CHEMICAL AND MOLECULAR PROPERTIES OF PROCESSED SOY PROTEINS

by

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INTRODUCTION

Numerous processed soy products, ranging from full-fat and defatted flours to highly processed isolates and spun fibers, have become commercially available in recent years (Alden, 1975). Although these products contain a high quantity of protein, the protein quality generally decreases with increased processing (Bressani, 1975). The reasons for this decreased quality have not been fully elucidated. Past efforts to explain the loss in nutritive value have concentrated on amino acid destruction (primarily sulfur-containing amino acids) and loss of lysine availability with heat treatment (Cogan et al., 1968; Badenhop and Hackler, 1971; Taira et al., 1969). However, neither of these changes fully account for the reduction in protein quality (Rios Iriarte and Barnes, 1966; Taira et al., 1969). For example, Longenecker et al. (1964) found that replacement of methionine destroyed during processing improved the protein efficiency ratio (PER) of a soy isolate, but not to the level of the flour from which the processed product was prepared. Thus, it appears that additional protein changes may occur during processing which also affect the nutritional value.

Other changes in soy proteins caused by processing have been studied to a limited extent. Processed soy products in general show protein aggregation, protein degradation and reduced protein solubility as compared with unprocessed soymeal (Ishino and Ikamoto, 1975; Cumming et al., 1973). It has been suggested that such processing methods as toasting and desolventizing may promote the oxidation of

sulfur amino acids (Walker et al., 1974). Recent research indicates that some oxidized forms of sulfur amino acids have little, if any, methionine-like activity (Walker et al., 1974; Cuq et al., 1973). Furthermore, measurement of amino acid composition changes may be complicated by misleading methodology. It has been suggested that amino acids from each kind of protein are released at different rates during acid hydrolysis (Webb, 1975; Kohler and Palter, 1967). Therefore, standard 18 or 24 hour hydrolysis times may result in inaccurate measurement of some amino acids. None of these changes have been related to nutritional changes in commercial soy products.

Therefore, this research measured processing-induced changes in soy protein solubility and molecular size distribution and changes involving sulfur amino acids, and attempted to improve the methodology for amino acid determination in soy products. In addition, an effort was made to correlate the measured physical and chemical properties of the products with their published protein nutritional quality.

REVIEW OF LITERATURE

Soybean Processing

The increased demand for soybeans in recent years can be attributed to their high protein quantity, good protein quality, low cost and unique functional characteristics (Rice, 1970). The functional properties of soy proteins permit the conversion of soybeans into a wide variety of processed products.

The current methods of processing soybeans were reviewed by Alden (1975). Defatted soy meal is usually prepared by hexane extraction of cracked, dehulled soybeans. The hexane is removed by evaporation or steam distillation. Heat treatment during this step may destroy the naturally occurring antinutritional factors, such as trypsin inhibitors, producing a soy meal suitable for animal feed. However, if low heat and pressures are used, a product of greater solubility, and therefore increased versatility, will result (Alden, 1975). This soy flake product, containing 50-53% protein, is the starting material for most other protein products.

Alden (1975) described the general processes by which soy flakes are converted to other soy products. Concentrates are prepared by acid precipitation (pH 4.5) of the protein which allows removal of the soluble carbohydrates. The dried, neutralized soy concentrate contains approximately 70% protein. Soy isolates are prepared by initially solubilizing the protein in alkali (pH$\sim$8) and removing the insoluble fiber. The protein is then acid precipitated, and the soluble carbohydrates are removed. When neutralized and dried, soy isolates

contain approximately 90% protein.

Textured soy products are prepared by two general methods: extrusion processing and spinning (Alden, 1975). The extrusion process subjects a soy flour and water slurry to high temperatures (280°F) and pressure (300-600 psi) (Alden, 1975). The slurry is then extruded through a nozzle to atmospheric pressure, causing the moisture to expand and texturize the product. Spun fibers are produced by forming the soy isolate into a "dope" with alkali (pH∿10). The dope is forced through spinnerettes into an acid bath which coagulates the protein. The resulting fibers are stretched, neutralized and dried (Alden, 1975).

Detailed discussions of soy processing procedures have also been presented by Wolf and Cowan (1975), Horan (1966) and Smith and Circle (1972).

Nutritional Effects of Processing

The nutritional effects of processing on soybeans have been observed and studied for many years. In 1917 Osborne and Mendel reported that although raw soybeans required mild heat treatment to obtain maximum nutritional value, more extensive heat treatment resulted in decreased nutritional value.

Antinutritive Factors and Amino Acid Destruction

The improvement of protein value obtained by mild heating has been attributed to inactivation of trypsin inhibitors and other biologically active components occurring naturally in soybeans (Liener, 1962; Rackis, 1966). However, recent studies indicate that

destruction of trypsin inhibitors is responsible for only about 40% of the growth improvement resulting from heat treatment of raw soybeans (Liener, 1976).

The impairment of nutritional value resulting from overheating is sometimes accompanied by amino acid destruction. Klose et al. (1948) found that the protein efficiency ratio (PER) of overheated soybeans could be improved by the addition of methionine, methionine plus lysine or methionine plus leucine. However, amino acid supplementation did not restore the PER to the level of mildly heated soybeans.

Similar nutritional impairment and amino acid destruction has been observed in a variety of processed soy products. Hackler and Stillings (1967) studied the effect of heat processing on the nutritional value of soy milk, and attempted to correlate PER's with amino acid composition. No correlation was found between in vitro estimation of quality and PER until antinutritional factors were destroyed. After this destruction, correlation was found within the range of processing temperatures studied up to 277°C. Cooking the milk from 15 minutes to 3 hours at 93°C had no significant effect on amino acid composition and no effect on PER. However, when milk was processed at 121°C, cystine and tryptophan contents decreased with increased heating time as did the PER. Increasing the spray-drying inlet temperature did not affect the cystine content, but an inlet temperature of 277°C or higher decreased the PER. The authors suggested that some other factor was influencing the utilization of the protein at high temperatures.

Hackler et al. (1967) also determined amino acid indexes (essential amino acid index, EAAI) and nutritional value (PER) of several soybean fractions, including soy milk, soy curd, whey protein and hulls. No correlation was found between chemical and biological indicators of protein quality for these soy fractions. Dry roasting of soybeans was found to result in destruction of cystine, tryptophan, and total and chemically determined available lysine, as well as a decrease in PER (Badenhop and Hackler, 1971).

Nutritional changes have also been observed in soy flour, concentrates, isolates and textured products. In general, nutritive value, as evaluated by PER assay, has been found to decrease with increased processing (Bressani, 1975; Central Soya, 1975).

The NER (nitrogen efficiency ratio) of soy flour was found to decrease with increased heating (Taira et al., 1969). Supplementation with amino acids destroyed during processing improved the nutritional value, but did not completely restore it to maximum (mildly heated) level.

Longenecker et al. (1964) studied the PER of three soy concentrates and four isolates before and after mild heat treatment. The processed products varied greatly in PER prior to heat treatment. All products had higher PER's following mild heat treatment. Soy flour had a higher PER than any of the processed, heat treated soy isolates and concentrates. The amino acid compositions of the isolates were reported to be similar to soy flour except for a lower sulfur amino acid content in the isolates. One isolate was found to contain 0.5 g cysteic acid per 100 g protein. The addition of 0.7% methionine

hydroxy-analog improved the PER of this isolate, but not to the level of soy flour.

Rios Iriarte and Barnes (1966) studied the effects of cooking on four commercially prepared soy products: uncooked full fat soy chips, steam-cooked full fat soy chips, steam-cooked and pressed soy chips and expeller-produced soy meal. Replacement of cystine destroyed during cooking improved the PER, but not to the level of the mildly heated product. Replacement of lysine, threonine, histidine and tryptophan in addition to cystine destroyed during processing did not improve the PER above that of meals supplemented with cystine alone. A decrease in nitrogen absorbability (usually called nitrogen digestibility) from overcooked products was also noted; however, these workers did not believe it sufficient to account for the low nutritive value of the product.

The effects of processing conditions of soy isolates on the PER, anti-tryptic activity, available lysine and methionine content was investigated by Cogan et al. (1968). The extraction mixture (soy dissolved in calcium hydroxide, pH 9.5-9.8) had a lower PER than the original meal, although no loss of chemically determined available lysine or microbially determined available methionine was found to result from this extraction step. The precipitation method (calcium salt or isoelectric precipitation, pH 4.5) had no effect on PER, available lysine or methionine in the products, if the products were properly heated. The PER of the spray-dried product was higher than that of the freeze-dried product. The improvement was probably due to mild heat treatment during the spray drying process, which resulted

in a reduced anti-tryptic content, or possibly in an improvement in digestibility. Although these researchers frequently attributed the reduced PER of the processed products to failure to completely inactivate trypsin inhibitors, their data fail to indicate a good correlation between the trypsin inhibitor content and the PER.

DeGroot and Slump (1969) studied the effects of alkali treatment on the nutritive value of various products. Decreased net protein utilization (NPU) of soy meal correlated with the length and severity of alkali treatment. Supplementation of treated proteins with methionine, the first limiting amino acid in soy protein, failed to restore the NPU to that of untreated proteins. Since the threonine content of the untreated and treated soy protein was similar, and threonine is the second limiting amino acid, the authors suggested that a decreased utilization of threonine in treated proteins was responsible for the lower nutritive value. A decreased rate of threonine absorption was observed in vitro using an everted sac method.

Sulfur Amino Acid Availability

Although destruction of cystine during processing has been well documented (Hacker and Stillings, 1967; Taira et al., 1969), and sulfur amino acids have been reported to be limiting in soy and processed soy products (Badenhop and Hackler, 1973; Liener, 1972), the total sulfur amino acid content does not always relate to the protein value of soy products (Rios Iriarte and Barnes, 1966; Liener, 1976). Therefore, some researchers have considered the possibility of the loss of sulfur amino acid availability during soy processing. Early research by Evans and coworkers (Evans and Butts, 1949; Evans and

McGinnis, 1948; Evans et al., 1950) indicated that around 40% of the cystine but none of the methionine was destroyed in autoclaved soybeans. However, both cystine and methionine were found to be less "available" in autoclaved meals. Availability was determined as the difference in sulfur amino acid liberation by enzymatic and acid hydrolysis, as measured by microbiological determination with Leuconostoc mesenteroides.

More recently, the availability of sulfur amino acids has been investigated in meat, fish and whale-meat meals (Miller and Carpenter, 1964). In a series of studies, Miller and coworkers (Miller and Carpenter, 1964; Miller et al., 1965a, b) noted that the sulfur amino acid content was not directly related to the net protein utilization (NPU) of these products, despite the fact that all were limiting in sulfur amino acids. Using Streptococcus zymogenes and chick assay procedures, they determined that 42 to 98% of the methionine was unavailable. Correlation of methionine availability determined by chick assay with that determined by the microbiological assay was 0.93.

The nutritional availability of various oxidized forms of sulfur amino acids in various foodstuffs has only recently been investigated. The chemical structures of some sulfur amino acids and their derivatives are presented in Appendix 1. Cysteic acid and methionine sulfone have been found to be completely unavailable to the chick and rat (Miller and Samuel, 1970; Kuzmicky et al., 1974; Anderson et al., 1976). The nutritional availability of methionine sulfoxide, on the other hand, is still subject to some dispute.

Miller and Samuel (1968) reported that free methionine sulfoxide could partially replace free methionine for Streptococcus zymogenes. Ellinger and Palmer (1969) confirmed that free methionine sulfoxide was almost completely available to S. zymogenes, but found that peptide-bound methionine sulfoxide had reduced availability.

Njaa (1962) found that free methionine sulfoxide had 75% the availability of methionine for the young rat. Miller and Samuel (1970) reported that free methionine sulfoxide was only 26% as active as methionine for rats (presumably adults). Both of these studies were of short duration.

Miller et al. (1970) used weanling rats to determine methionine sulfoxide availability. Growth rates from a short term (17 day) study indicated that free methionine sulfoxide was clearly less well utilized than methionine. However, long term studies (132 days) indicated no significant difference in ability to utilize methionine or methionine sulfoxide. It was suggested that either an adaptive mechanism for utilization of methionine sulfoxide developed with age, or that older rats had a lower methionine requirement.

Similar results for free methionine sulfoxide utilization by weanling rats were reported by Anderson et al. (1976). Analysis of rat plasma sulfur amino acids suggested that rats had a limited ability to reduce methionine sulfoxide to methionine.

Pieniazek et al. (1975) studied the effects of heat and performic acid oxidation on the nutritive value and chemical composition of casein. Rats fed oxidized casein had lower weight gain than rats fed untreated casein. Cysteic acid and methionine sulfone were found in

the oxidized casein. Enzymic hydrolysis with pancreatopeptidase E of oxidized casein released 23% less methionine sulfone and 100% less cysteic acid than did acid hydrolysis. It was concluded that oxidized sulfur amino acids may result in the formation of proteolytic resistant complexes.

Cuq et al. (1973) reported that the amount of methionine released by enzymatic digestion with Pronase was lower in hydrogen peroxide treated casein than in non-treated casein. It was suggested that bonds involving methionine sulfoxide were resistant to hydrolysis by pronase.

Chemical and Molecular Effects of Processing

Although the reduction in protein nutritive value as a result of processing is well documented, the causes of such a reduction are not well understood. Destruction of amino acids has frequently been cited (Liener, 1972); however, most of the previously discussed studies indicate that this is only a partial answer. Although several studies have investigated various other chemical and molecular changes in soy protein as a result of processing, most of these studies have been directed at understanding the unique functional properties of soy proteins rather than at relating measured changes to nutritional value.

Nitrogen Solubility and Protein Molecular Weight

Solubility changes in heated soybeans were investigated by Balloun et al. (1953). Water solubility of soy was reported to decrease with autoclaving up to 20 minutes. Little solubility change was noted with additional heat treatment to 90 minutes. Water solubility was recommended as an index of underheating of soy meals. Fukushima (1959)

found that the solubility of autoclaved soybean meal-water mixtures decreased to a minimum and then increased again with continued heating.

In an effort to identify changes in soy proteins during heating, Mann and Briggs (1950) heated water extracts of defatted soy meal. They found that the amount of precipitate increased with increased temperature and time of heating. Starch gel electrophoresis patterns of heated protein indicated that heat precipitation involved primarily the non-globulin proteins. There was a reduction in the number of peaks in heated extracts. They also observed that the protein precipitated by heat was the same as that which precipitated at room temperature after 5-7 days. Thus, these researchers suggested that the heat treatment accelerated the same denaturation reaction as occurred at room temperature.

Cumming et al. (1973) determined the effects of thermoplastic extrusion of soy protein on protein solubility and molecular weight using sodium dodecyl sulfate electrophoresis. As the temperature of processing was increased, the amount of higher molecular weight proteins decreased while the amount of faster migrating, lower molecular weight proteins increased. It was noted that the concentration of the various protein bands changed at different rates, indicating that the protein fractions were unequally affected by processing. Solubility decreased with increased texturization. Cumming et al. (1973) noted that the changes caused by extrusion cooking were similar to those noted in heated soy solutions.

Ishino and Okamoto (1975) treated water extractable acid precipitated soy protein with various concentrations of sodium

hydroxide. Electrophoretic analysis indicated that when soy was treated with pH 11.5 NaOH, slow moving bands disappeared while several bands appeared between the fastest and slowest bands. Cystine content remained constant with treatment up to pH 10.7, but thereafter decreased with increased pH. Solubility studies with n-heptane indicated that a hydrophobic region in the protein was exposed at pH 11.0, but was destroyed at pH greater than 12.9.

Saio et al. (1975) investigated the effect of high temperature on soybean 7S and 11S proteins. The S designation refers to the Svedberg unit, 1×10^{-13} sec, which is a measure of the ultracentrifugal sedimentation rate (Lehninger, 1970). The larger "S" designation represents a larger molecular weight. Both proteins showed minimum solubility at higher temperatures. Solubility was increased by the addition of urea or urea plus mercaptoethanol at all temperatures investigated. Electrophoretic analysis indicated that the 7S and 11S proteins were affected similarly by heat. Both proteins remained relatively unchanged when heated to 120-130°C, but at 150°C no bands were observed, indicating protein degradation to low molecular weight substances.

Sulfhydryl and Disulfide Interactions

The insolubility of heated soy and soy isolates has been attributed to disulfide polymers (Nash and Wolf, 1967). It was suggested that the soy proteins existed in the flour as polymers, some of which are dissolved during extraction. During isoelectric precipitation the polymers aggregate, resulting in insoluble protein. This protein can be resolubilized by the addition of mercaptoethanol (Nash and Wolf, 1967).

Sulfhydryl and disulfide content and interactions have been studied in several soy products in an effort to understand functional properties and processing changes. Fukushima and Van Buren (1970a,b) studied the role of disulfide bonds in the insolubilization of soy milk during drying. Small levels of disulfide cleaving reagents (cysteine, mercaptoethanol) and chelating agents increased the dispersibility of soy milk after drying. Larger concentrations of disulfide (SS) splitting reagents decreased dispersibility. Hydrogen peroxide and sulfhydryl (SH) blocking reagents also increased dispersibility. Based on this evidence, it was concluded that the insolubilization probably took place through protein unfolding and exposure of SH groups, followed by the formation of intermolecular SS bonds during drying. They also suggested that with heat treatment of milk for over 30 minutes at 120^{o}C the active SH groups were deactivated, possibly through the formation of intramolecular SS bonds, sulfinic acid, sulfonic acid or sulfenic acid.

Sulfhydryl groups have been found to affect the physical characteristics of soybean tofu-gel (Saio et al., 1971). Heat was found to have a more rapid effect on the SH groups in 11S than in 7S protein. The texture of tofu-gels prepared from 11S and 7S became softer and more adhesive when SH blocking groups were added. As the number of SH groups was increased through the addition of SS cleaving reagents, 11S tofu-gels became harder and more cohesive, whereas the 7S tofu-gels were unaffected by such treatment. It was suggested that tofu made from 11S proteins contained more SS bonds than that from 7S proteins.

SH and SS interaction has been cited as partly responsible for soy fiber formation (Kelley and Pressey, 1966). Viscosity of a soy-alkali "dope" was found to be affected by substances which influenced SH and SS groups. Acid-precipitated protein had increased nitrogen solubility and increased concentration of 7S and 11S protein fractions when extracted in buffer containing mercaptoethanol as compared with extraction in buffer alone. They suggested that treatment of the proteins with strong alkali caused the native proteins to unfold and dissociate, and favored SH-SS interchange. This interchange was thought to occur prior to or during acidification of the protein, and would result in the formation of new disulfide groups. Acidification of the sample was also thought to favor the formation of hydrogen and ionic bonds during extrusion. It was concluded that "many of the properties which the spun fibers possess may be attributed to the action of hydrogen, ionic and disulfide linkages."

Chiang and Sternberg (1974) reported that SH and SS interactions were responsible for the toughening of soy fibers during aging. A decrease in SH groups was found to correlate with fiber aging. SS crosslinking and protein aggregation were studied by electrophoresis. As the fiber aged, the fastest moving protein band disappeared, but could be restored by adding mercaptoethanol to the sample. It was suggested that the changes in soy fibers on aging resulted from protein compacting through SS formation.

Sulfur Amino Acid Oxidation

Processing of soy proteins not only causes SH-SS interactions, but procedures such as solvent removal, toasting and various modifi-

cations may also promote the oxidation of sulfur amino acids to methionine sulfoxide, methionine sulfone or cysteic acid (Walker et al., 1974; Cuq et al., 1973). This possibility is consistent with data indicating lack of correlation between nutritional value and total sulfur amino acid content of processed soy products (Rios Iriarte and Barnes, 1966; Cogan et al., 1968).

