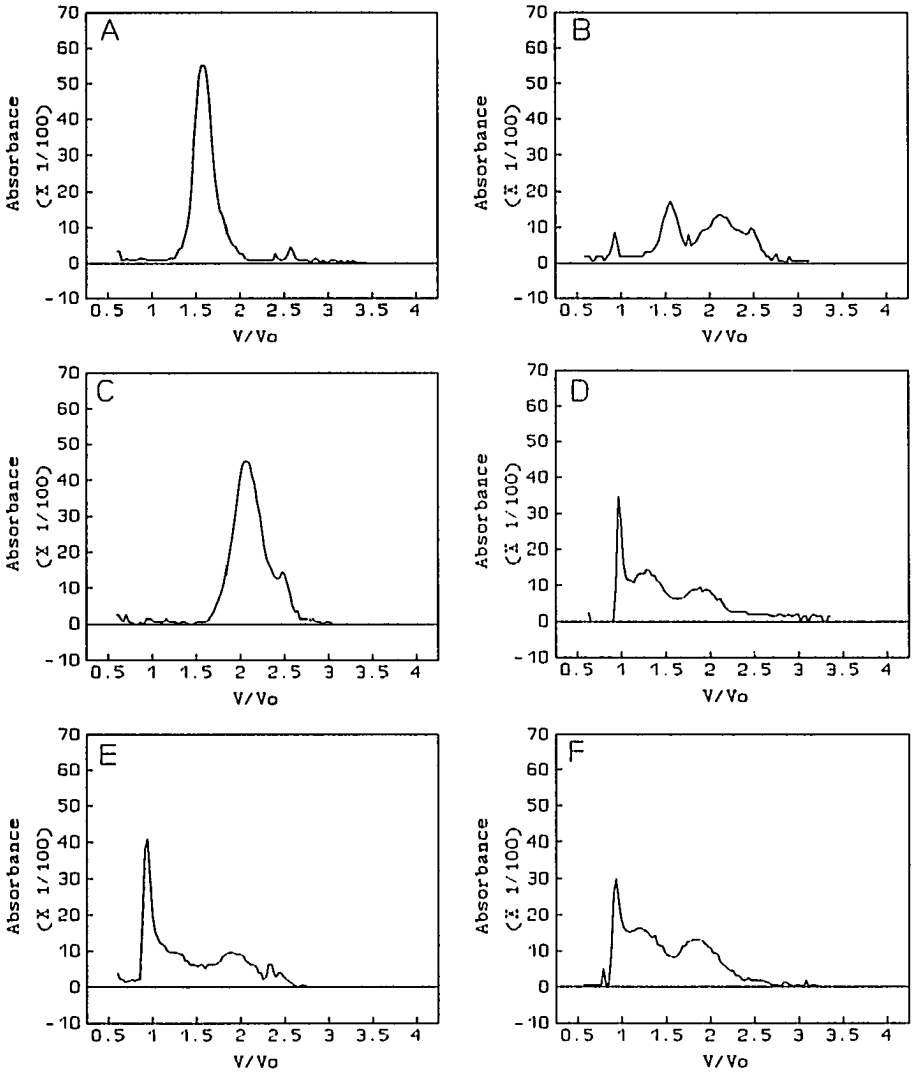


Figure 5. Effects of HTC processing on gel filtration patterns of aqueous - alcolhol-washed soy protein concentrate proteins (A, glycinin standard; B, no heat; C, HTC processed at 154°C for 11 sec; D, HTC processed at 154°C for 25 sec; E, HTC processed at 154°C for 31 sec; and F, HTC processed at 154°C for 42 sec).

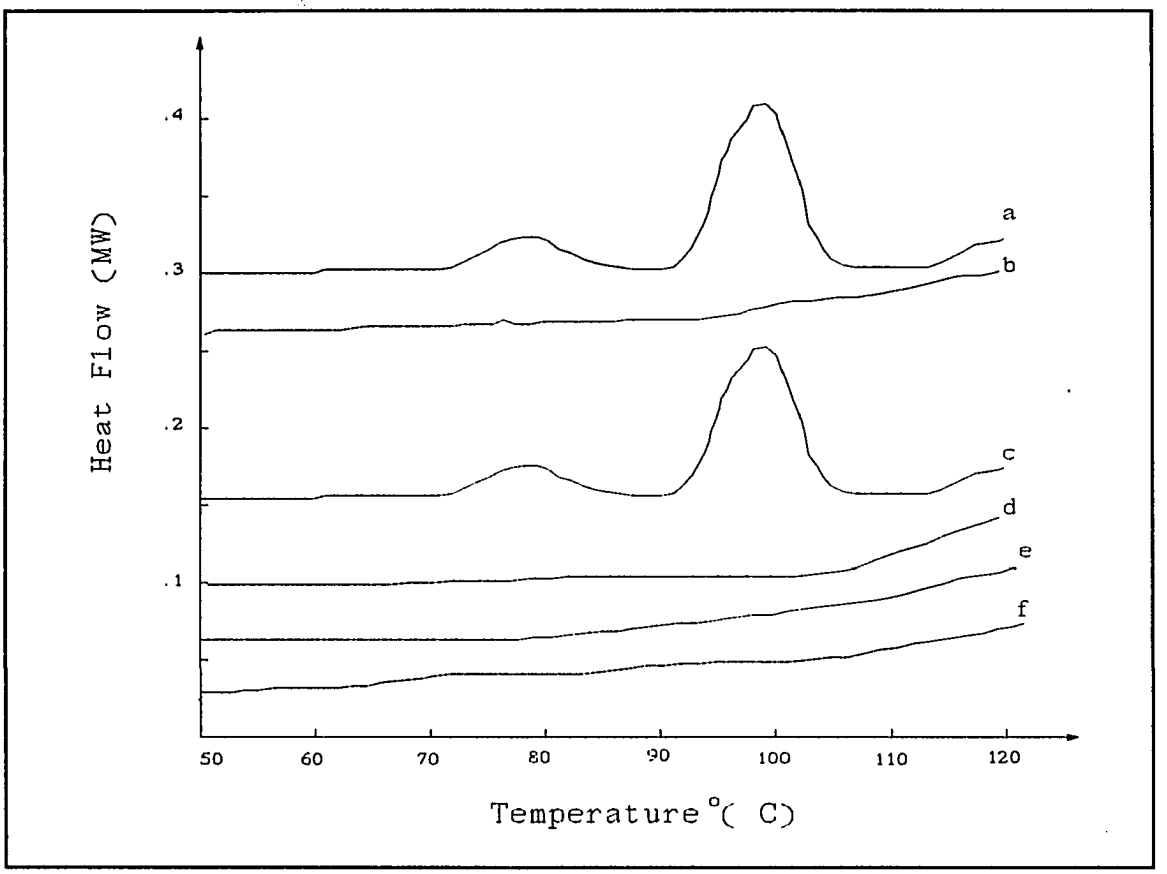


Figure 6. Effects of HTC on DSC patterns of soy protein products (a, spray-dried soy flour; b, soy flour HTC processed at 154°C for 11 sec; c, unheated spray dried soy protein isolate; d, soy protein isolate HTC processed at 154°C for 11 sec; e, unheated spray-dried soy protein concentrate; and f, soy protein concentrate HTC processed at 154°C for 11 sec)