The quantitative measurement of the sulfur amino acids and their oxidized forms presents numerous problems especially in foods. Acid hydrolysis of plant protein materials was found to result in a 30-59% loss of methionine (Jennings and Lewis, 1968). On the other hand in some food products, erroneously high methionine values could result if the protein contains methionine sulfoxide, since this oxidized form may be partially reduced to methionine during acid hydrolysis (Floyd et al., 1963). Total sulfur amino acid content (methionine plus methionine sulfoxide and methionine sulfone, and cystine plus cysteic acid) can be accurately determined following performic acid oxidation (Moore, 1963). This method oxidizes methionine, methionine sulfoxide and cystine to acid stable compounds, methionine sulfone and cysteic acid respectively. The procedure, therefore, fails to differentiate between the oxidized forms of sulfur amino acids which may be present in foods and thus does not indicate nutritionally available methionine (Walker et al., 1974).

Several methods have been proposed for the quantitative measurement of methionine sulfoxide, methionine sulfone and cysteic acid. Cysteic acid and methionine sulfone are stable to 6N HCl hydrolysis and therefore can be measured directly (Blackburn, 1968; Slump and

Schreuder, 1973). Treatment of methionine sulfoxide with 6N HCl, however, results in the formation of methionine, homocystine, homocysteic acid and methionine methyl sulfonium salt among other products (Njaa, 1962; Floyd et al., 1963).

Since acid hydrolysis has been found inadequate for methionine sulfoxide determination in proteins, several alternate methods of analysis have been proposed. Direct measurement of methionine sulfoxide following basic hydrolysis has been proposed and modified (Neumann, 1967; Blackburn, 1968). However, chromatographic separation difficulties with this amino acid have been noted (Walker et al., 1974). In addition, reports of the use of alkaline hydrolysis fail to indicate recovery data for methionine sulfoxide (Lunder, 1972). Sodium hydroxide and barium hydroxide hydrolysis may result in silicate formation, and protein losses during the removal of barium from barium hydroxide hydrolyzates have been noted (Hugli and Moore, 1972).

Neumann (1967) proposed indirect measurement of methionine sulfoxide by alkylation of methionine with iodoacetic acid. Methionine sulfoxide, which is resistant to alkylation, is measured following performic acid oxidation to methionine sulfone. Unfortunately, erroneous results may be obtained because of a failure to completely alkylate inaccessible methionine (Walker et al., 1974). These methods are diagrammed in Appendix 2.

Several other methods for the determination of oxidized sulfur amino acids have been suggested but require further study. Walker et al. (1974) investigated the use of X-ray photoelectron spectroscopy

for the determination of sulfur oxidation states. Lipton et al. (1974) investigated the use of dimethyl sulfoxide in two different procedures for the determination of nutritionally available methionine, but the procedures are not yet developed for quantitative use. Lunder (1972) developed a procedure for measuring methionine sulfoxide in intact protein. The method is based on reaction between methionine sulfoxide and acetic anhydride followed by determination of released formaldehyde by colorimetry.

Slump and Schreuder (1973) used a combination of the previously discussed analytical methods to investigate sulfur amino acid oxidation in hydrogen peroxide treated foods. Cysteic acid and methionine sulfone were measured following acid hydrolysis. Methionine sulfoxide plus methionine sulfone were measured using the iodoacetic acid method of Neumann (1967), and methionine sulfoxide was then calculated by difference. Total sulfur amino acids were measured following performic acid oxidation. Results indicated that hydrogen peroxide treatment of casein and fish meal caused significant conversion of methionine to methionine sulfoxide. Methionine sulfone was detected in peroxide-treated casein. NPU decreased in peroxide-treated samples, and the percent decrease corresponded to the percent of sulfur amino acids found to be oxidized. Supplementation of the peroxide-treated casein with methionine increased its nutritive value.

The direct method (alkaline hydrolysis) was reported to be more accurate than the indirect method (iodoacetic acid) for the determination of methionine sulfoxide in hydrogen perioxide treated casein and milk (Cuq et al., 1973). Mild hydrogen peroxide treatment

resulted in significant methionine sulfoxide formation in casein and milk. Enzymatic treatment with Pronase indicated a reduced rate of release of methionine in treated casein. The reduced rate was attributed to the decreased ability of Pronase to split bonds involving methionine sulfoxide. The rate of release of some other amino acids was unaffected by hydrogen peroxide treatment; however, tryptophan, cysteic acid, and cystine were not measured. Hydrogen peroxide treated milk samples had a 70% reduction in total methionine release by Pronase, but like casein, peroxide treatment had no effect on Pronase release of other measured amino acids.

Rate of Protein Hydrolysis

A change in the rate of enzymatic release of amino acids in processed soybeans has been reported by several groups (DeGroot and Slump, 1969; Taira et al., 1969). However, the effects of processing on the rate of acid hydrolysis of proteins has received little attention. Most studies reporting amino acid content of soy products have used an 18 or 24 hour hydrolysis time in 6N HCl. Reports on other protein products have indicated that the optimum hydrolysis time for individual amino acids may be quite variable, and that these optimum times differ from protein to protein (Webb, 1975; Hill, 1965; Kohler and Palter, 1967).

Hill (1965) reviewed the literature concerning protein hydrolysis. Hydrolysis at 110^{o}C for 24 hours in 6N HCl was recommended as the best method for total acid hydrolysis for most amino acids. However, Hill (1965) stated that accurate analysis of a protein must also include alternate methods of analysis for amino acids destroyed

during acid hydrolysis, and also should include measurement of the rate of release and destruction of amino acids during acid hydrolysis.

The effect of hydrolysis time on the measured amino acid composition of α-chymotrypsinogen was studied by Wilcox et al. (1957). Proline, isoleucine and valine increased from 24 to 72 hours of hydrolysis. Serine and threonine decreased between 12 and 48 hours of hydrolysis. A least-squares method was used to extrapolate serine and threonine to zero time.

Studies involving the rates of hydrolytic release of various amino acids in proteins from food products are limited. Davies and Thomas (1973) studied the rate of amino acid release from casein. They found that serine, threonine, tyrosine and phenylalanine were fully released after 20 hours of hydrolysis and were degraded by further acid treatment. Proline, glycine, alanine and isoleucine required 36 hours of hydrolysis for complete liberation, and remained stable to 60 hours.

The hydrolytic rates of release of amino acids from hard and soft wheats were investigated by Kohler and Palter (1967). Correction factors were developed for amino acids which were either unstable to acid or not released within 24 hours. These included serine, threonine, isoleucine and valine. The correction factors for several varieties of wheat and wheat products did not vary greatly and thus the authors suggested the use of average factors for calculation of amino acid content in determinations in crude food and feeds.

A variety of cereals and oilseed meals were analyzed by Tkachuck and Irvine (1969). Proteins were hydrolyzed at 110°C for 24, 48 and

72 hours. They found that serine and threonine were progressively destroyed with hydrolysis and therefore the amounts of these amino acids were calculated by linear extrapolation to zero time. Isoleucine and valine increased to 72 hours of hydrolysis, but appeared completely liberated at this point. Tyrosine reached a maximum at 24 hours of hydrolysis. The amounts of all other acid-stable amino acids were constant from 24 to 72 hours. Based on these findings, correction factors were developed for amino acids not maximally determined at 24 hours. Comparison of the correction factors for each amino acid indicated a small range between factors from different samples, and thus average correction factors for each amino acid were recommended for use in analysis of food samples.

OBJECTIVES

The factors responsible for the reduced nutritive value of soy concentrates and isolates as compared with soy flours have not been fully identified (Taira et al., 1969; Rios Iriarte and Barnes, 1966). Therefore, this study measured chemical and molecular properties of several commercially processed soy products in an attempt to relate properties of soy proteins with reported nutritive value. Raw and heat-treated soy meal were also investigated to characterize changes in soy proteins resulting from heat treatment. Properties investigated and related methodology included:

--Nitrogen solubility: extraction followed by Kjeldahl nitrogen determination

--Protein molecular weights: gel filtration and polyacrylamide gel electrophoresis

--Sulfhydryl and disulfide content: Ellman's reagent

--Sulfur amino acid oxidation: measurement of methionine, methionine sulfoxide, methionine sulfone, cystine and cysteic acid

--Rate of acid hydrolysis of proteins: measurement of amino acids released after 12 to 72 hours of hydrolysis.

These properties were selected on the basis of the literature review as the most likely to change as a result of processing and to be related to nutritive value.

In summary, the objectives were:

(1) To measure several molecular and chemical properties and identify physical and chemical changes resulting from processing

or heat treatment in two soy flours, two isolates, one soy concentrate, three textured soy products and raw and heat-treated soy meal;

(2) To relate measured chemical and molecular properties with product nutritional value reported by the manufacturers.

EXPERIMENTAL DESIGN

Products

Commercially processed soy products were obtained locally or from the manufacturers. Processed products studied included Soya Fluff 200W, Soya Fluff 200T (flours, Central Soya, Chicago, Illinois), Promosoy 100 (concentrate, Central Soya, Chicago, Illinois), Promine F, Promine D (isolates, Central Soya, Chicago, Illinois) and three textured products Plus Meat (J. H. Filbert, Inc., Baltimore, Maryland), Ultra Soy (FarMarCo, Hutchinson, Kansas) and Burger Bonus (A. E. Staley Co., Oak Brook, Illinois).

Raw York soybeans were donated by Dr. S. A. Tolin from crops grown locally in 1974. Beans were ground to 2 mm in a Wiley mill.

For some experiments raw soy meal was autoclaved from 5 to 20 minutes following the procedure described by Klose et al. (1948). Meal was placed in 200 ml glass beakers to a depth of 1 inch and autoclaved uncovered at 15 psi. Following heat treatment, the meal was spread to dry at room temperature.

Materials

All chemicals used were reagent grade. Acrylamide, bisacrylamide, mercaptoethanol and tetraethylaminediamine (TEMED) were obtained from BioRad, Richmond, California. Methionine sulfoxide, methionine sulfone and cysteic acid were purchased from Sigma Chemical Company, Saint Louis, Missouri.

Ninhydrin, methyl cellosolve, thiodiglycol and amino acid standards (Type H) were obtained from Pierce Chemical Company,

Rockford, Illinois.

Methods

Protein Determination

Each product was analyzed for total nitrogen by a boric acid modification of the macro-Kjeldahl method (Scales and Harrison, 1920). Duplicate determinations were made using 0.5 to 1.0 g of sample per analysis. Crude protein was calculated by multiplying the nitrogen content by a factor of 6.25 and is expressed on a wet weight (as is) basis.

Nitrogen Solubility

Nitrogen extractability of all products was measured by dissolving 6.0 g of meal in 60.0 ml of solvent. Textured products were finely ground with a mortar and pestle prior to extraction. The extraction slurry was mixed with a magnetic stirrer for 60 minutes. The slurry was then transferred to a 150 ml bottle and centrifuged at 10,000 x G for 30 minutes. The supernatant was filtered through Whatman 541 filter paper. All extractions were carried out at room temperature.

Solvents used for extraction included deionized water; pH 7.6 potassium phosphate buffer, 0.5μ (Briggs and Wolf, 1957), designated standard buffer (SB); standard buffer containing 0.5% sodium dodecyl sulfate (SDS); standard buffer containing 0.1 M mercaptoethanol (MCE); standard buffer containing 0.1 M MCE and 8 M urea; standard buffer containing 0.5% SDS and 0.1 M MCE; and pH 8.6 Tris-glycine buffer containing 0.1 M MCE (Lowry et al., 1974). Not every product was extracted with every solvent.

Extracts were analyzed for nitrogen by the boric acid modification of the macro-Kjeldahl method (Scales and Harrison, 1920). Crude protein content was calculated by multiplying nitrogen values by 6.25. Prior to nitrogen determination, extracts containing urea or Tris-glycine were dialyzed for 48 hours at room temperature against three changes of buffer containing no urea or Tris-glycine. An aliquot of buffer containing urea or Tris-glycine but no protein was also dialyzed as a control.

Protein Molecular Weight Characterization

Column Chromatography

Chromatographic separation of proteins from the three textured products (Burger Bonus, Plus Meat and Ultra Soy) and from raw York soy meal was conducted following the method described by Obara and Kimura (1967). Proteins were extracted with water following the procedure described under solubility studies, except that a 1:5 sample-to-water ratio was used in order to increase the nitrogen concentration per ml of extract.

Extracts were chromatographed on a 93 X 3 cm Sephadex G-200 (Pharmacia) column, equilibrated with pH 7.6 potassium phosphate buffer (0.0325 M K_2HPO_4, 0.0026 M KH_2PO_4, 0.4 M NaCl), 0.5μ, containing 0.1 M MCE (Briggs and Wolf, 1957). Microbial deterioration was prevented by the addition of 0.02% NaN_3 to the buffer.

The Sephadex was hydrated by heating a 1:50 Sephadex:buffer slurry for 5 hours. The cooled slurry was allowed to settle and the excess buffer was removed. Fresh buffer was then added and the slurry

mixed gently. This washing procedure was repeated until the absorbance at 280 nm of the excess buffer versus unused buffer was less than 0.120. Following the final washing the slurry was deaerated. The column was filled approximately one third full with buffer and the slurry was poured slowly into the column. The column was allowed to stand for thirty minutes before the outlet was opened. The process was repeated until the desired resin height was achieved.

Column void volume was determined by chromatographing 1 ml of Blue Dextran (2 mg/ml, BioRad) and measuring the volume eluted prior to the elution of the blue dye. Void volume was found to be 120 ml.

Five ml of protein extract was applied to the column and eluted with pH 7.6 potassium phosphate buffer using a flow rate of 10 ml per hour. Fractions (∿5 ml) were collected every thirty minutes. Absorbance of the fractions was measured at 280 nm against unused column buffer. The soy proteins were completely eluted by 500 ml of buffer. The column was regenerated by thorough washing with the above phosphate buffer.

The Lowry test was used to estimate relative protein concentration in the column fractions (Lowry et al., 1951). Absorbance was measured at 750 nm. Fractions showing the highest absorbance at 280 nm were also examined to determine the wavelength of maximum absorbance between 200-350 nm using a Unicam SP 8000 ultraviolet spectrophotometer.

Gel Electrophoresis: Textured Soy Products, Raw and Autoclaved Soy Meal

Gel electrophoresis was used for separation, characterization and differentiation of extractable proteins from the three textured soy

products, raw soy meal and autoclaved ground soy meal. The autoclaved soy meal was produced as described previously.

Two solvents, in a ratio of 1:10 with sample, were used to extract the protein following the extraction procedure described under solubility studies. Solvents used were water and Tris-glycine buffer, pH 8.6, containing 0.1 M mercaptoethanol (Lowry et al., 1974).

Two electrophoresis procedures were used to analyze the various extracts (Appendix 3). Sodium dodecyl sulfate (SDS) gel electrophoresis was carried out using 7.5% polyacrylamide gels at pH 7.9 in the presence of sodium phosphate buffer containing 0.2% mercaptoethanol. The methods of Weber and Osborn (1969) were followed in general. Electrophoretic analysis of water and Tris-glycine extracts was also conducted using 7.5% polyacrylamide gels in a pH 8.6 Tris-HCl buffer system (Appendix 3).

The gel buffer and acrylamide-bisacrylamide solution were deaerated prior to the addition of the catalysts, persulfate and TEMED. The gel solution was transferred to 5 x 5/16 inch extruded acrylic tubes (Commercial Plastics and Supply Corp., Cornwells Heights, Pa.) using a syringe fitted with a short length of plastic tubing. Gel tubes were filled to within 1/4 inch of the top and carefully overlayered with water to provide a flat surface. Gels were allowed to polymerize for 30 minutes.

Proteins were layered on top of the gels in solutions consisting of 0.1 ml of 50% sucrose solution containing 0.05% bromophenol blue (BioRad) tracking dye, 0.2 ml of gel buffer, and 0.1 ml of protein extract. The volume of protein extract was increased to 0.4 ml when

preparing layering solutions for autoclaved soy meal having low protein extractability. Twenty to 40 ml of layering solution was applied to each gel. A 2 mg/ml solution of lysozyme (Schwarz Mann), bovine serum albumin (Schwarz Mann), ovalbumin (Sigma) and carbonic anhydrase (Sigma) was applied to one gel. The proteins served as standards for molecular weight determinations, as described by Weber and Osborn (1969). Electrophoresis of SDS gels in sodium phosphate running buffer was carried out at 7 ma per tube for 5 hours using a Hoefer Scientific Instruments D5102 electrophoresis chamber and an ISCO Model 490 Electrophoresis Power Supply. Electrophoresis in Tris-HCl buffer was run at 5 ma per tube for 35 minutes.

Gels were stained for 2 hours with Coomassie Brilliant Blue dye (Sigma) and diffusion destained overnight in 7.5% acetic acid containing 5% methanol (Weber and Osborn, 1969). The destained gels were scanned at 590 nm using a Schoeffel Model SD3000 Spectrodensitometer. Approximate molecular weights of proteins separated in the presence of SDS were calculated as described by Weber and Osborn (1969).

Gel Electrophoresis: Soy Flours, Isolates, and Concentrate

Electrophoretic analysis of extracts of soy flours, isolates and concentrate was performed following the procedure developed by Neville (1971) and detailed in Appendix 4. The procedure uses a 2% stacking gel and 7% running gel and a discontinuous buffer system containing SDS. Cold gel buffer and the acrylamide solution were deaerated prior to addition of the persulfate and TEMED solutions. An automatic pipet was used to place 1.0 ml of lower gel solution in each of twelve 4 x 5/16 inch plastic tubes. The gel solution was immediately

overlayered with water. After 45 minutes the water was removed and 0.2 ml of upper gel solution per tube was added using an Eppendorf pipet.

Proteins were extracted using a 1:10 sample to buffer ratio in pH 7.6 potassium phosphate buffer, 0.5μ (Briggs and Wolf, 1957) containing 8M urea and 0.1M mercaptoethanol. Layering solutions were prepared by mixing 0.05 ml protein extract with 0.5 ml dialysis buffer (upper gel buffer containing 0.1% SDS and 0.1% MCE, Appendix 4) and 0.1 ml sucrose-bromophenol blue dye. A standard protein mixture was prepared as previously described. Electrophoresis was carried out at 1.0 ma/tube for 10 minutes to allow the tracking dye to enter the lower gel, followed by 1.5 ma per tube for 75 minutes. Gels were stained, destained and scanned at 590 nm as previously described.

Sulfhydryl and Disulfide Determinations

Sulfhydryl and disulfide contents were determined in extracts from the three textured soy products and from raw soy meal. The four soy products were extracted with three different solvents. Extraction solvents used were: deionized water; pH 7.6 potassium phosphate buffer (0.0325M K_2HPO_4, 0.0026M KH_2PO_4), containing 0.4M NaCl; pH 7.6 potassium phosphate buffer containing 0.4M NaCl and 0.5% sodium dodecyl sulfate; and pH 7.6 potassium phosphate buffer containing 0.4M NaCl, 0.5% SDS and 0.1M 2-mercaptoethanol.

Sulfhydryls (SH) and disulfides (SS) were measured using Ellman's reagent, following in general the colorimetric method described by Beveridge et al. (1974). Solutions used in Ellman's determination were:

Tris-glycine buffer, pH 8.3 (designated Tris-gly; containing 10.4 g Tris, 6.9 g glycine and 1.2 g EDTA per liter); 0.5% sodium dodecyl sulfate in Tris-gly (SDS in Tris-gly); Ellman's reagent, DTNB (5,5' dithio-bis-2-nitrobenzoic acid) obtained from Aldrich Chemical Company and used in a 4 mg/ml solution in Tris-gly; and 25% trichloroacetic acid (TCA).

Experimental variables were chosen following a series of preliminary studies. Accuracy of the method was checked by using a protein of known amino acid composition, ovalbumin. The amount of Ellman's reagent used in the procedure was judged not to be limiting since an increase of 40% in protein sample volume produced a proportional increase in final absorbance. Completeness of protein precipitation by TCA was checked by varying the concentration of TCA added to the reaction mixture (Figure 1). Time for completion of Ellman's reaction was determined by following the change in measured absorbance over time (Figure 2). All reactions appeared to reach maximum color development 30 minutes after adding all reagents, and color was stable for 90 minutes.

For SH determination in textured products and unprocessed soy meal, 0.40 ml of protein extract was mixed with 5.80 ml SDS in Tris-gly and 0.05 ml Ellman's reagent. Absorbance was measured at 412 nm after 30-45 minutes. Disulfide content (SS) and total sulfhydryl content (2SS + SH) were measured by mixing 0.50 ml protein extract from textured products (or 0.50 ml of a 1:10 dilution (with Tris-gly) of unprocessed soy meal extract) with 2.0 ml SDS in Tris-gly. Then 0.05 ml 2-mercaptoethanol was added as a disulfide reducing agent, and the mixture was incubated for one hour at room temperature.

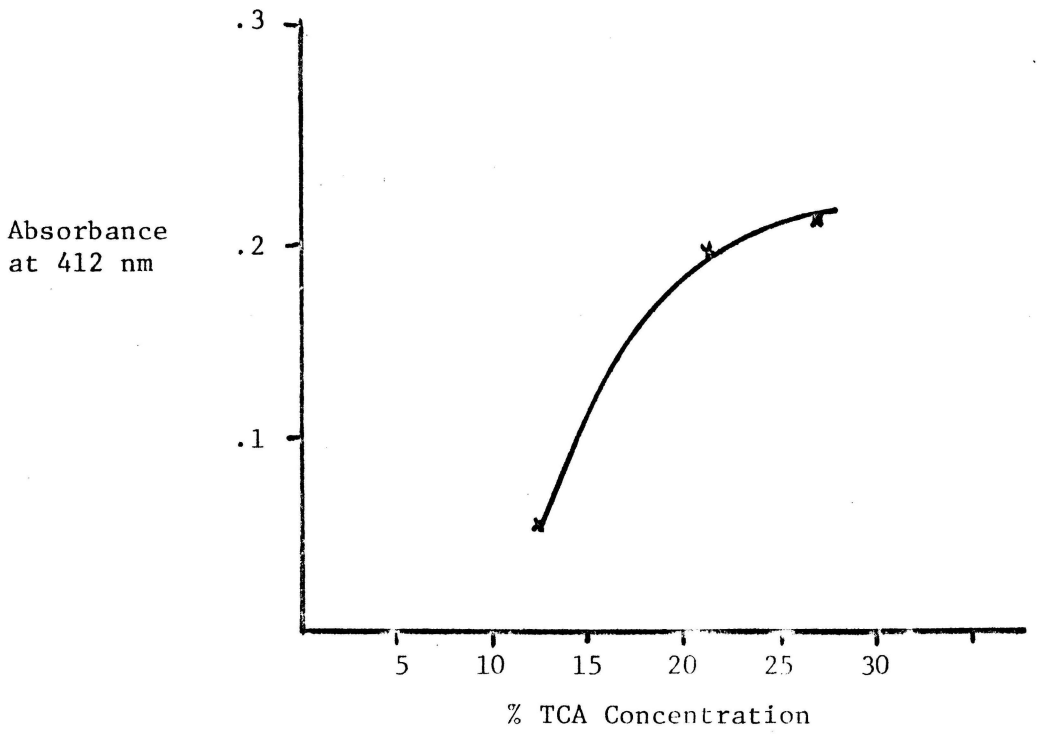


Figure 1. Effect of concentration of trichloroacetic acid (TCA) on measured absorbance at 412 nm of soy meal water extracts reacted with Ellman's reagent.

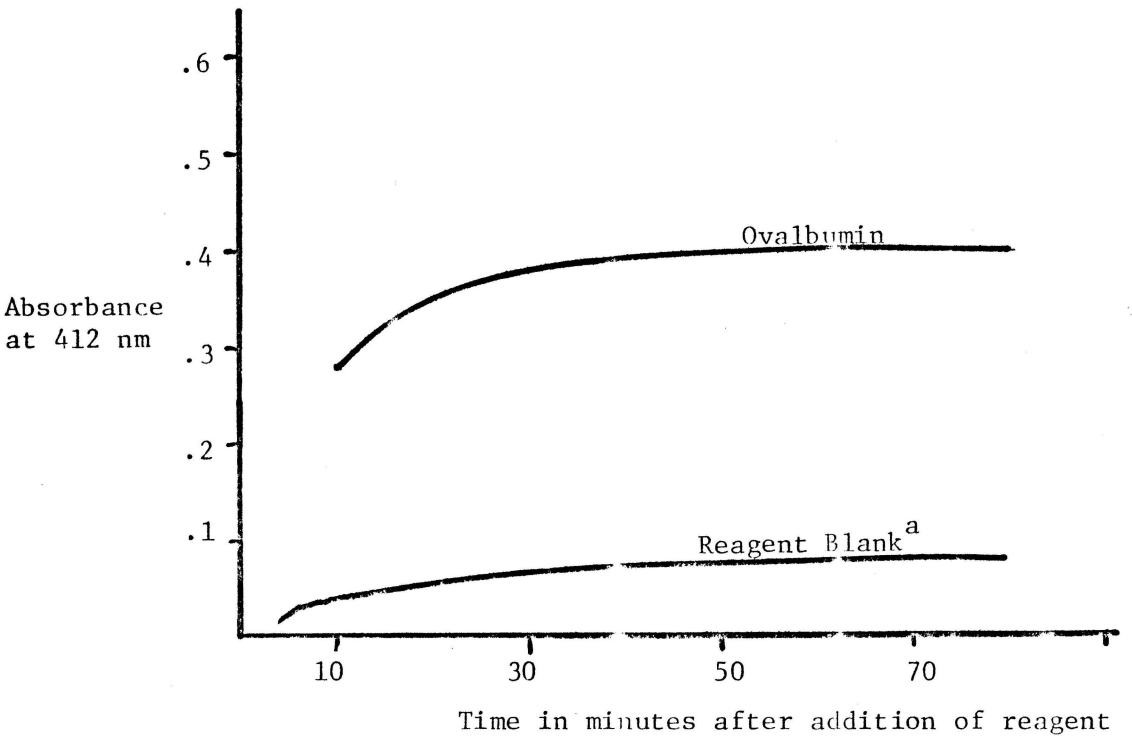


Figure 2. Effect of time on absorbance at 412 nm of ovalbumin and a reagent blank reacted with Ellman's reagent.

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[a]Reagent blank contains all reagents; protein solution was replaced by buffer.

Protein extracts in pH 7.6 phosphate buffer containing mercaptoethanol were also incubated with 0.05 ml 2-mercaptoethanol since disulfide reduction during the extraction process appeared incomplete.

Following disulfide reduction, mercaptoethanol was removed by precipitating the protein with trichloroacetic acid (TCA). Five ml of 25% TCA was added to each tube. Following an hour of incubation, tubes were centrifuged at 5000 x G for 10 minutes. The precipitates were twice resuspended in 2.5 ml 25% TCA and centrifuged. Blank tubes containing no protein were carried throughout the procedure to insure complete MCE removal. Precipitates from water and buffer extracts were redissolved in 5.0 ml of SDS in Tris-gly. MCE-buffer extract precipitates were redissolved in 10.0 ml of SDS in Tris-gly. Color was developed by adding 0.05 ml Ellman's reagent. Absorbance was measured after 45 minutes. Ellman's reagent blanks (buffer replacing protein extract) and protein blanks (buffer replacing Ellman's reagent) were included in both SH and SS determinations. These blanks were treated exactly like samples, and were carried through the entire procedure. Extracts were refrigerated 24 hours prior to the second SH and SS or total SH assay.

The calculation of SH values is described by Beveridge et al. (1974), and a sample calculation appears in Appendix 5. Disulfides were determined as sulfhydryls following reduction to SH's with MCE as previously described. SS level was calculated as one-half the difference between SH values before and after reduction. This method of determination prevents calculation of separate SS or SH values for extracts prepared with MCE in buffer since some reduction does occur

on extraction. For these extracts the SH value after reduction is reported, which is designated total SH level and is equivalent to

$$\frac{\mu \text{ moles SH}}{\text{g extracted nitrogen}} + 2\frac{\mu \text{ moles SS}}{\text{g extracted nitrogen.}}$$

Similar total SH values can be calculated for water and buffer extracts from the SS and SH levels reported.

The SH and SS values (or total SH values for MCE-buffer extracts) for each sample were compared to those for other samples treated under the same conditions using Tukey's test (Snedecor and Cochran, 1967). For a given sample and set of experimental conditions, values for days 1 and 2 were compared using the paired Student's t-test. SH and SS results for water and buffer extracts of a given sample on either day 1 or 2 were compared by a two-sample Student's t-test.

Oxidized Sulfur Amino Acids

Processed soy flours, isolates, concentrate and three textured products were analyzed for the presence of oxidized forms of sulfur amino acids. Total content of sulfur amino acids was measured using the performic acid procedure of Moore (1963). A sample containing two to five mg of protein was oxidized overnight with 2.0 ml of performic acid in a 10 ml glass ampoule (Scientific Products). Excess performic acid was removed by adding 0.3 ml 48% HBr and evaporating the sample on a rotary evaporator. Five ml of 6N HCl was then added, the ampoule flushed with N_2 and sealed under a slight vacuum (Webb, 1975). Samples were hydrolyzed for 18 to 36 hours at 110°C. No change in the measured content of total sulfur amino acids over this

time range was noted. Following hydrolysis, the sample was filtered through Pyrex superfine glass wool, and a 2.0 ml aliquot was dried over NaOH in a vacuum desiccator which was evacuated using a vacuum pump. Samples appeared dry in 3-4 hours and were chromatographed within 3 days of drying. Samples were redissolved in 4.0 ml pH 2.0 sodium citrate buffer (Cartridge Equilibrating Buffer, Technicon TSM Manual), and chromatographed on a Technicon NC-1P Amino Acid Analyzer using conditions described in Appendix 5. Recoveries of pure free sulfur amino acid mixtures were 96% of cystine as cysteic acid and 101% of methionine as methionine sulfone.

Cysteic acid and methionine sulfone already present in the samples were measured by hydrolyzing 0.04 to 0.10 g of sample in 10.0 ml of 6N HCl for 18 hours at 110°C (Slump and Schreuder, 1973). Vials were flushed with N_2 and sealed under a slight vacuum before hydrolysis. Following hydrolysis, samples were filtered, and a 2.0 ml aliquot was dried and then redissolved in 5.0 ml pH 2.0 sodium citrate buffer as previously detailed. Samples were chromatographed on a Technicon NC-1P Amino Acid Analyzer (Appendix 6, 7). Recoveries of cysteic acid and methionine sulfone were 96 and 101% respectively for a pure amino acid test mixture carried through the same procedure. Neither cysteic acid nor methionine sulfone were detected in pure ovalbumin (Sigma) subjected to the procedure.

Two procedures were used for determination of methionine sulfoxide. Hydrolysis with barium hydroxide was attempted using a modification of the procedure described by Blackburn (1968). Two ml of a mixture of free amino acids (20 mg of each sulfur-containing amino acid per 25 ml

water) and 1.26 g $Ba(OH)_2 \cdot 8H_2O$ were placed in a heavy wall Pyrex test tube which was then flushed with N_2 and sealed in an oxygen-gas torch. Hydrolysis was carried out at 110°C for 16 hours. Cooled sample was quantitatively transferred to a plastic centrifuge tube using 10 ml of hot H_2O. Barium was precipitated by directing at the surface a stream of CO_2 (generated from dry ice and washed with H_2O) for 10 minutes. The sample was centrifuged for 15 minutes at 10,000 x G and the supernatant removed. The precipitate was washed three times with distilled water. The supernatant and washings were filtered through a medium pore sintered glass funnel, quantitatively transferred to a 100 ml volumetric flask and brought to volume. A 10.0 ml aliquot was dried in a vacuum desiccator, redissolved in 5.0 ml of pH 2.0 citrate buffer, and chromatographed on a Technicon NC-1P Amino Acid Analyzer (Appendix 6, 7).

The second procedure used for the determination of methionine sulfoxide was a modification of the procedure described by Neumann (1967). Five mls of protein was weighed into a polypropylene tube which was shortened to fit inside a 18 x 150 mm heavy wall Pyrex ignition tube. Seven-tenths ml H_2O and 0.30 ml 50% NaOH were added. The tube was flushed with nitrogen and sealed. Following hydrolysis at 110°C for 16 hours, the contents were acidified with 0.95 ml 6N HCl, quantitatively transferred to a 5.0 ml volumetric flask and brought to volume with pH 2.0 sodium citrate buffer. Contents were then filtered through superfine Pyrex glass wool and chromatographed on a Technicon NC-1P Amino Acid Analyzer (Appendix 6, 7).

Rate of Protein Hydrolysis

The rate of liberation of essential amino acids during acid hydrolysis was determined for the soy flours, isolates and concentrate. Fifty to 100 ml of sample was hydrolyzed with 10.0 ml 6N HCl in 20 ml glass ampoules. Ampoules were flushed with N_2 and sealed under a slight vacuum (Webb, 1975). Hydrolysis was conducted at 110°C for 12 to 72 hours. Following hydrolysis, cooled samples were filtered through Pyrex superfine glass wool, and a 1.0 ml aliquot was dried over NaOH in a vacuum desiccator. Dried samples were redissolved in 10.0 ml of pH 2.0 sodium citrate buffer and analyzed on a Technicon NC-1P Amino Acid Analyzer. Two or three replications of each sample at each hydrolysis time were carried out. Cystine and tryptophan are unstable to acid hydrolysis and, therefore, were not measured by this procedure. Arginine and proline were not measured because of separation or detection difficulties.

Data were analyzed by standard linear and curvilinear regression techniques (Snedecor and Cochran, 1967) using a computer program developed by Dr. K. P. Bovard, Department of Animal Science, Virginia Polytechnic Institute and State University. Student's t-test was used to test differences between predicted and determined maximum values and manufacturer's reported values.

RESULTS

Selected chemical and molecular properties of several commercially processed soy products and raw and heat-treated soy meal were investigated. Products studied, their protein quantity (N x 6.25) and their reported protein efficiency ratios are listed in Table 1. Crude protein content ranged from 33.1% in raw soy meal to 86.7% in processed soy isolate.

Solubility Studies

Nitrogen solubility of the products was determined using a variety of extraction solvents, with and without denaturing agents. Nitrogen solubility of textured products and raw soy meal is presented in Table 2. Ultra Soy had higher nitrogen solubility than Plus Meat and Burger Bonus in all extraction solvents. Nitrogen extractability of all textured products increased when 2-mercaptoethanol (MCE) was present in the extraction media. Extractability of raw soy decreased when 0.5% sodium dodecyl sulfate (SDS) was present in the extraction media; the presence of SDS did not affect the nitrogen extractability of the textured products.

The nitrogen extractability of raw and autoclaved soy meal given in Figure 3. Autoclaving the soy meal resulted in decreased extractability by both water and buffer containing MCE. Buffer containing mercaptoethanol extracted slightly more nitrogen in unheated or slightly heated (5-10 minutes) soy meal than did water; however, little difference in nitrogen extractability by the two solvents was observed after 15 minutes of autoclaving.

Table 1. Percent protein content and PER values of processed soy products and raw soy meal.

PRODUCT	% PROTEIN[a]	PER[b]
Raw soy meal York, fullfat	33.1	--
Soya Fluff 200T toasted flour Central Soya Chicago, Illinois	49.5	∿2.3
Soya Fluff 200W flour Central Soya Chicago, Illinois	49.6	∿2.3
Promosoy 100 concentrate Central Soya Chicago, Illinois	64.6	2.27
Promine F isolate Central Soya Chicago, Illinois	86.7	1.6
Promine D isolate Central Soya Chicago, Illinois	86.6	1.1-1.2
Burger Bonus textured soy A. E. Staley Co. Oakbrook, Illinois	51.0	--
Plus Meat textured soy J. H. Filbert, Inc. Baltimore, Maryland	51.2	--
Ultra Soy textured soy FarMarCo Hutchinson, Kansas	49.0	--

[a]Protein = Kjeldahl N x 6.25; expressed on wet weight of sample.

[b]PER values reported by the manufacturer.

Table 2. Nitrogen concentration, in mg per ml, of extracts of textured soy products and unprocessed soymeal.[a]

PRODUCT	EXTRACTION MEDIA			
	H_2O	SB[b]	SB + 0.5%[c] SDS	SB + 0.5% SDS[d] + 0.1M MCE
Burger Bonus	0.8	0.8	0.9	1.3
Plus Meat	0.8	0.8	0.8	1.5
Ultra Soy	1.1	0.9	1.0	1.6
raw soymeal	1.6	1.9	0.7	0.9

[a]Results represent averages of duplicate determinations on each of six extracts per sample per extraction media.

[b]SB represents standard buffer, pH 7.6 potassium phosphate, 0.5μ (Briggs and Wolf, 1957).

[c]SDS represents sodium dodecyl sulfate.

[d]MCE represents 2-mercaptoethanol.

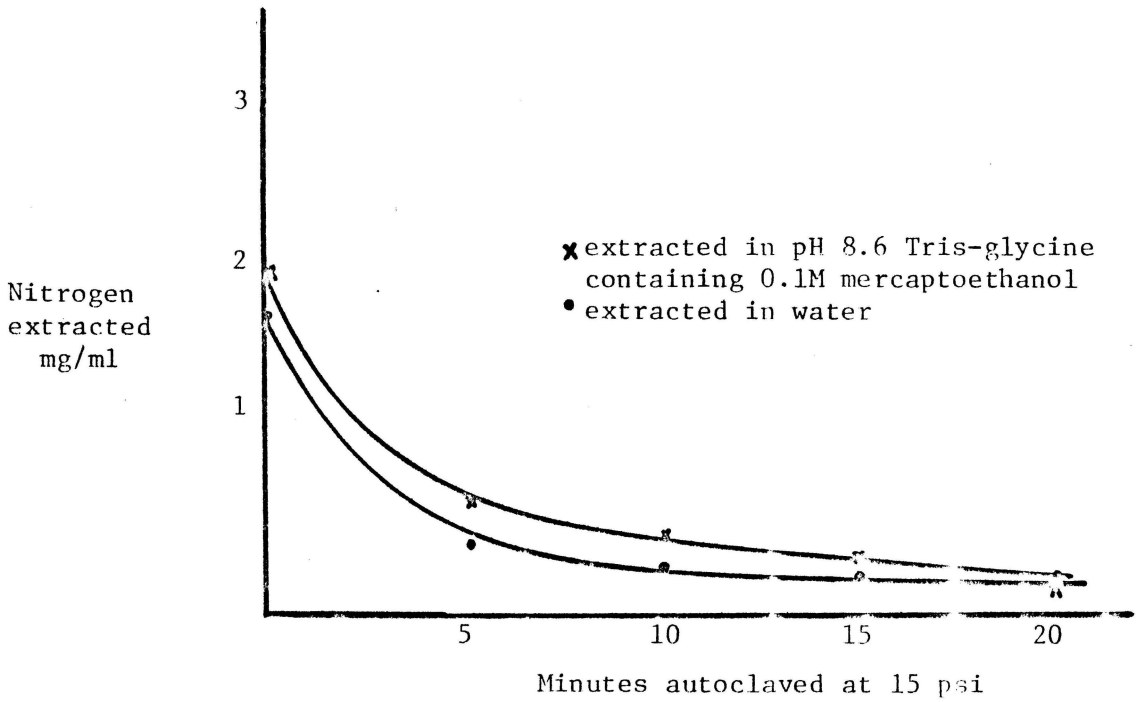


Figure 3. Nitrogen extractability, in mg/ml, of raw and heat treated soy meal.[a]

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[a]Results represent averages of duplicate determinations on each of two extractions per sample per extraction media.