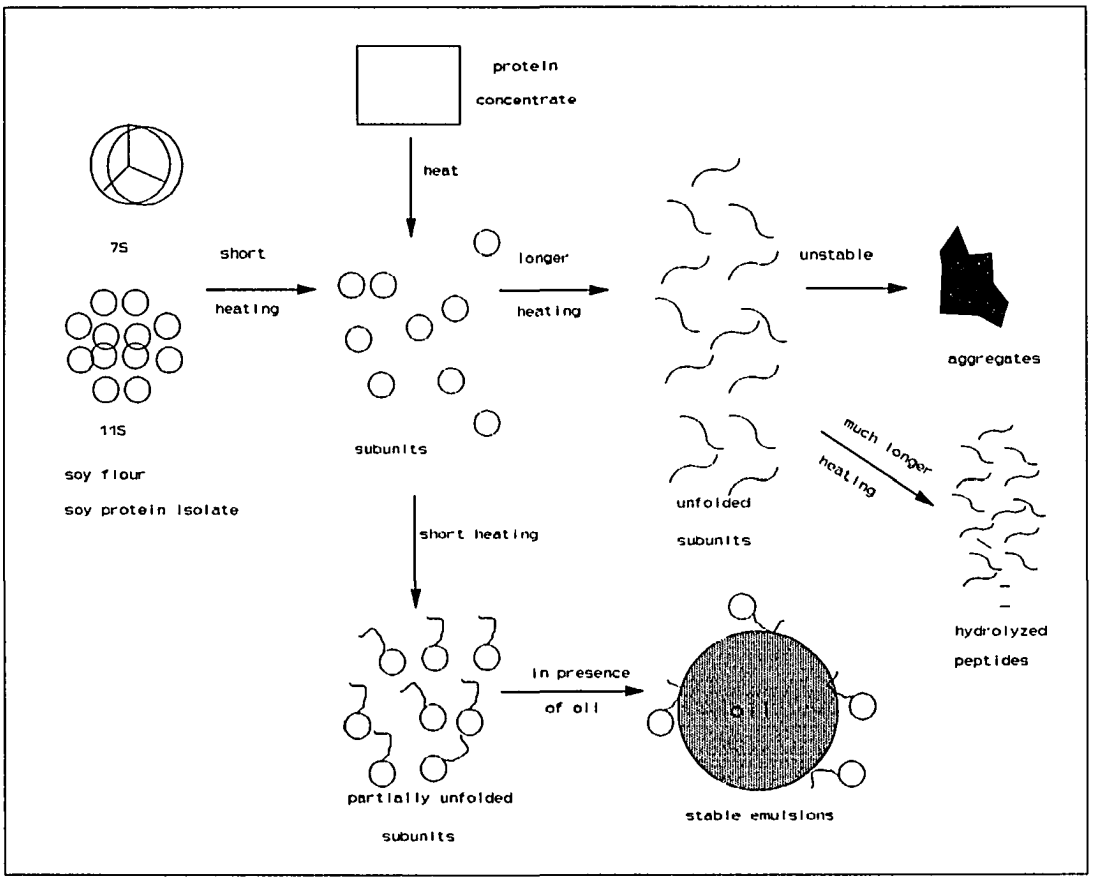


Figure 7. Hypothesized mechanisms of HTC processing on soy protein ultrastructure