Data from extractability experiments with a series of processed soy products is presented in Table 3. All products had increased nitrogen solubility when the extraction media contained 8 M urea plus 0.1 M MCE.

Protein Molecular Weight Characterization

Column Chromatography

Water extractable proteins from three textured soy products were separated by column chromatography using Sephadex G-200 (Pharmacia). The absorbance at 280 nm of fractions collected from raw soy meal and Ultra Soy extracts are presented in Figures 4 and 5. Results for raw soy meal are similar to those reported by Obara and Kimura (1967) which are reproduced in Figure 6. Although peak E measured in this study is considerably larger than the corresponding peak measured by Obara and Kimura (1967), the soy meal used in this study had not been defatted, and therefore differences in the size of the last peak were attributed to absorbance resulting from pigments still present in the soy meal. No difference in absorbance patterns at 280 nm was found between the three textured soy products, Burger Bonus, Plus Meat and Ultra Soy. Absorbance patterns from the textured products did clearly differ from that of raw soy meal (Figures 4, 5). Raw soy meal separated into five distinct absorbance peaks (Figure 4, A-E) whereas textured soy products separated into only four absorbance peaks (Figure 5: A, C, D, E), only two of which were major and distinct (Figure 5: A, E). Peak A in all products was

Table 3. Nitrogen concentration, in mg per ml, of extracts of processed soy products.[a]

PRODUCT	EXTRACTION MEDIA			
	H_2O	SB[b]	SB + 0.1M MCE[c]	SB + 0.1M MCE + 8M UREA
Soya Fluff 200T	1.6	2.2	3.9	6.0
Soya Fluff 200W	4.7	4.7	5.8	6.9
Promosoy 100	0.6	0.5	1.1	7.9
Promine D	7.2	4.0	8.4	12.3
Promine F	6.7	2.5	4.8	11.3

[a] Results represent averages of duplicate determinations on each of two extracts per sample per extraction media.

[b] SB represents standard buffer, pH 7.6 potassium phosphate, 0.5μ (Briggs and Wolf, 1957).

[c] MCE represents 2-mercaptoethanol.

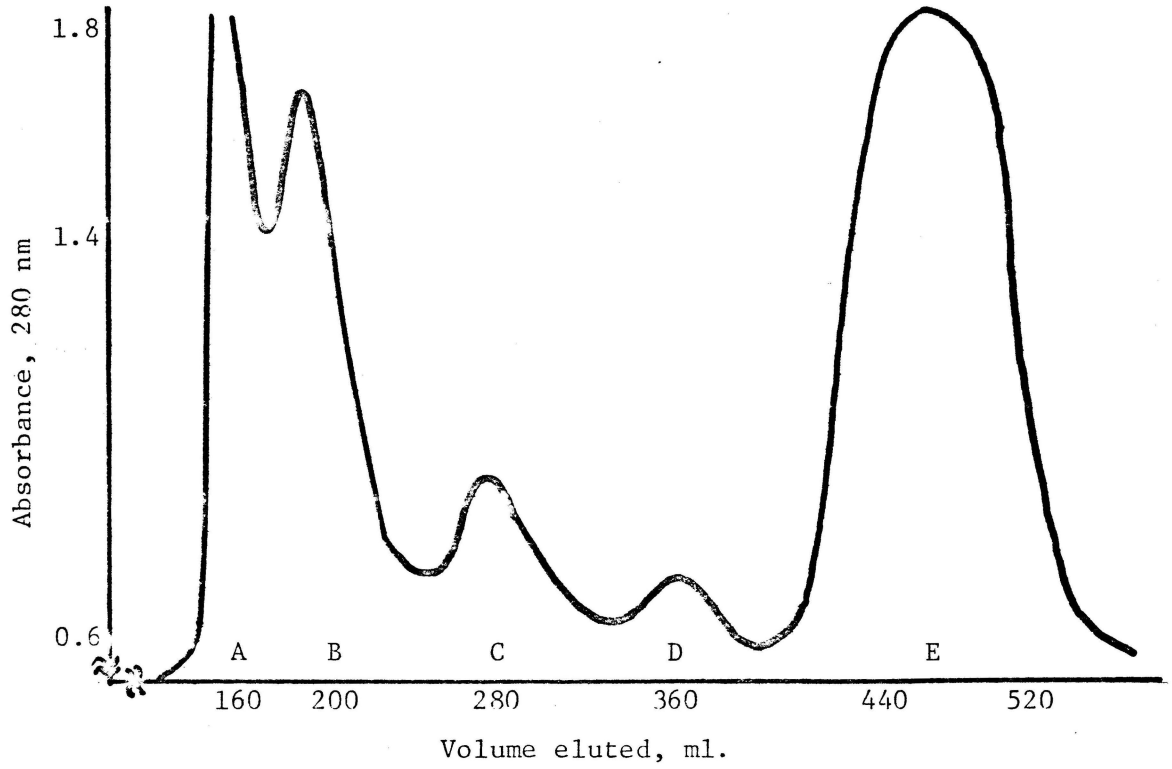


Figure 4. Absorbance at 280 nm of fractions of water extracted raw soy meal on Sephadex G-200.

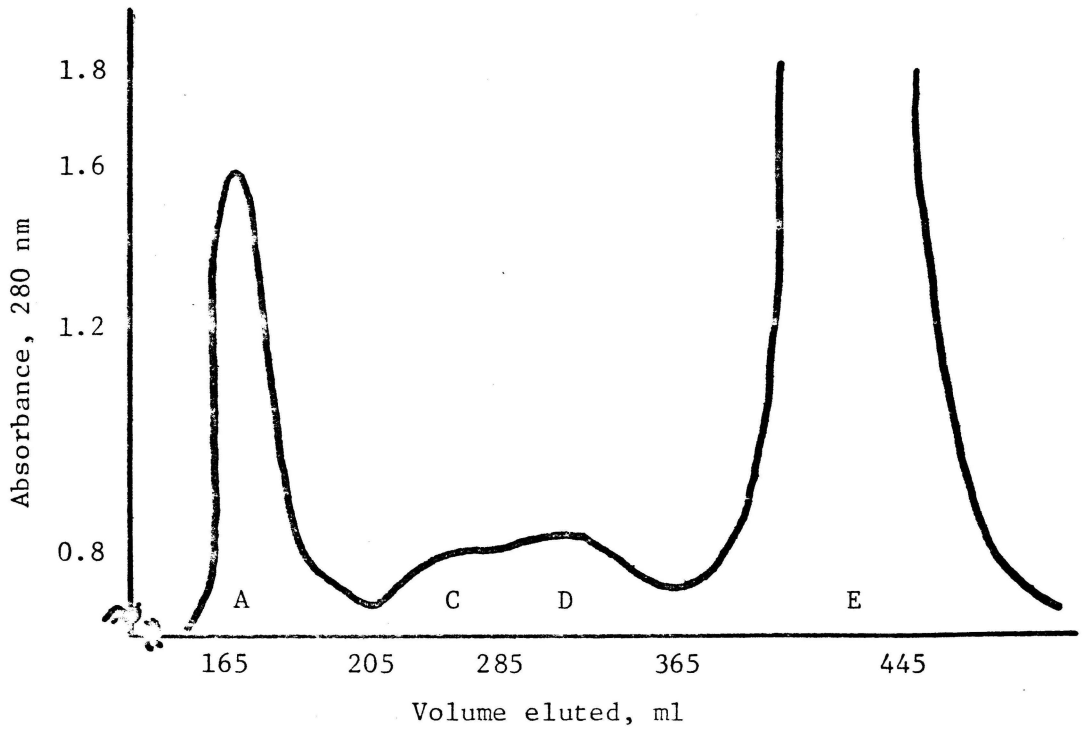


Figure 5. Absorbance at 280 nm of fractions of water extracted Ultra Soy separated on Sephadex G-200.

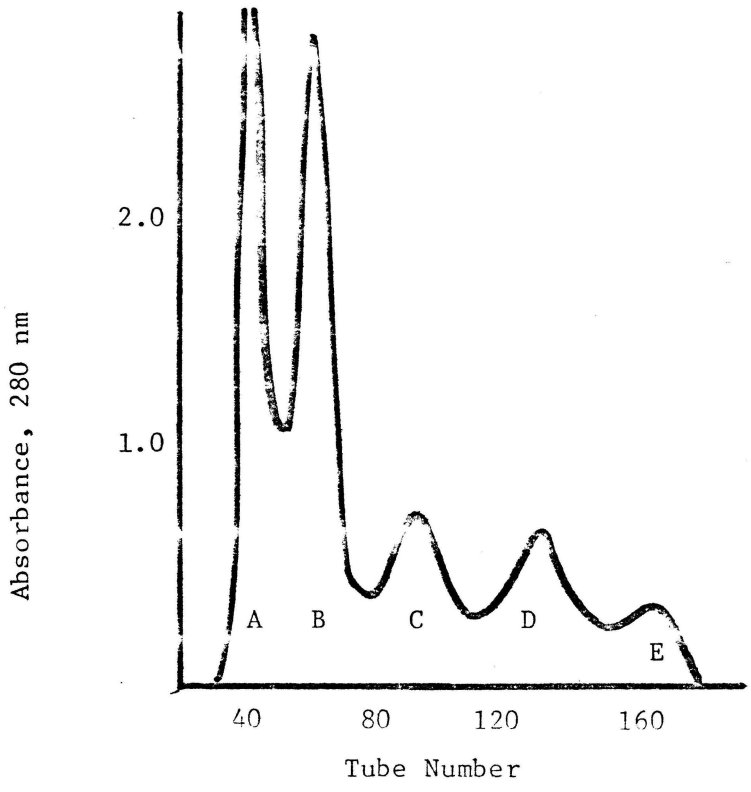


Figure 6. Sephadex G-200 separation of water extracted soy protein (Obara and Kimura, 1967).

turbid, peak E in all products was slightly yellow in color, and all other fractions were clear.

Since absorbance at 280 nm does not necessarily represent only protein, but also includes absorbance from such substances as nucleic acids, the Lowry protein method (Lowry et al., 1951) was also used to estimate the protein content of fractions. Again, no difference in absorbance patterns was found between the fractions from the three textured soy products (Figure 7). Judging from the Lowry absorbance at 750 nm, the major protein fraction of soy was in peak B (Figure 8) whereas the major protein fractions of textured soy appeared to correspond with peaks C, D and E (Figure 7).

Table 4 presents the ultraviolet wavelength of maximum absorbance of peak fractions (determined by absorbance at 280 nm) collected from all products. Results for soy meal are similar to those reported by Obara and Kimura (1967).

Gel Electrophoresis: Textured Soy Products and Raw and Autoclaved Soy Meal

Water extractable proteins of three textured products and from raw and heat treated soy meal were separated using a Tris-HCl electrophoresis separation system (Appendix 3). Densitometer traces of stained gels from extracts of Plus Meat and raw soy meal are presented in Figure 9. Water extracts of the three textured soy products were identical to each other but clearly differed from extracts of raw soy meal. Textured products had a greater amount of very slow and very fast moving proteins but had fewer sharp and distinct protein peaks than did the extract of raw soy meal. Separation of water

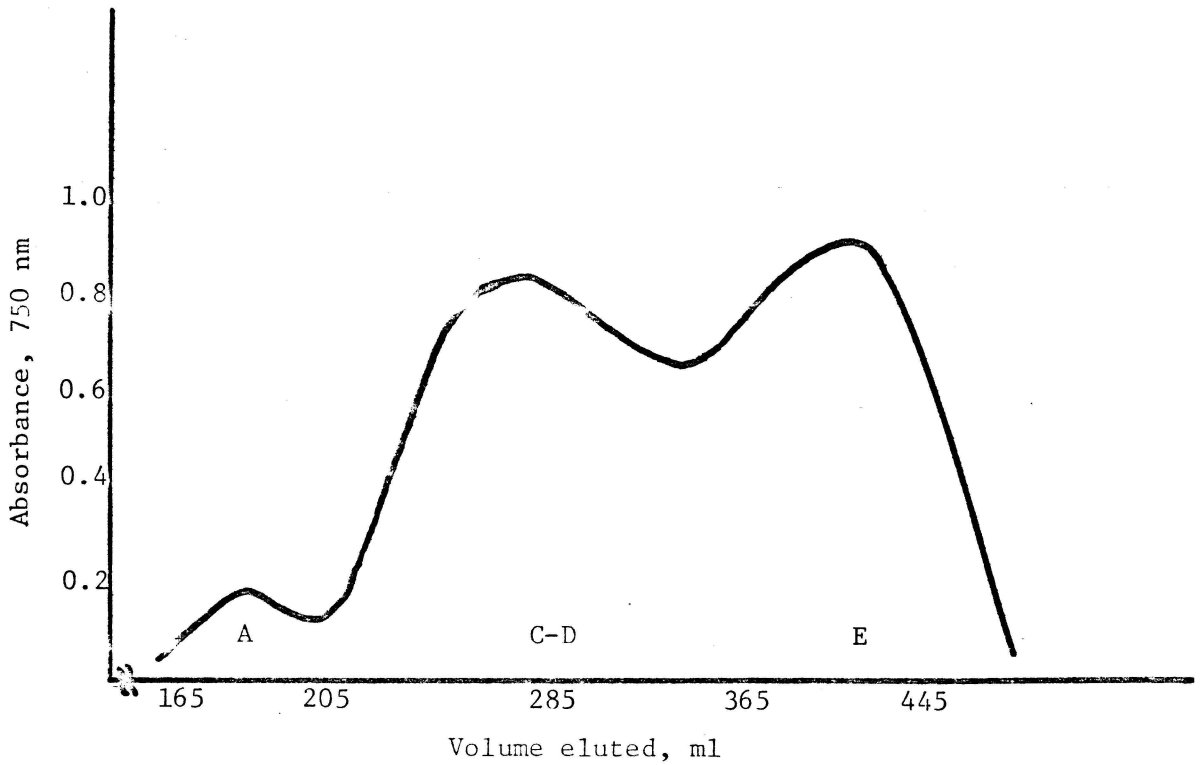


Figure 7. Lowry protein absorbance at 750 nm of fractions of water extracted Ultra Soy separated on Sephadex G-200.

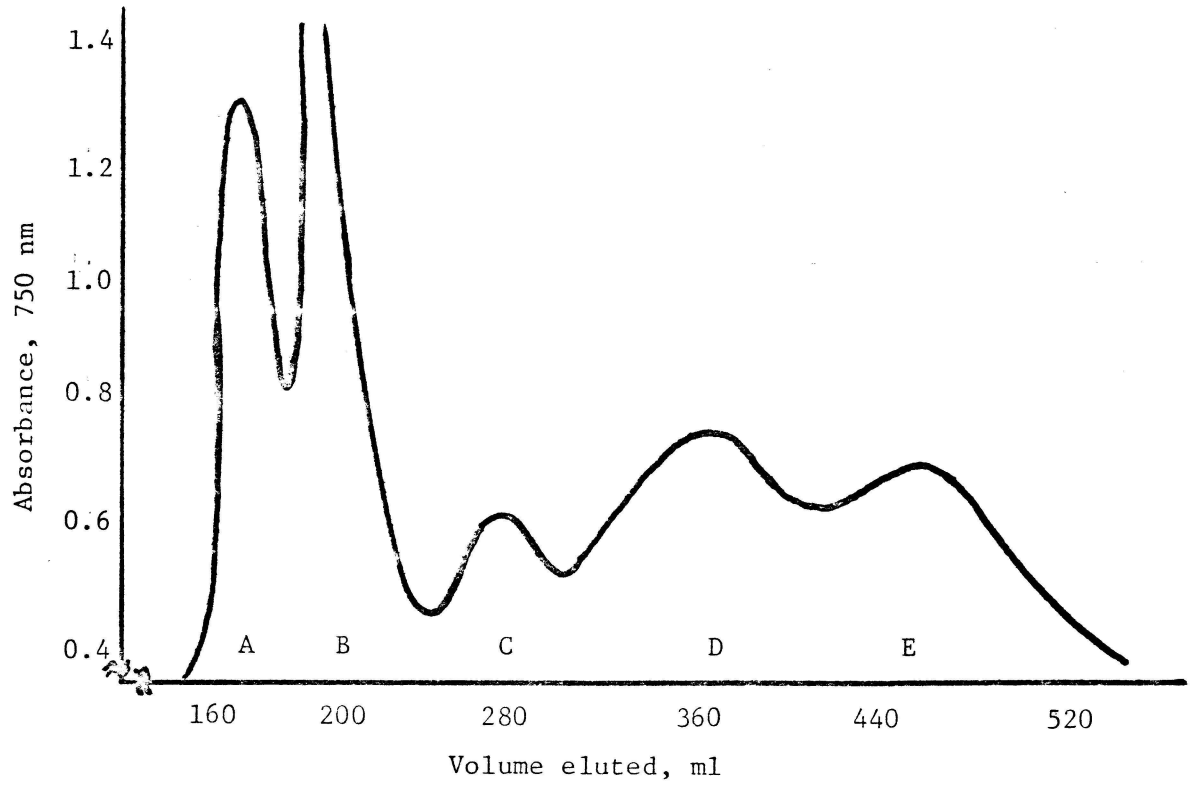


Figure 8. Lowry protein absorbance at 750 nm of fractions of water extracted soy meal separated on Sephadex G-200.

Table 4. Wavelength of maximum ultraviolet absorbance (200-350 nm) of peak fractions of water extracted soymeal and textured soy protein.

	PRODUCT			
PEAK[a]	Soymeal	Ultra Soy	Plus Meat	Burger Bonus
A	268	270	268	268
B	275	---	---	---
C	265	262	261	263
D	264	262	261	263
E	268	273	276	280

[a]Peaks correspond with labeled peaks determined by absorbance of fractions at 280 nm (Figures 5, 6).

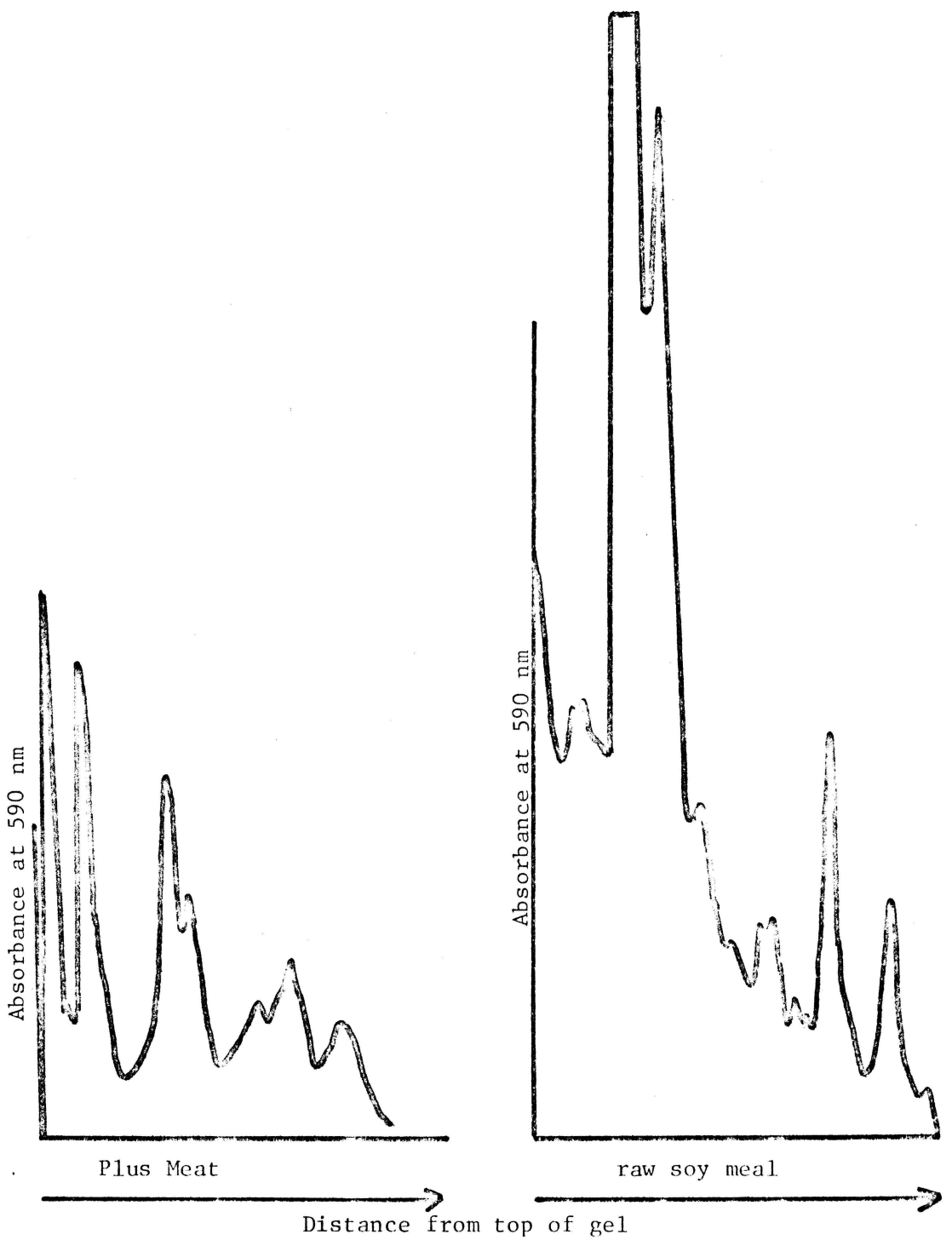


Figure 9. Densitometer traces of Tris-HCl gel electrophoresis separation of water extracts of raw soy meal and Plus Meat.

extracts of soy meal autoclaved 5 to 20 minutes are shown in Figures 10 and 11. With increased length of autoclaving there was an increase in the number of slow moving peaks.

Proteins from textured soy and raw and heat treated soy meal were also extracted in pH 8.6 Tris-glycine buffer containing 0.1 M MCE and separated using a Tris-HCl system (Appendix 3). Textured products did not differ in electrophoretic patterns from each other but did differ from raw soy meal (Figure 12). The densitometer traces of Tris-glycine extracts of autoclaved soy meal are shown in Figures 13 and 14. Length of autoclaving resulted in fewer and fewer distinct protein peaks in the faster moving section.

Sodium dodecyl sulfate (SDS) electrophoresis was used to separate extractable proteins from textured soy and raw and autoclaved soy meal, and to estimate the molecular weights of extractable proteins (Appendix 3). The densitometer trace of four pure proteins separated by this method are presented in Figure 15. Relative mobility of each protein to lysozyme was calculated and plotted against the log of the protein molecular weight (Figure 16). Results yielded a linear plot where $y = -1.13x + 5.29$, with a regression coefficient of 0.999. Molecular weights of proteins separated from extracts of soy meal and processed soy were then calculated by determining mobility of each protein to relative lysozyme (x) and solving for y, the log of the molecular weight. Molecular weights determined by this method must be considered approximate since apparent molecular weight may be affected by several conditions, including the gel strength used for separation (Neville, 1971).

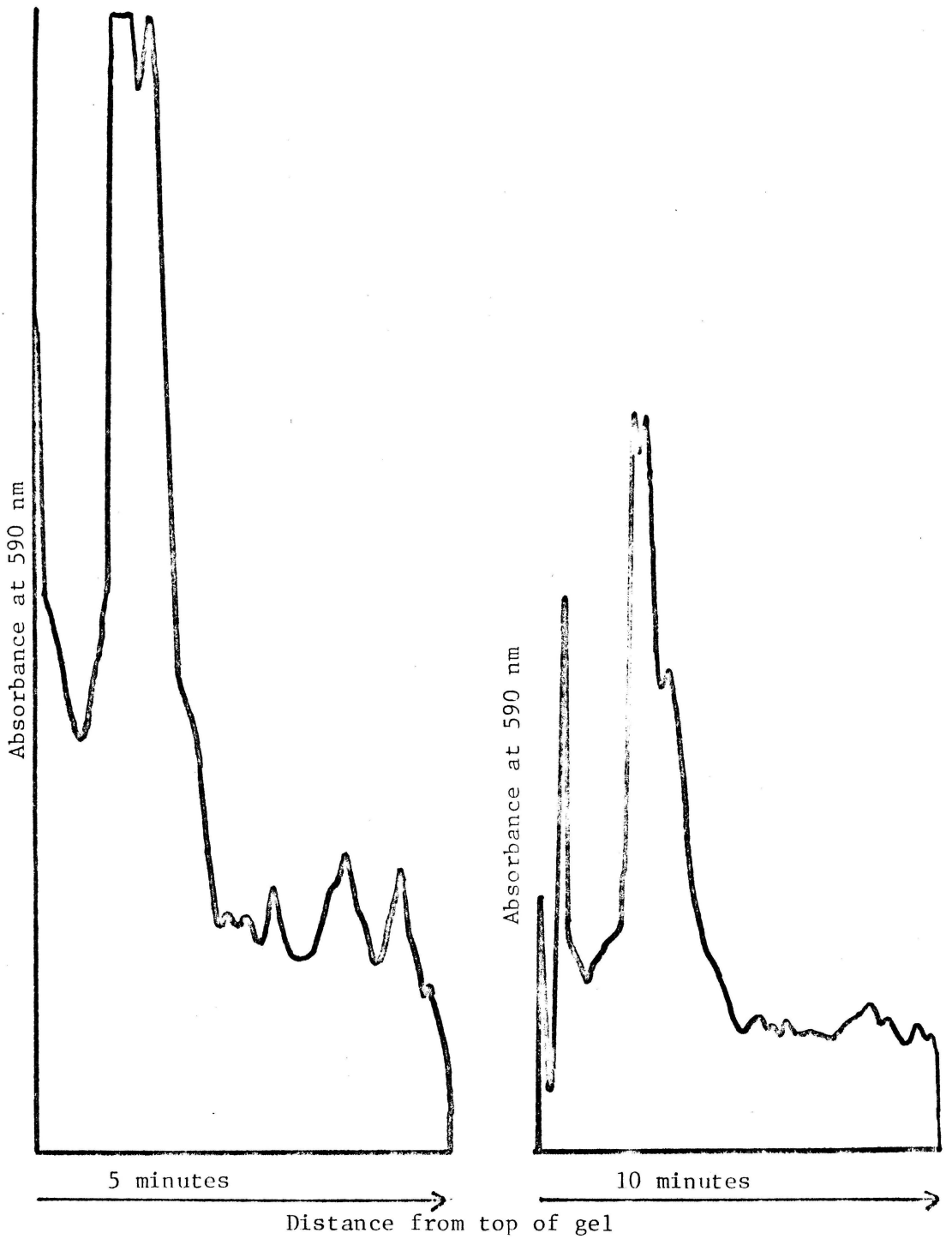


Figure 10. Densitometer traces of Tris-HCl gel electrophoresis separation of water extracts of soy meal autoclaved at 15 psi for 5 and 10 minutes.

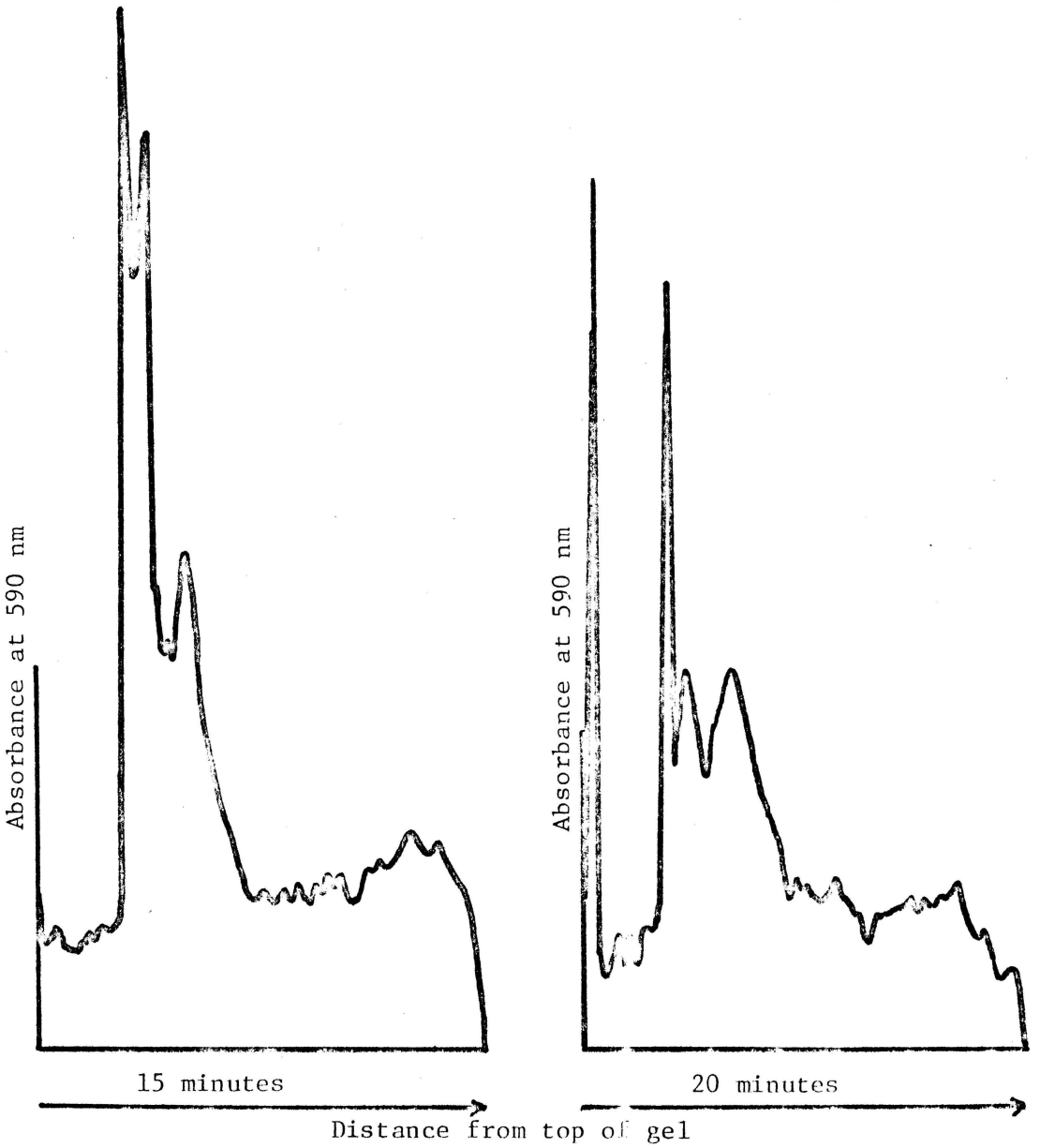


Figure 11. Densitometer traces of Tris-HCl gel electrophoresis separation of water extracts of soy meal autoclaved at 15 psi for 15 and 20 minutes.

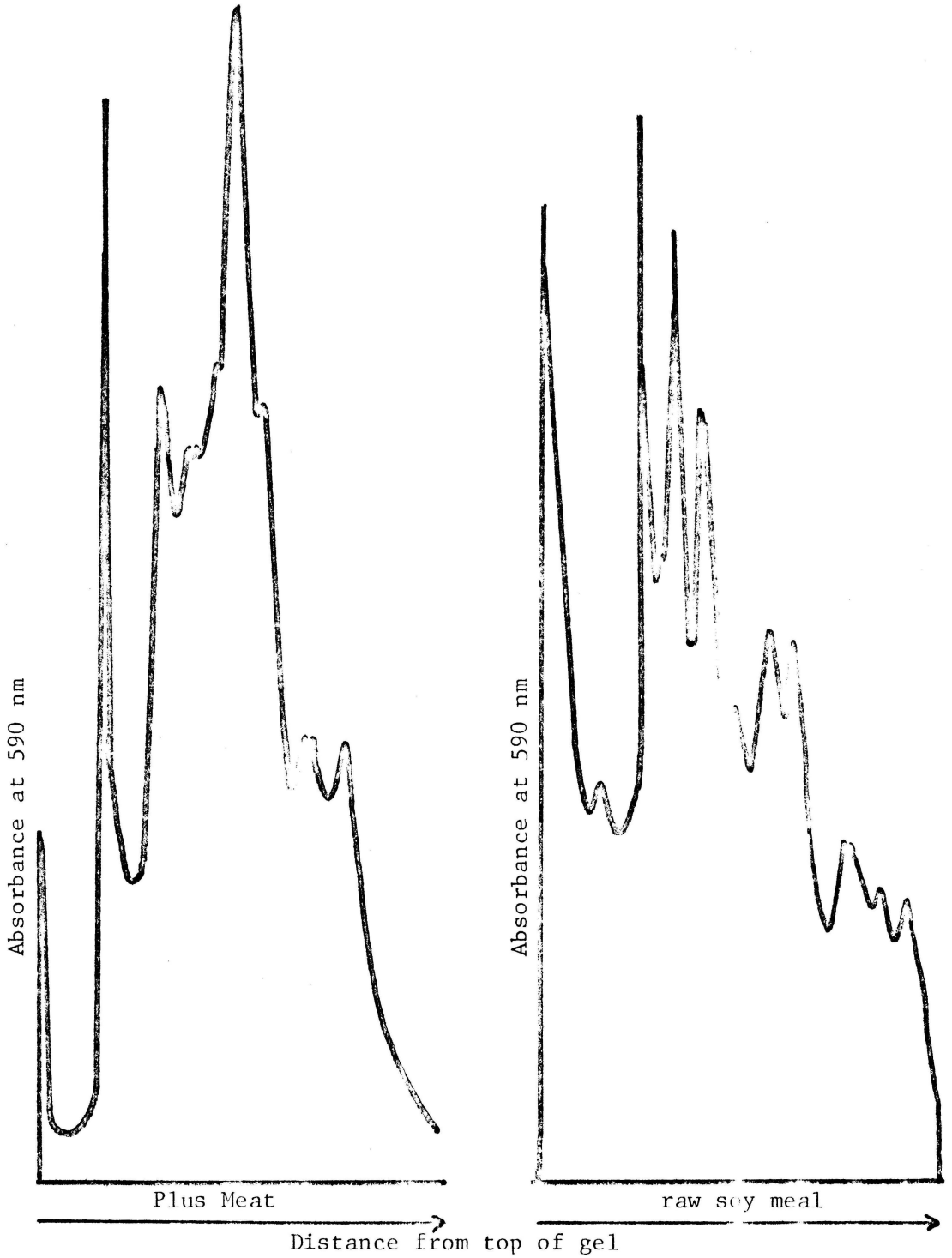


Figure 12. Densitometer traces of Tris-HCl gel electrophoresis separation of Plus Meat and raw soy meal extracted in pH 8.6 Tris-glycine containing 0.1M mercaptoethanol.

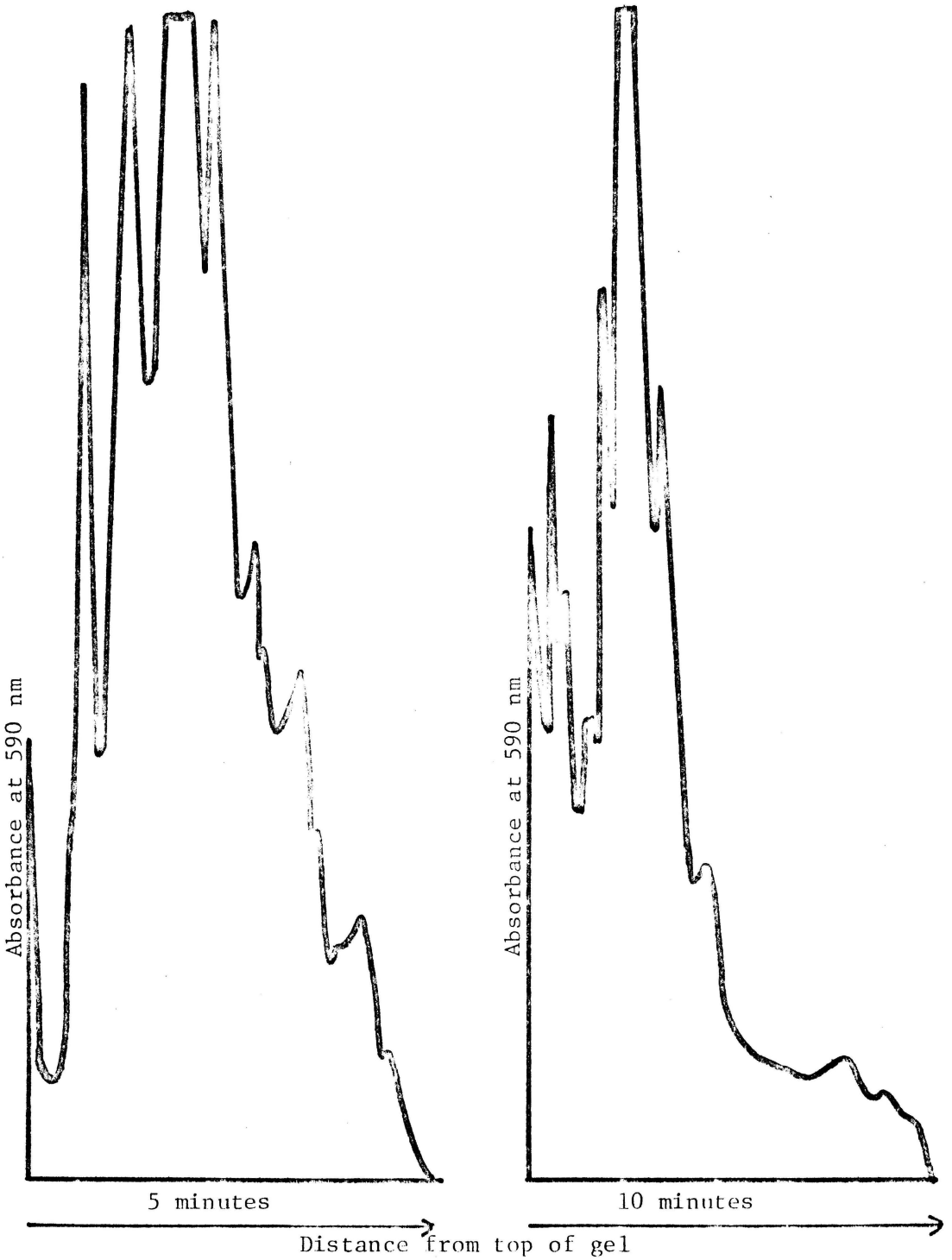


Figure 13. Densitometer traces of Tris-HCl gel electrophoresis separation of soy meal autoclaved at 15 psi for 5 and 10 minutes and extracted in pH 8.6 Tris-glycine containing 0.1M mercaptoethanol.