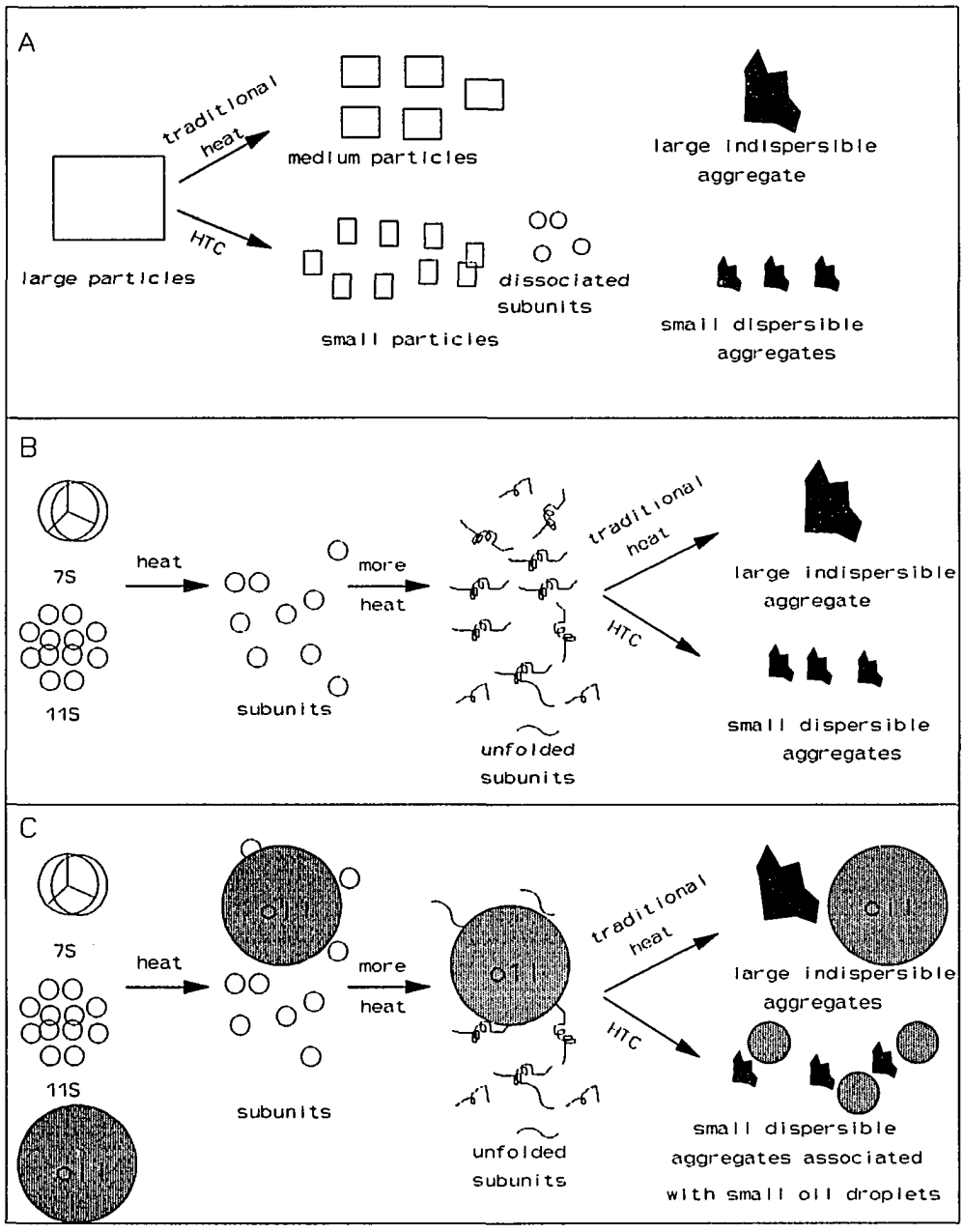


Figure 8. Mechanisms of HTC processing on soy protein ultrastructure (A, soy protein concentrate; B, soy flour and soy protein isolate; and C, full-fat soymilk)

## GENERAL SUMMARY

HTC processing increased the NSI of soy protein concentrates by approximately four times (from 15 to 56%). HTC changed the solubility profile of soy protein concentrate from a flat profile to one typical of undenatured soy protein. It also improved their foaming and emulsifying properties. For soy protein isolates, HTC also improved NSI, and foaming and emulsifying properties, although the effects were less dramatic. NSI and emulsifying properties of soy flour were also improved when processed for longer times than 19 sec. The foaming properties of soy flour were not improved by HTC.

Tofu yield and recoveries of solids and protein in the coagulated curd all followed a common trend with increasing $CaCl_2$ concentration, namely, an initial increase rising to a peak followed by a decrease. HTC-processed soymilk, especially those processed at high temperature (162°C), gave lower tofu yields and lower solids and protein recoveries in tofu. HTC-processed soymilks, especially those processed at high temperature (132, 154, and 162°C), resulted in tofu with inferior texture characteristics, namely, very soft, fragile, and adhesive. Both the mass balance and tofu texture data indicated that HTC-processed soymilk was not suitable for tofu

manufacturing. However, the characteristic of high calcium salt tolerance of HTC-processed soymilk might be utilized to improve dispersion stability of calcium-fortified soy-based dairy analogs.

Light microscopy and particle size analysis showed that HTC processing disrupted large particles from aqueous alcohol-washed soy protein concentrates, which might lead to solubilization of proteins in the soy protein concentrate. HTC also reduced particle size of soy flour dispersions by both disrupting and preventing formation of large protein aggregates during heating, which may account for the increased protein solubility of soy flour by HTC processing. Transmission electron microscopy indicated that HTC processing transformed proteins into protein aggregates, which were still dispersible. The gel filtration chromatography also showed proteins formed aggregates after HTC processing. Rocket immunoelectrophoresis indicated the proteins (glycinin and ß-conglycinin) were no longer in their biologically native forms. DSC verified that soy proteins were no longer in native conformation after processed by HTC.