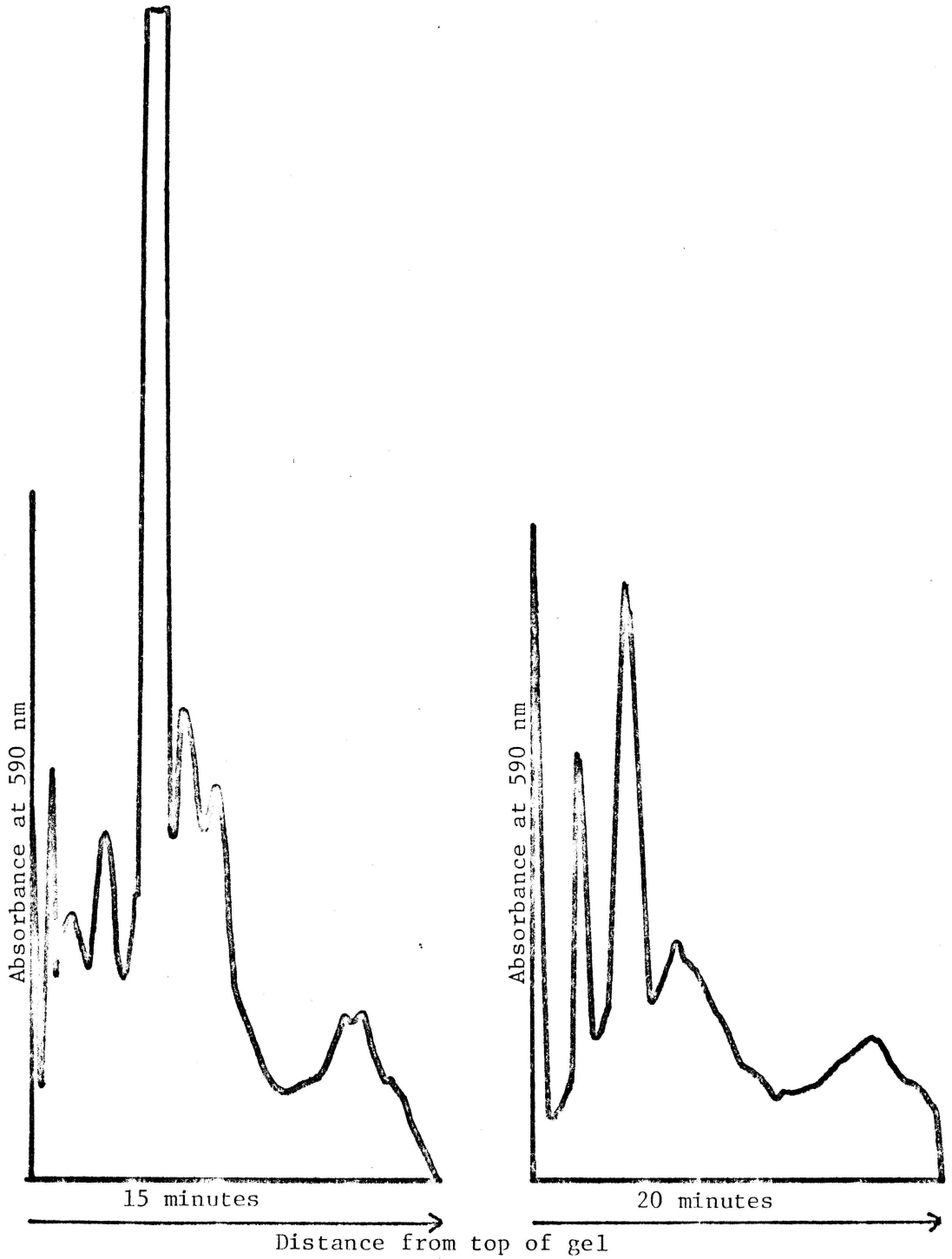


Figure 14. Densitometer traces of Tris-HCl gel electrophoresis separation of soy meal autoclaved at 15 psi for 15 and 20 minutes and extracted in pH 8.6 Tris-glycine containing 0.1 M mercaptoethanol.

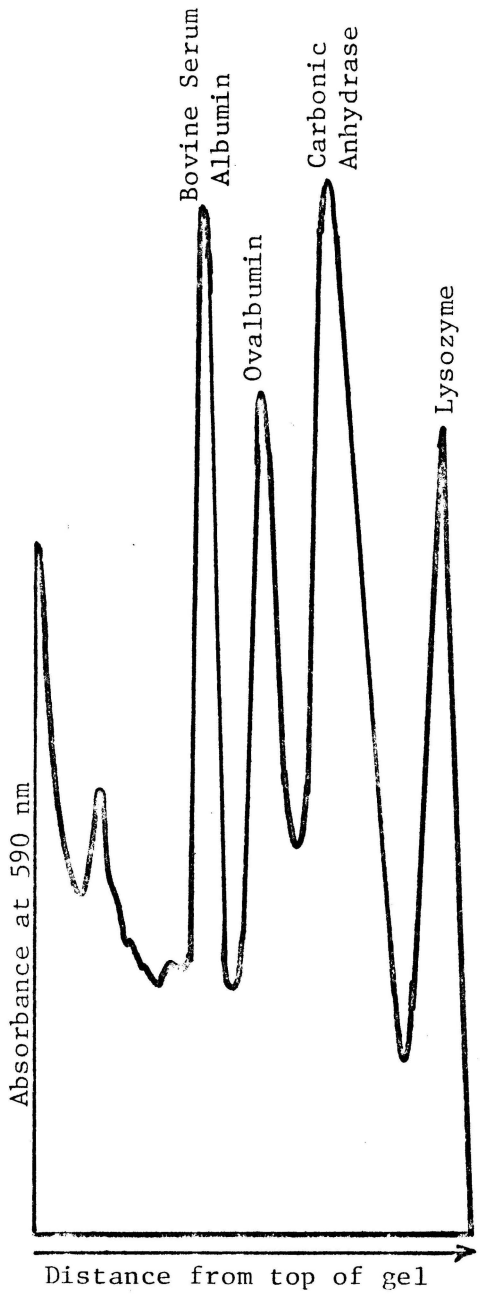


Figure 15. Densitometer trace of SDS gel electrophoresis separation of purified proteins.

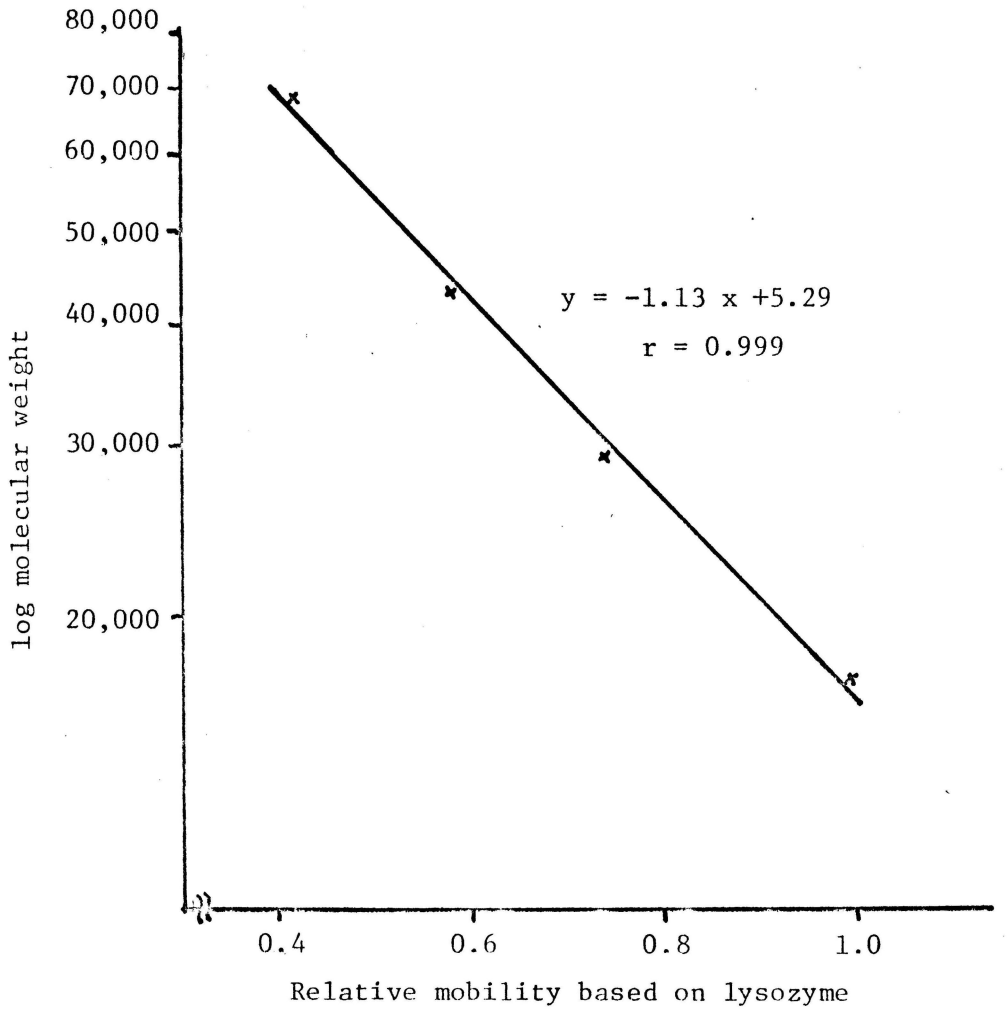


Figure 16. Relative mobility versus logarithm of molecular weight of pure proteins separated by sodium dodecyl sulfate gel electrophoresis.

SDS electrophoresis of water extracts of Ultra Soy and raw soy meal are diagrammed in Figure 17. No difference in gel patterns was found among the three textured products. Proteins from the textured products ranged in molecular weight from 81,000 to 8,500. Major peaks occurred at 50,000, 27,000 and 17,000. Extracts of raw soy separated into 12 distinct peaks which ranged in molecular weight from 170,000 to 15,000. Two major bands occurred at approximate molecular weights 100,000 and 81,000.

Densitometer scans of SDS electrophoresis gels separating water extracts of autoclaved soy meal are given in Figures 18 and 19. Higher and lower molecular weight peaks became less distinct with increasing heat treatment, and the major peak at 100,000 had virtually disappeared after 10 minutes of heat treatment. After 20 minutes of heat treatment a major band at molecular weight 81,000 in raw soy meal also disappeared.

The electrophoretic scans of Tris-glycine extracts of Plus Meat and raw soy meal are presented in Figure 20. Again, no differences were found between extracts of the three textured products. Approximate molecular weight range of the major peaks of textured soy extracts was 81,000 to 8,500 and for raw soy meal was 170,000 to 8,500. Major peaks for soy again occurred at 100,000 and 81,000 while major peaks for textured soy occurred at 25-35,000. Densitometer scans of gels of autoclaved soy extracts are given in Figures 21 and 22. Heat treatment resulted in the loss or decrease in protein bands at high and low molecular weights. Protein peaks at 100,000 and 81,000

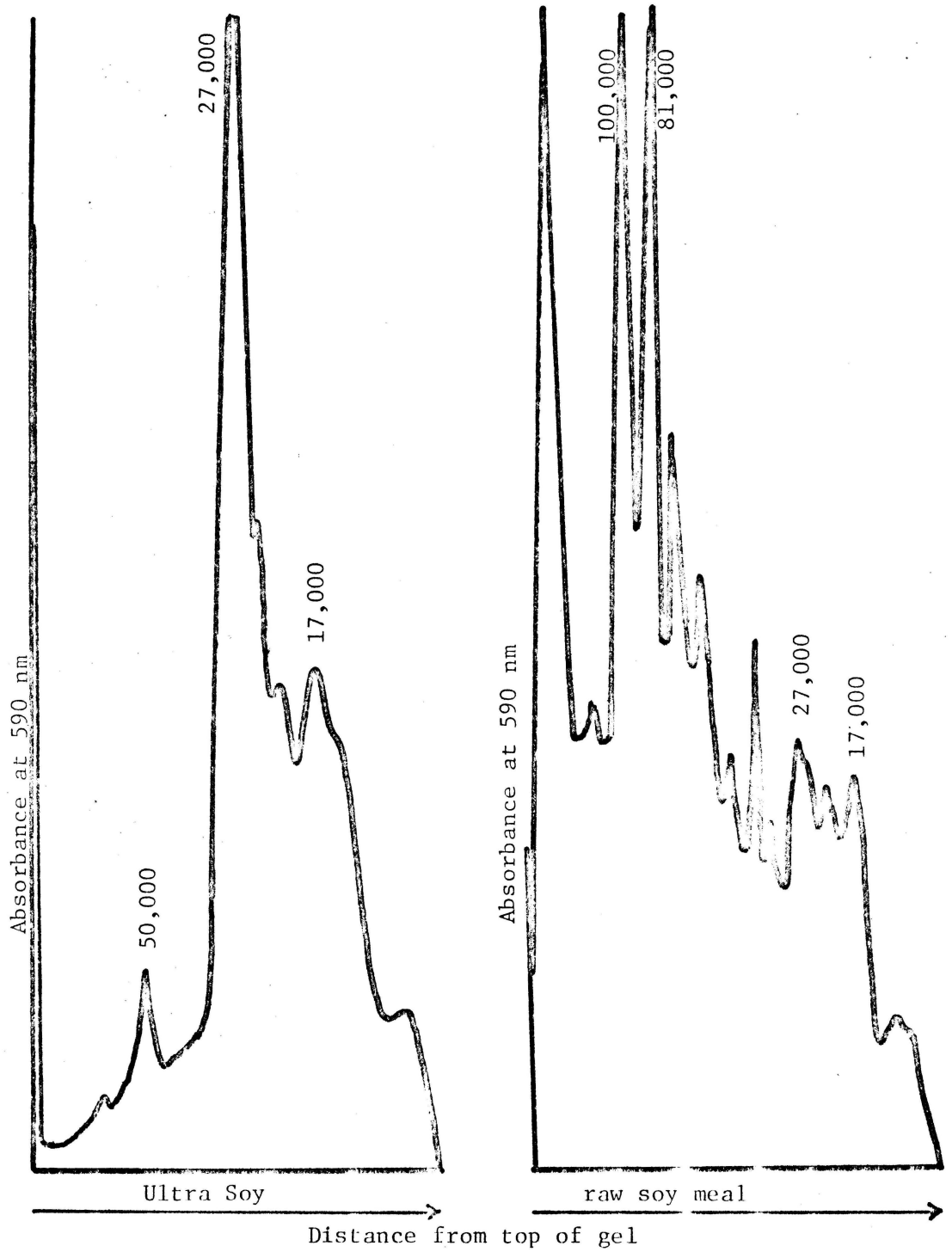


Figure 17. Densitometer traces of SDS gel electrophoresis separation of water extracts of Ultra Soy and raw soy meal. Numbers represent approximate molecular weights of proteins in various bands.

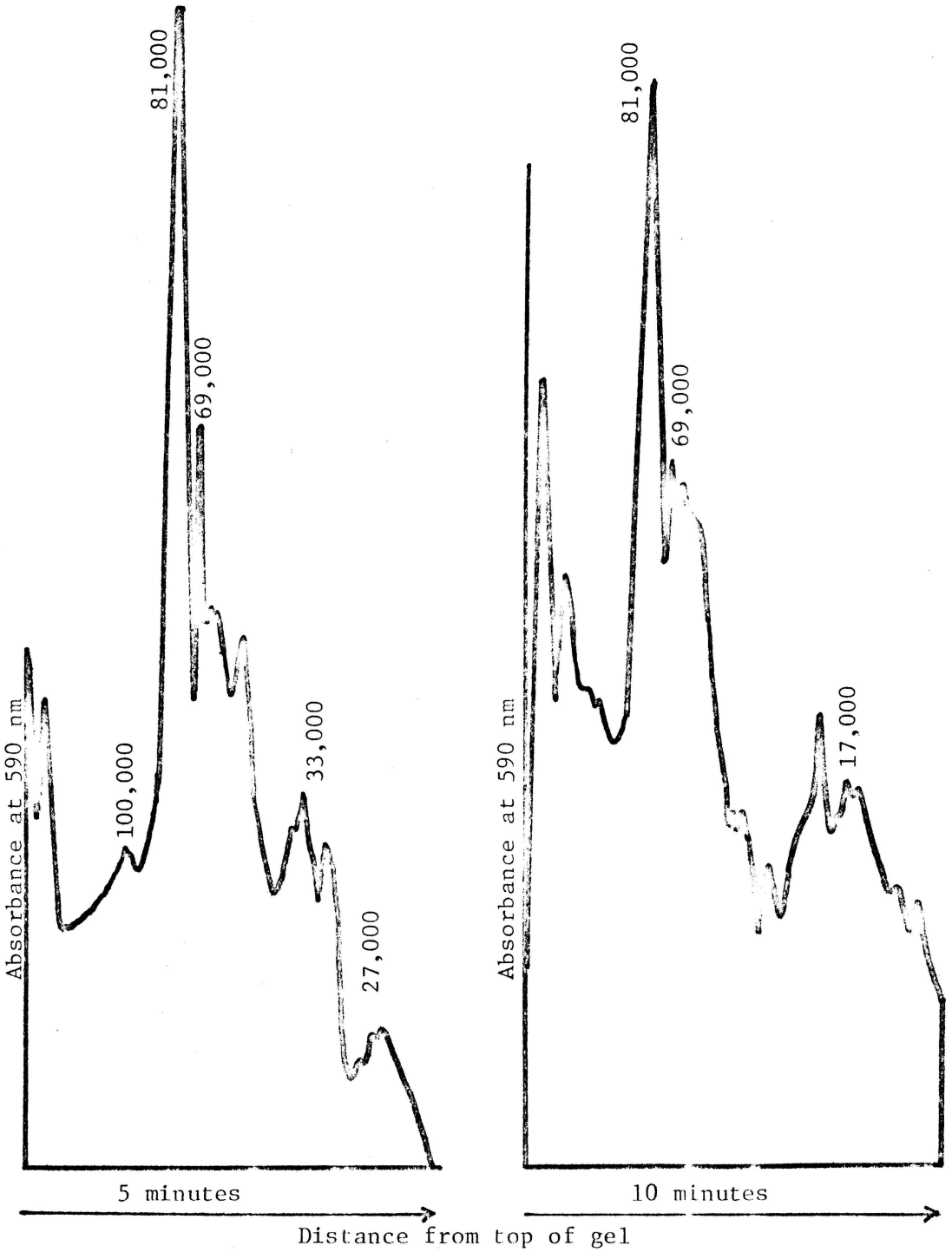


Figure 18. Densitometer traces of SDS gel electrophoresis separation of water extracts of soy meal autoclaved at 15 psi for 5 and 10 minutes. Numbers represent approximate molecular weights of proteins in various bands.

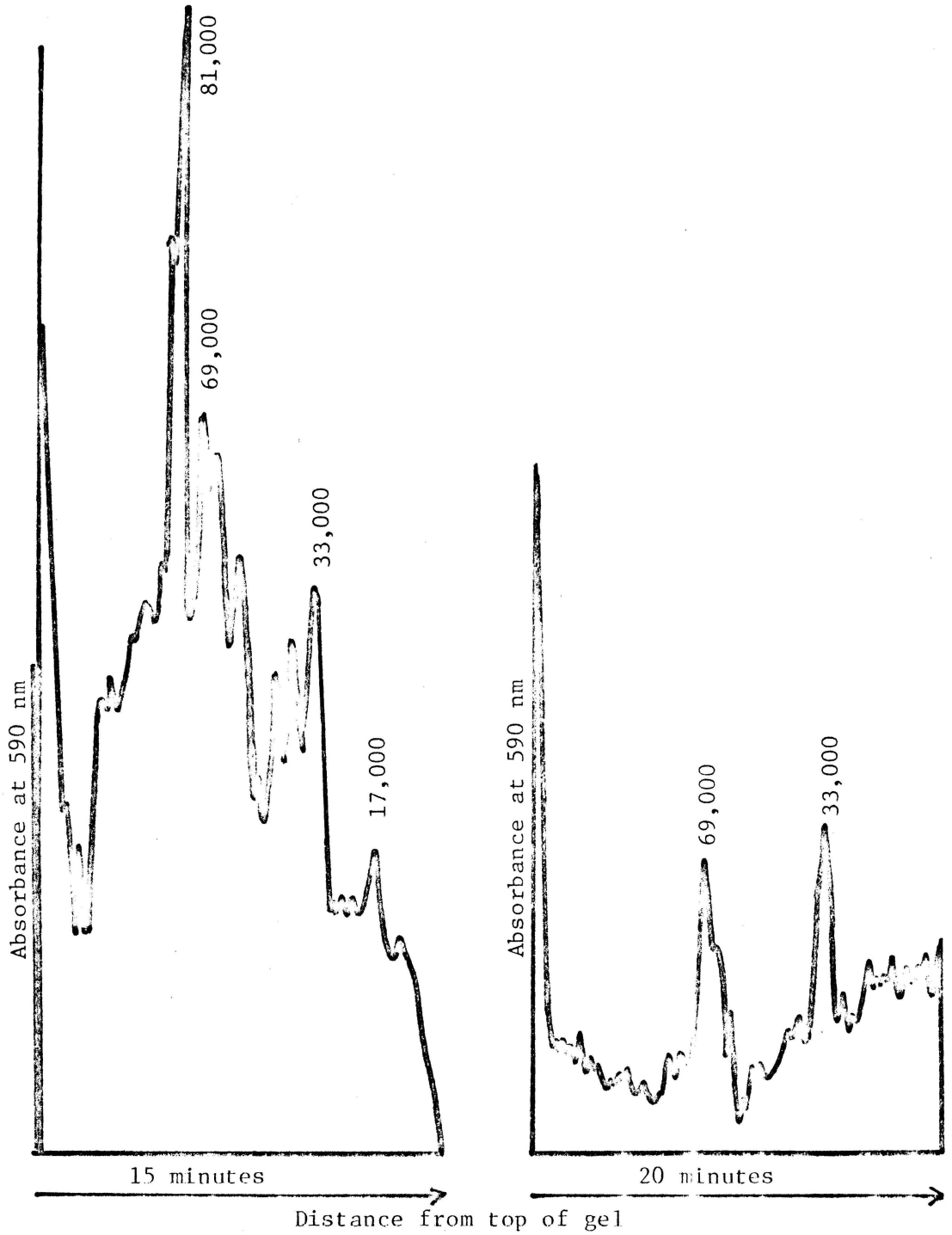


Figure 19. Densitometer traces of SDS gel electrophoresis separation of water extracts of soy meal autoclaved at 15 psi for 15 and 20 minutes. Numbers represent approximate molecular weights of proteins in various bands.

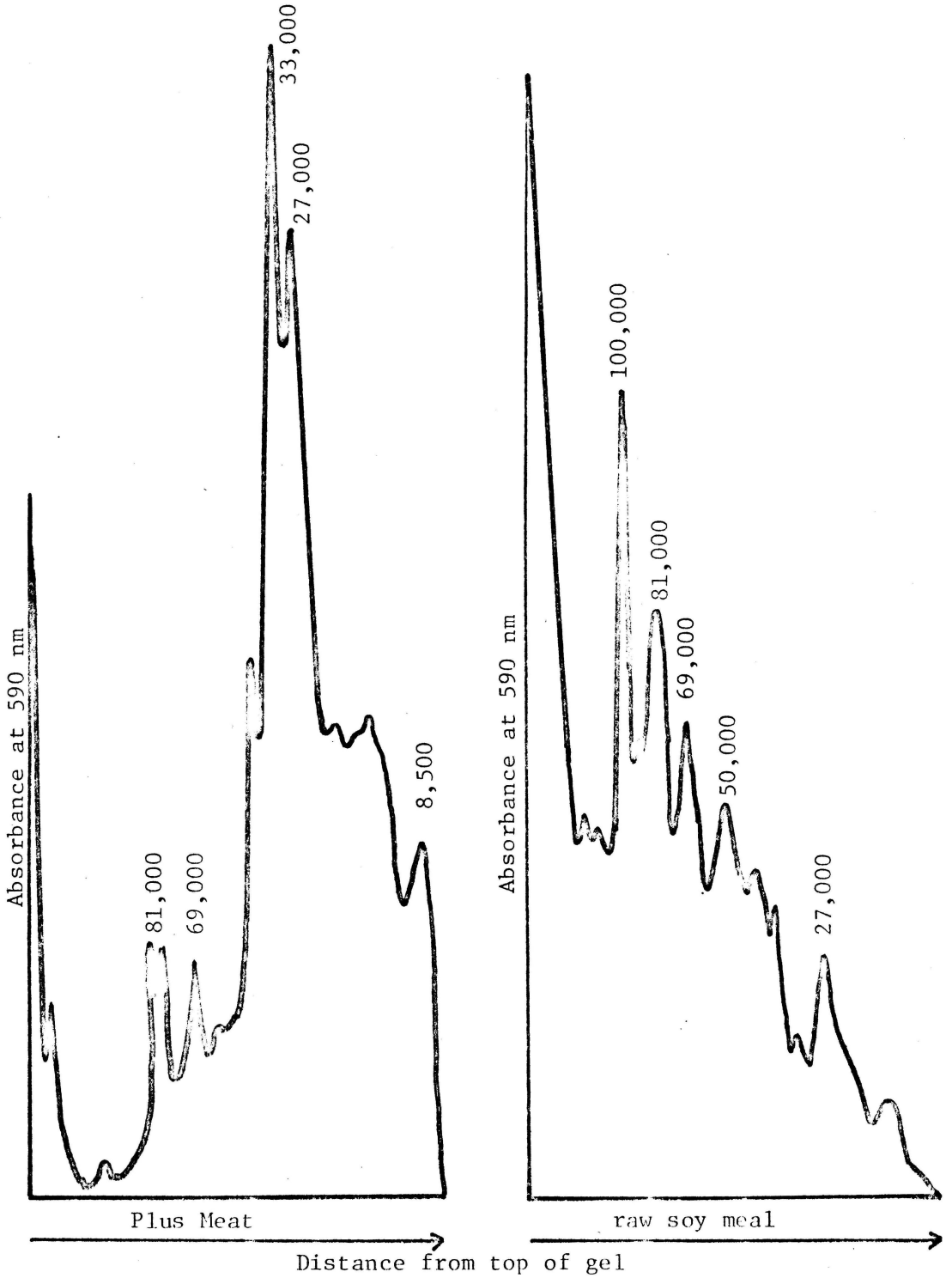


Figure 20. Densitometer traces of SDS gel electrophoresis separation of Plus Meat and raw soy meal extracted in pH 8.6 Tris-glycine containing 0.1M mercaptoethanol. Numbers represent approximate molecular weights of proteins in various bands.

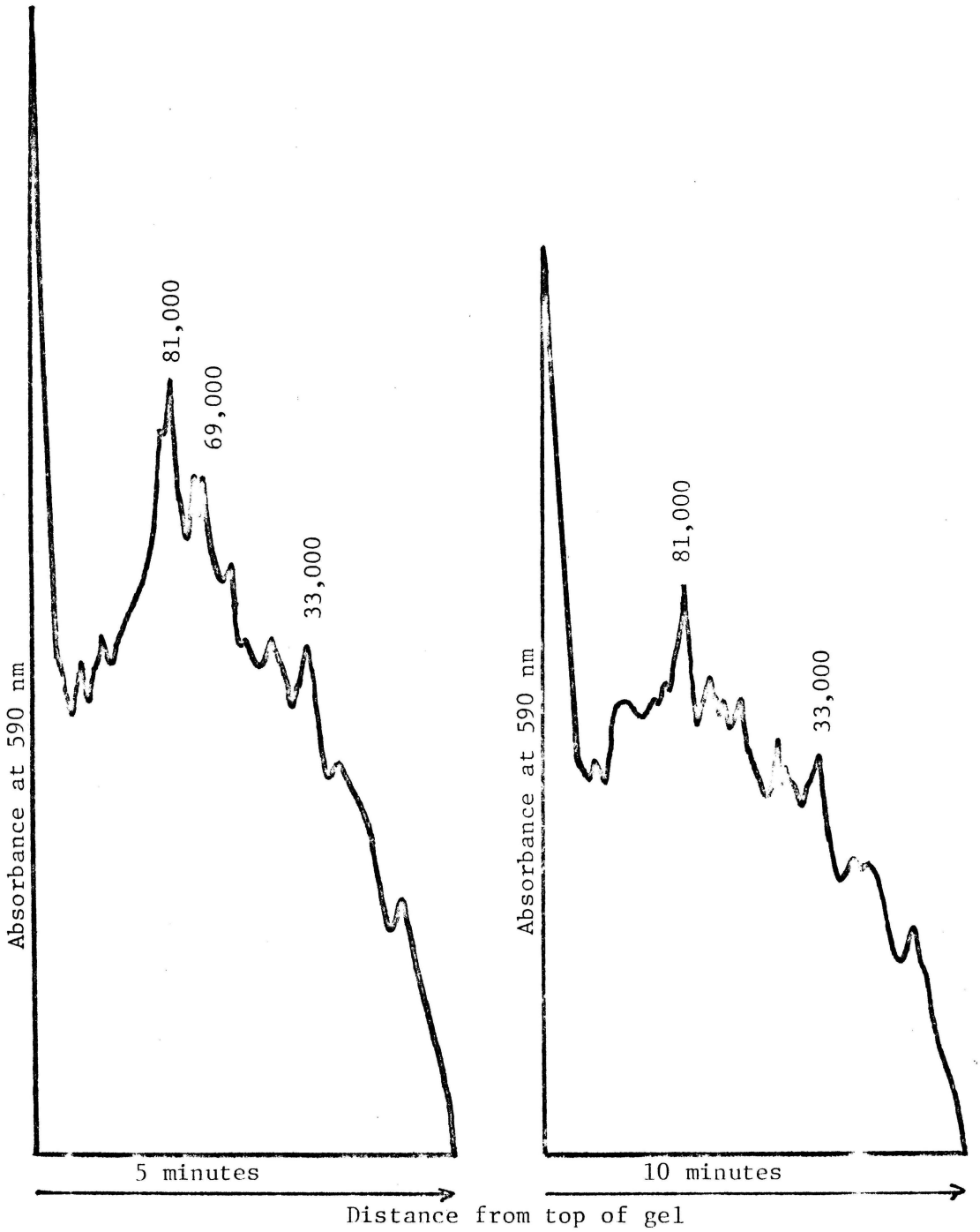


Figure 21. Densitometer traces of SDS gel electrophoresis separation of soy meal autoclaved at 15 psi for 5 and 10 minutes and extracted in pH 8.6 Tris-glycine containing 0.1M mercapto-ethanol. Numbers represent approximate molecular weights of proteins in various bands.

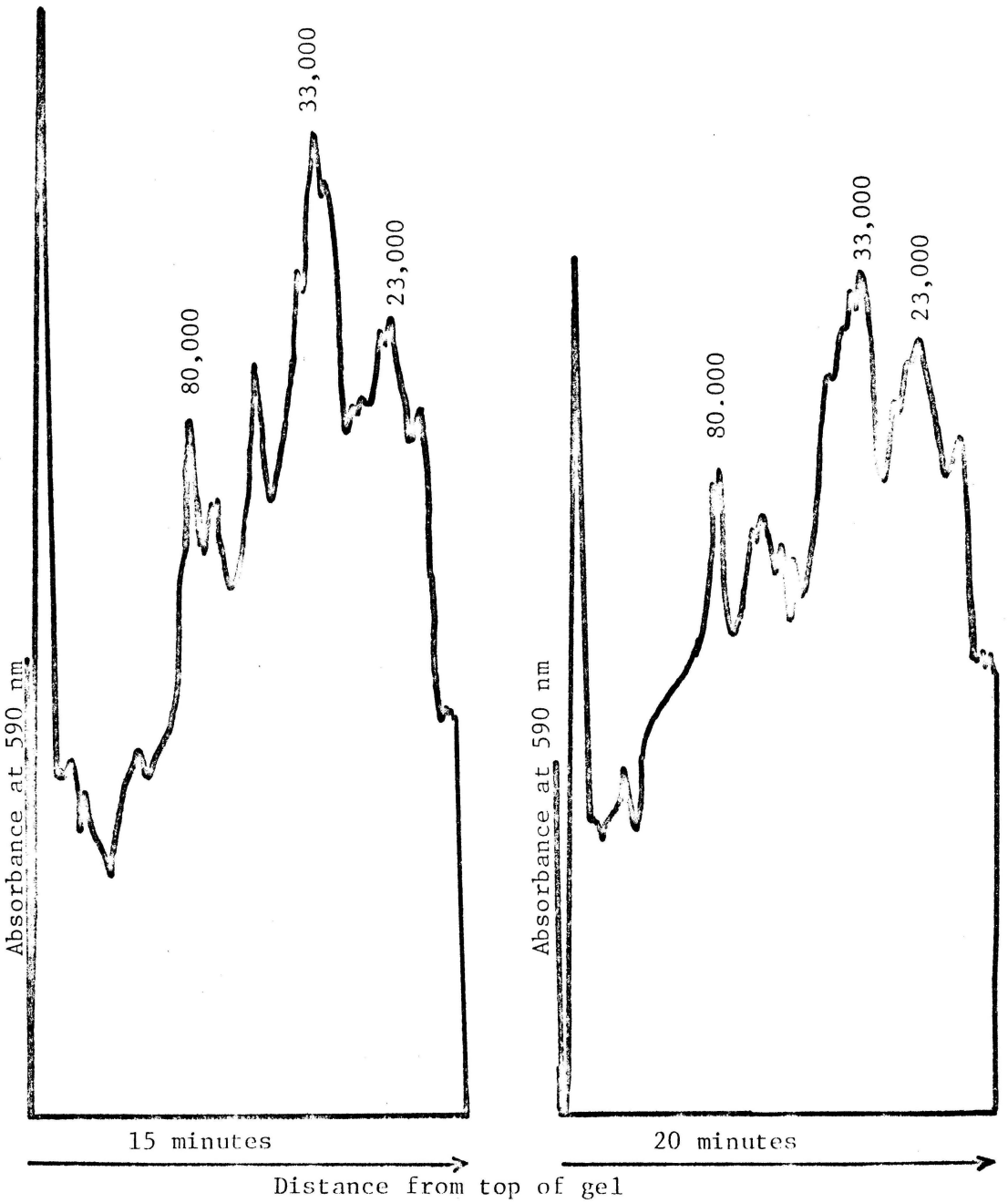


Figure 22. Densitometer traces of SDS gel electrophoresis separation of soy meal autoclaved at 15 psi for 15 and 20 minutes and extracted in pH 8.6 Tris-glycine containing 0.1M mercaptoethanol. Numbers represent approximate molecular weights of proteins of various bands.

diminished in size relative to other extractable proteins with increasing length of heat treatment.

Gel Electrophoresis: Soy Flours, Concentrate and Isolates

SDS electrophoresis of processed soy flours, concentrate and isolates was conducted using the system described by Neville (1971). Excellent separation of standard proteins was achieved using this system (Figure 23). The resulting plot of relative mobility versus log of protein molecular weight yielded a correlation coefficient of 0.999, where $y = -1.21x + 5.36$. Processed soy was extracted in pH 7.6 buffer containing 8 M urea and 0.1 M MCE.

The densitometer trace of separated proteins from Soya Fluff 200W is shown in Figure 24. Surprisingly, none of the product extracts differed in pattern when extracted in SB containing urea and mercaptoethanol and separated by this system.

Sulfhydryl and Disulfide Determination

Extracts from three textured soy products and from raw soy meal were analyzed for disulfide (SS), sulfhydryl (SH) and total SH (2SS + SH) content. Results are presented in Tables 5-7 and statistical comparisons are presented in Table 8. No statistical comparisons were made for proteins extracted in standard buffer.

When analyzed immediately after extraction in water (day 1) Ultra Soy had a significantly lower SH content than Plus Meat, Burger Bonus and raw soy meal. Ultra Soy also had a significantly lower day 1 SH content than Plus Meat and Burger Bonus when extracted in standard buffer containing SDS, as well as the lowest measured

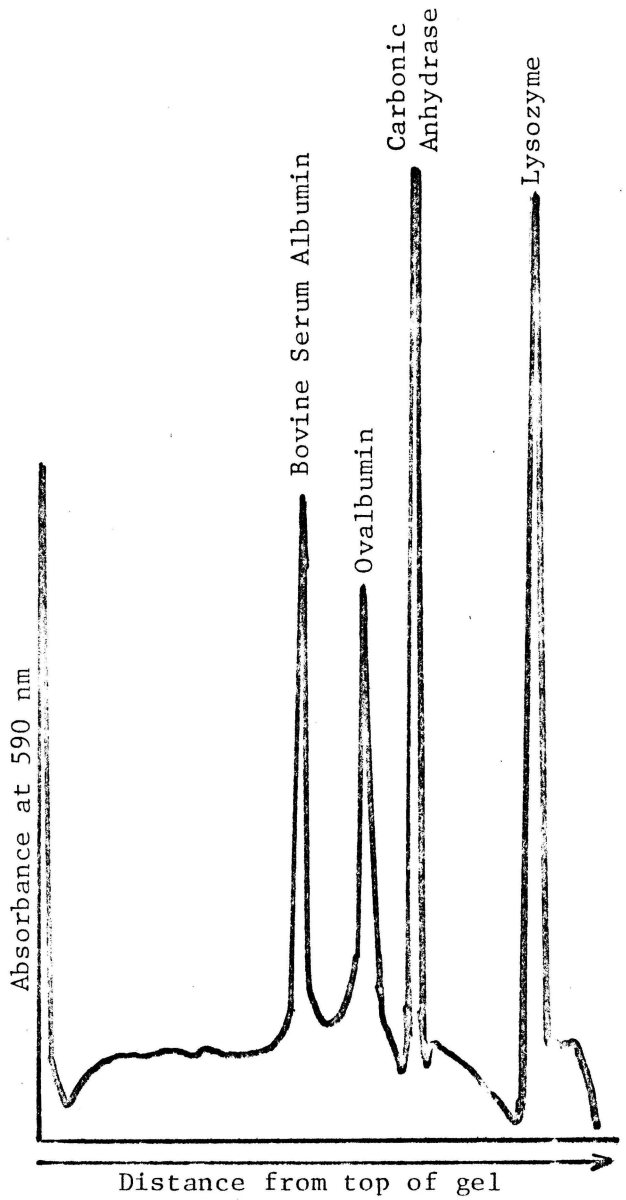


Figure 23. Densitometer trace of SDS gel electrophoresis separation of purified proteins using a discontinuous buffer system.