The overall conclusions were that HTC processing was a very effective means to improve functional properties of soy protein products and it has potential application in calcium fortification of soymilk. HTC

processing transformed proteins into aggregates which were dispersible in water.

## REFERENCES


1. Smith, A.K, and S.J. Circle. In *Soybeans: Chemistry and Technology*, edited by A.K. Smith and S.J. Circle, AVI Publishing Co., Westport, CN, p. 339, 1976.
2. Slavin, J. Nutritional benefits of soy fiber. *J. of Am. Diet. Assoc. 91*:816 (1991).
3. Carrol, K.K. Review of clinical studies on cholesterol-lowing response to soy protein. *J. of Am. Diet. Assoc. 91*:820(1991).
4. Jacues, H., D. Laurin, S. Moorjani, F.H. Steinke, C. Gagne, D. Brun, and P.J. Lupien. Influence of diets containing cow's or soy protein beverage on plasma lipid in children with familial hypercholesterolemia. *J. of Am. Col. of Nutr. 11*:69S (1992).
5. Johnson, L.A., D.J. Myers and D.J. Burden. Soy protein's history, prospects in food, feed. *INFORM 3*:429 (1992).
6. Nielsen, N.C. In *New Protein Foods, Vol 5* edited by A.M. Altschul, and H.L. Milcke, Academic Press, New York p. 27, 1985.
7. Kinsella, J.E., S. Damodaran, and B. German. In *New protein foods. Vol 5*, edited by A.M. Altschul, and H.L. Milcke, p. 107, Academic

Press, New York, 1985.

8. Hermansson, A.M. *J. Am. Oil Chem. Soc. 56*:595 (1979).

9. Sheard, P.R., A. Follows, D.A. Ledward, and J.R. Mithchell, *J. Food Tech. 51*:65 (1986).

10. Wolf, W.J., and T. Tamura. *Cereal Chem. 46*:331 (1969).

11. Lillford, P.J. In *Plant Proteins,* edited by G. Norton, p. 298. Butterworths, London, 1978.

12. Iwabuchi, S., H. Watanabe, and F. Yamauchi. *J. Agric. Food Chem. 39*:27 (1991).

13. Anderson, R.L. *J. Am. Oil Chem. Soc.* In Press (1992).

14. Murphy, P.A., and A.P. Resurreccion. J. Agric. *Food Chem. 32*:911 (1984).

15.. Shen, J.L. *J. Agric. Food Chem. 24*(4):784 (1976).

16. Patel, P.D., and J.C. Fry. in *Developments in food proteins-5,* edited by B.J.F. Hudson, Elsevier Applied Science, London and New York, p. 299 (1987).

17. Johnson, L.A. *Soy protein: Chemistry, processing, and food applications*. Presented at 70th Annual Meeting of American

Association of Cereal Chemists. Sept. 22-26th, 1985. Orlando, FL (1985).

18. Hashizume, K., K. Kakiuchi, E. Koyama, and T. Watanabe. *Agr. Biol. Chem. 35*:449 (1971).

19. Johnson, L.A., C.W. Deyoe, and W.J. Hoover. *J. Food Sci.* 46:239 (1981).

20. Hung, J.S. 1984. *Studies on processing, functional characteristics and nutritional quality of hydrothermal extracts of soybeans*, Ph.D. Dissertation, Kansas State University, Manhattan, KS, 1984.

21. Hackler, L.R., D.B. Hand, K.H. Steinkraus, and J.P. Van Buren. *J. Nutr. 80*:205 (1963).

22. Kim, C.J. *Physico-chemical, nutritional, and flavor properties of soybean extracts processed by rapid-hydration hydrothermal cooking*, Ph.D. Dissertation, Iowa State University, Ames, IA, 1988.

## ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my major professor, Dr. Lawrence A. Johnson for his guidance and patience during the entire course of this study and my graduate career at Iowa State University. Dr. Johnson spent countless evenings and weekends reading this dissertation. He has set an excellent example for a graduate student who is going to have his own graduate students in the future.

I am also very grateful to the other members of my graduate committee: Dr. Charles Hurburgh, Dr. Mark Love, Dr. Pat Murphy, and Dr. Lester Wilson for their extremely helpful suggestions. Dr. Jay-lin Jane and Dr. Deland J. Myers have also been very supportive.

I would like also to thank my groupmates, Mark Reuber, Maide Raeker, Reyna Luz Vidal-Quintanar, Kathrine Miller, Steve Fox and Mila Evangelista, for their frendship.

My family has been extremely supportive during the program. My wife Li came to join me in February of 1989, after conquering numerous obstacles. She has been working exceptionally hard both at her career and at home to give me the extra time needed to complete this project. I am forever in her debt. On August 18, at the midpoint of the research program,

our first child and lovely daughter, Lucy Ming-Jane was born. I am grateful to her for taking long naps, telling me "Mao" and "Tao" every time when I was about to go out to the cold; most of all, for giving me the joy of raising her. My parents also have been very understanding and supportive, I would like to thank them for raising me the way they did.

# APPENDIX

Table A1.

Moisture Contents of HTC-Processed Soy Protein Products[1]

| Products | Cooking Time (sec) | | | | |
|---|---|---|---|---|---|
| | 0 | 11 | 19 | 30 | 42 |
| Flour | 1.95 | 1.60 | 2.23 | 1.63 | 1.89 |
| Concentrates | 0.56 | 3.22 | 2.60 | 2.23 | 1.31 |
| Isolates | 1.67 | 2.40 | 1.70 | 1.37 | 1.37 |

[1] Least significant difference was 0.40%.

Table A2.

Protein Contents of HTC-Processed Soy Protein Products[1]

| Products | Cooking Time (sec) | | | | |
|---|---|---|---|---|---|
| | 0 | 11 | 19 | 30 | 42 |
| Flour | 63.2 | 57.3 | 56.6 | 55.5 | 54.7 |
| Concentrates | 63.8 | 77.0 | 75.0 | 70.2 | 68.8 |
| Isolates | 82.8 | 76.8 | 78.1 | 78.4 | 79.0 |

[1] Foaming tests were performed at the same level of protein. Least significant difference was 1.72.

Table A3.

Effects of Hydrothermal Cooking on Foaming Capacity (%) of Soy Protein Products (based on weight)[1]

| Products | Cooking Time (sec) | | | | |
|---|---|---|---|---|---|
| | 0 | 11 | 19 | 30 | 42 |
| Flour | 449 | 323 | 282 | 256 | 295 |
| Concentrates | 186 | 528 | 485 | 575 | 613 |
| Isolates | 920 | 1023 | 1234 | 1147 | 1182 |

[1] Foaming tests were performed at the same level of solids content. Least significant difference was 152%.

Table A4.

Effects of Hydrothermal Cooking on Foam Stability (%) of Soy Protein Products (based on weight)[1]

| Products | Cooking Time (sec) | | | | |
|---|---|---|---|---|---|
| | 0 | 11 | 19 | 30 | 42 |
| Flour | 80.4 | 26.2 | 58.7 | 64.0 | 71.1 |
| Concentrates | 6.6 | 86.3 | 85.8 | 87.2 | 88.2 |
| Isolates | 85.8 | 90.0 | 55.2 | 77.0 | 64.4 |

[1] Foaming tests were performed at the same level of solids. Least significant difference was 15.8%.

Table A5.

Effects of Hydrothermal Cooking on Emulsifying Capacity (%) of Soy Protein Products (based on weight)[1]

| Products | Cooking Time (sec) | | | | |
|---|---|---|---|---|---|
| | 0 | 11 | 19 | 30 | 42 |
| Flour | 650 | 400 | 450 | 713 | 753 |
| Concentrates | 100 | 300 | 687 | 720 | 728 |
| Isolates | 710 | 853 | 883 | 837 | 847 |

[1] Emulsifying tests were performed at the same level of solids. Least significant difference was 77.9.