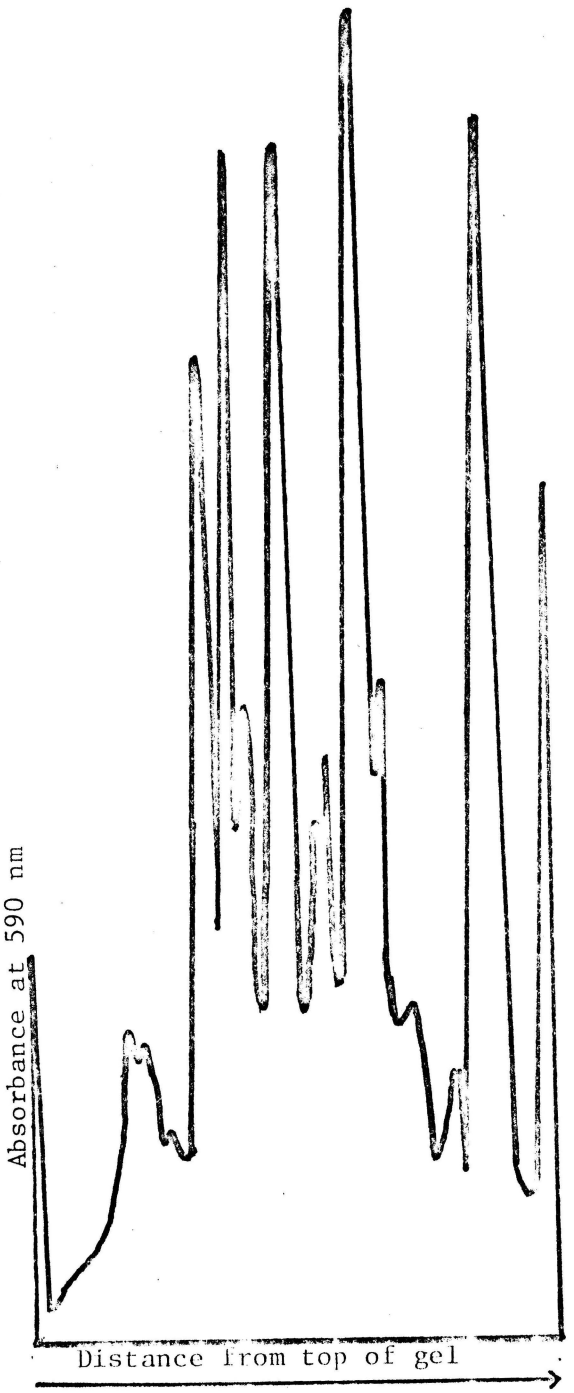


Figure 24. Densitometer trace of SDS gel electrophoresis separation of Soya Fluff 200W extracted in pH 7.6 potassium phosphate buffer, 0.5μ, containing 8M urea and 0.1M mercaptoethanol.

Table 5. Sulfhydryl content, in μmoles per gram nitrogen, of textured soy products and raw soy meal extracted in water, standard buffer, and standard buffer containing 0.5% SDS.[a,b]

SAMPLE	DAY	EXTRACTION SOLVENT		
		H_2O	Buffer	Buffer + 0.5% SDS
Burger Bonus	1	95	81	68
	2	53	54	50
Plus Meat	1	103	58	85
	2	66	37	51
Ultra Soy	1	61	52	58
	2	36	24	19
Soymeal	1	107	107	0
	2	40	93	0

[a]Six separate extractions were performed on each sample for each solvent. Duplicate Ellman's determinations were performed on each extract. Reported values are the resulting averages.

[b]Standard buffer was pH 7.5 potassium phosphate, 0.5μ (Briggs and Wolf, 1957).

Table 6. Disulfide content, in μmoles per g nitrogen, of textured soy products and raw soy meal extracted in water, standard buffer and standard buffer containing 0.5% SDS.[a,b]

SAMPLE	DAY	EXTRACTION SOLVENT		
		H_2O	Buffer	Buffer + 0.5% SDS
Burger Bonus	1	39	49	64
	2	76	45	64
Plus Meat	1	57	86	70
	2	74	88	73
Ultra Soy	1	65	71	64
	2	72	76	78
Soymeal	1	234	212	256
	2	259	253	349

[a]Six separate extractions were performed on each sample for each solvent. Duplicate Ellman's determinations were performed on each extract. Reported values are the resulting averages.

[b]Standard buffer was pH 7.6 potassium phosphate, 0.5μ (Briggs and Wolf, 1957).

Table 7. Total sulfhydryl content (SH + reduced SS), in μmoles/gN, of textured soy products and unprocessed soybeans extracted in water standard buffer, standard buffer containing 0.5% SDS or standard buffer containing 0.5% SDS and 0.1M mercaptoethanol.[a,b]

PRODUCT	DAY	EXTRACTION MEDIA			
		H_2O	Buffer	Buffer + 0.5% SDS	Buffer + 0.5% SDS + 0.1M MCE
Burger Bonus	1	173	178	197	552
	2	205	144	179	572
Plus Meat	1	217	230	224	669
	2	214	233	197	663
Ultra Soy	1	191	178	186	610
	2	179	176	174	684
Soymeal	1	575	531	511	693
	2	558	600	698	620

[a] Six separate extractions were performed on each sample for each solvent. Duplicate Ellman's determinations were performed on each extract. Reported values are the resulting averages.

[b] Standard buffer was pH 7.6 potassium phosphate, 0.5μ (Briggs and Wolf, 1957).

Table 8. Statistical comparison of measured sulfhydryl (SH) and disulfide (SS) content of extracts of Burger Bonus (BB), Plus Meat (PM), Ultra Soy (US) and raw soy meal (SOY).[a,b]

TYPE OF COMPARISON	FACTORS HELD CONSTANT	(Day)	RESULT SH	SS
BB vs PM	H_2O	1	ns	ns
	H_2O	2	*	ns
	SB + SDS	1	*	ns
	SB + SDS	2	ns	ns
	SB + SDS + MCE	1	--	ns
	SB + SDS + MCE	2	--	ns
BB vs US	H_2O	1	*	*
	H_2O	2	*	ns
	SB + SDS	1	ns	ns
	SB + SDS	2	**	ns
	SB + SDS + MCE	1	--	ns
	SB + SDS + MCE	2	--	ns
PM vs US	H_2O	1	**	ns
	H_2O	2	ns	ns
	SB + SDS	1	**	ns
	SB + SDS	2	**	ns
	SB + SDS + MCE	1	--	ns
	SB + SDS + MCE	2	--	ns
BB vs SOY	H_2O	1	ns	**
	H_2O	2	ns	**
	SB + SDS	1	**	**
	SB + SDS	2	--	--
	SB + SDS + MCE	1	--	**
	SB + SDS + MCE	2	--	ns
PM vs SOY	H_2O	1	ns	**
	H_2O	2	ns	**
	SB + SDS	1	**	**
	SB + SDS	2	--	--
	SB + SDS + MCE	1	--	ns
	SB + SDS + MCE	2	--	ns

Table 8 (continued)

TYPE OF COMPARISON	FACTORS HELD CONSTANT	(Day)	RESULT SH	RESULT SS
US vs SOY	H_2O	1	**	**
	H_2O	2	ns	**
	SB + SDS	1	**	**
	SB + SDS	2	--	--
	SB + SDS + MCE	1	--	ns
	SB + SDS + MCE	2	--	ns
Day 1 vs Day 2	H_2O	BB	**	**
	H_2O	PM	**	*
	H_2O	US	**	**
	H_2O	SOY	**	*
	SB + SDS	BB	*	ns
	SB + SDS	PM	**	ns
	SB + SDS	US	**	**
	SB + SDS	SOY	--	--
	SB + SDS + MCE	BB	--	ns
	SB + SDS + MCE	PM	--	ns
	SB + SDS + MCE	US	--	**
	SB + SDS + MCE	SOY	--	ns
H_2O vs SB + SDS	BB	1	*	**
	BB	2	ns	ns
	PM	1	*	ns
	PM	2	*	ns
	US	1	ns	ns
	US	2	*	ns
	SOY	1	--	--
	SOY	2	--	--

[a]Extraction media included: water (H_2O); pH 7.6 potassium phosphate buffer, 0.5μ + 0.5% sodium dodecyl sulfate (SB + SDS); pH 7.6 potassium phosphate buffer, 0.5μ + 0.5% sodium dodecyl sulfate + 0.1% mercaptoethanol (SB + SDS + MCE).

[b]Tuckey's test or Student's t-test: ** = significant at 1% level; * = significant at 5% level; ns = non-significant; -- = no test done.

SH content of all products extracted in standard buffer. All textured products had significantly higher day 1 SH content than raw soy meal when extracted in standard buffer containing SDS. When each product is compared to itself, all products, regardless of extraction media, had lower measured SH content on day 2 as compared with day 1 with the exception of soy meal extracted in buffer containing SDS. The measured SH contents of Burger Bonus and Plus Meat extracted in water on day 1 were significantly higher than their corresponding SH content measured from buffer containing SDS extracts, whereas Ultra Soy day 1 SH content measured in water and buffer plus SDS extractc did not differ.

The disulfide content of buffer containing SDS extracts of the three textured products did not differ significantly when measured on day 1 (Table 6). Water extracted Burger Bonus had lower SS content than water extracted Ultra Soy, although neither product differed in SS content from that of water extracted Plus Meat. All extractions of textured products had significantly lower day 1 SS levels than did raw soy meal. Measured SS level from water extractions of all products increased significantly from day 1 to day 2. The SS content of buffer containing SDS extracts of Plus Meat, Ultra Soy and raw soy meal remained unchanged from day 1 to day 2.

Total SH content (SH + 2SS) is presented in Table 7. Soy meal extracted in water, buffer or buffer containing SDS had higher total SH's than did extracts of the textured products on day 1. However, when extracted in buffer containing SDS and MCE, only Burger Bonus had a significantly lower total SH content than did raw soy meal on day 1.

Sulfur Amino Acid Oxidation

Total sulfur amino acid content of processed soy products, as measured by performic acid oxidation, is given in Table 9. The measured half-cystine content of each product was close to or identical with that reported by the manufacturer except for Soya Fluff 200T. The measured methionine content was slightly higher than that reported by the manufacturer in all cases.

Table 10 presents the cysteic acid content of each product. Products contained from 2.9 to 23.2% of total half-cystine content as cysteic acid. Isolates contained higher cysteic acid content than flours and concentrate. Methionine sulfone was not found in any of the soy products analyzed.

The methionine sulfoxide content of soy products was investigated by two procedures. Barium hydroxide hydrolysis of a mixture of free amino acids resulted in average aspartic acid and methionine sulfoxide recoveries of 18.8 and 23.9% respectively. Large variation between duplicate samples occurred. Published reports using barium hydroxide for methionine sulfoxide analysis fail to indicate recovery data or whether recovery was based on recovery of a "stable" amino acid (Neumann, 1967; Cuq et al., 1973). This procedure was judged to be inadequate for the accurate determination of methionine sulfoxide in soy samples, and no further experiments using barium hydroxide hydrolysis were conducted.

Efforts were also made to determine the methionine sulfoxide content of soy proteins using a modification of the sodium hydroxide hydrolysis procedure described by Neumann (1967). Percent recovery

Table 9. Results of performic acid determination of total sulfur amino acid content of processed soy expressed as measured products (methionine sulfone and cysteic acid) and as methionine and cystine equivalents.[a]

PRODUCT	methionine sulfone[b]	cysteic acid[b]	methionine[c] measured	methionine[c] reported[d]	1/2 cystine[c] measured	1/2 cystine[c] reported[d]
Soya Fluff 200T	93.8	117	1.4	1.3	1.4	1.6
Soya Fluff 200W	93.8	133	1.4	1.3	1.6	1.6
Promosoy 100	93.8	125	1.4	1.3	1.5	1.5
Promine F	87.1	108	1.3	1.0	1.3	1.2
Promine D	87.1	108	1.3	0.9	1.3	1.2
Ultra Soy	93.8	133	1.4	---	1.6	---
Burger Bonus	87.1	133	1.3	---	1.6	---
Plus Meat	80.4	133	1.2	---	1.6	---

[a] Results represent averages of at least three independent determinations.

[b] µmoles/g protein.

[c] g/16g N.

[d] Product data sheets, Central Soya, Chicago, Illinois.

Table 10. Cysteic acid content, in μmoles/g protein, and % cysteic acid relative to total 1/2 cystine, of processed soy products.[a]

Product	Cysteic Acid	% Cysteic Acid[b]
Soya Fluff 200T	4.9	4.2
Soya Fluff 200W	5.9	4.4
Promosoy 100	3.6	2.9
Promine F	15.5	14.3
Promine D	25.1	23.2
Ultra Soy	6.4	4.8
Burger Bonus	6.0	4.5
Plus Meat	6.6	5.0

[a] Results represent averages of at least two independent determinations.

[b] $\frac{\mu\text{moles cysteic acid}}{\mu\text{moles 1/2 cystine}} \times 100$.

and average deviation of pure free leucine and methionine sulfoxide treated with sodium hydroxide in simulated hydrolysis were 88 ± 2% and 37 ± 6% respectively. Average recovery of leucine from sodium hydroxide hydrolyzed soy flours and concentrate as compared with acid hydrolyzed samples was 69 ± 1%. Since soy isolates contained approximately 90% protein, sample size of these products was too small (∿5 mg) to weigh accurately, and therefore leucine recovery from these samples could not be determined. Leucine has been reported stable to alkaline hydrolysis, and therefore leucine concentration is frequently used to calculate sample size during the determination of tryptophan (Blackburn, 1968).

Although sodium hydroxide hydrolysis had obvious inadequacies, the procedure was relatively simple to conduct and more reproducible than barium hydroxide hydrolysis. Results using this procedure cannot be considered quantitative, however, the procedure was considered adequate for demonstration of the presence, if not the exact amount, of methionine sulfoxide in protein samples. Methionine sulfoxide recovery from soy protein samples was assumed to be 100% since no pure proteins of known methionine sulfoxide content were available for determination of methionine sulfoxide recovery from intact protein. Therefore, measured methionine sulfoxide content of sample proteins may underestimate the true methionine sulfoxide content.

Using sodium hydroxide hydrolysis, methionine sulfoxide was found to be present in only one of the processed soy products, Promine D, a soy isolate. Duplicate samples of Promine D hydrolyzed with sodium hydroxide were found to contain 0.294 mg and 0.238 mg leucine.

Since Promine D hydrolyzed with hydrochloric acid was found to contain 8.7 g leucine per 16 g nitrogen, or 75.3 mg leucine per gram sample, sample weights for the duplicate sodium hydroxide hydrolyzed Promine D samples could be calculated. Assuming 100% leucine recovery, sample weights were 3.9 mg and 3.2 mg respectively. Assuming 100% methionine sulfoxide recovery, the methionine sulfoxide content was 31 ± 7 μmoles per gram protein. If methionine sulfoxide recovery is assumed to be only 37%, the methionine sulfoxide content of Promine D is 84 ± 7 μmoles per gram protein. If leucine recovery is assumed to be 69% (based on recovery from soy flours and isolate), Promine D sample weights were 5.8 mg and 4.7 mg respectively, and the methionine sulfoxide concentration of Promine D, assuming 100% methionine sulfoxide recovery, was 19 ± 4 μmoles per gram protein. When expressed as a percentage, 22-97% of the measured methionine in Promine D was present as methionine sulfoxide, depending on the method of sample size calculation and assumed recovery of methionine sulfoxide.

Rate of Protein Hydrolysis

Amino acid composition was determined as a function of hydrolysis time for two soy flours, one soy concentrate and two soy isolates. Average contents of each essential amino acid determined at each hydrolysis time for the five soy products are presented in Tables 11-15.

Three general types of hydrolytic release of essential amino acids were observed for the five soy products. The first type of release behavior is illustrated by phenylalanine for Promosoy 100 in Figure 25.

Table 11. Effect of length of acid hydrolysis on the measured essential amino acid content (g/16g nitrogen) of Soya Fluff 200T.

	AMINO ACID							
HOURS	Thr	Val	Ile	Leu	Tyr	Phe	Lys	Met
18	4.35	4.96	4.92	7.59	3.75	5.22	6.57	1.33
24	4.00	4.52	5.07	7.53	3.69	5.34	6.36	1.13
36	4.39	4.73	4.73	7.84	3.70	5.49	6.17	1.08
48	4.08	4.99	5.38	8.02	3.62	5.26	6.38	1.12
60	4.35	5.49	4.96	7.72	3.49	5.32	6.62	1.05
72	4.46	5.71	4.79	7.75	3.24	5.07	6.49	1.00

[a]Results represent the average value of at least three independent hydrolyses.

Table 12. Effect of length of acid hydrolysis on the measured essential amino acid content (g/16g nitrogen) of Soya Fluff 200W.[a]

	AMINO ACID							
HOURS	Thr	Val	Ile	Leu	Tyr	Phe	Lys	Met
12	4.24	4.36	4.13	7.87	3.39	5.24	6.43	1.13
18	4.38	4.82	4.33	7.37	3.39	5.18	6.61	0.97
24	4.19	4.79	4.69	7.58	3.31	5.01	--	0.97
36	4.13	4.92	5.14	8.01	3.39	5.09	6.55	0.95
48	4.22	5.13	4.89	8.28	3.64	5.34	6.60	0.92
60	3.96	5.24	4.97	7.85	3.36	4.72	6.00	0.97
72	3.94	5.48	5.01	7.66	3.52	5.06	6.57	0.96

[a]Results represent averages of at least three independent hydrolyses.

Table 13. Effect of length of acid hydrolysis on the measured essential amino acid content (g/16g nitrogen) of Promosoy 100.[a]

	AMINO ACID							
HOURS	Thr	Val	Ile	Leu	Tyr	Phe	Lys	Met
12	4.53	4.01	3.96	7.67	3.59	4.81	5.99	1.42
18	4.04	4.94	4.38	7.91	3.66	5.12	6.80	1.36
24	4.12	4.88	4.46	7.47	3.49	4.90	6.88	1.15
36	3.97	4.92	4.35	7.35	3.24	5.00	6.66	1.05
48	3.92	5.09	4.60	7.52	3.35	4.63	6.85	1.06
60	4.21	5.08	5.05	7.71	3.44	5.13	6.51	1.03
72	3.91	5.25	4.84	7.56	3.42	4.95	6.63	1.04

[a]Results represent averages of at least three independent hydrolyses.

Table 14. Effect of length of acid hydrolysis on the measured essential amino acid content (g/16g nitrogen) of Promine F.[a]

	AMINO ACID							
HOURS	Thr	Val	Ile	Leu	Tyr	Phe	Lys	Met
12	4.02	4.36	4.65	8.20	3.81	5.41	6.35	1.13
18	3.74	4.41	4.49	8.01	3.46	5.22	5.97	1.04
24	3.89	4.91	4.82	8.18	3.97	5.80	6.15	1.12
36	3.72	5.30	5.46	8.65	3.49	5.61	6.26	1.13
48	3.80	5.12	5.08	7.93	3.76	5.49	6.41	1.08
60	3.79	5.46	4.73	8.62	3.88	5.59	6.32	1.00
72	3.82	5.42	5.17	8.04	3.61	5.59	5.22	0.98

[a]Results represent averages of at least three independent hydrolyses.

Table 15. Effect of length of acid hydrolysis in the measured essential amino acid content (g/16g nitrogen) of Promine D.[a]

	AMINO ACID							
HOURS	Thr	Val	Ile	Leu	Tyr	Phe	Lys	Met
12	4.29	4.62	4.26	8.25	3.78	5.13	6.19	1.25
18	4.00	4.79	4.97	8.47	4.07	5.81	6.67	0.94
24	3.83	4.83	4.91	8.41	3.79	6.09	6.56	0.87
48	4.03	5.32	5.39	8.32	4.20	6.16	6.83	1.06
60	3.90	5.53	5.03	8.13	3.81	5.83	6.61	1.11
72	3.96	4.90	5.44	6.86	--	5.61	6.14	--

[a]Results represent averages of at least three independent hydrolyses.

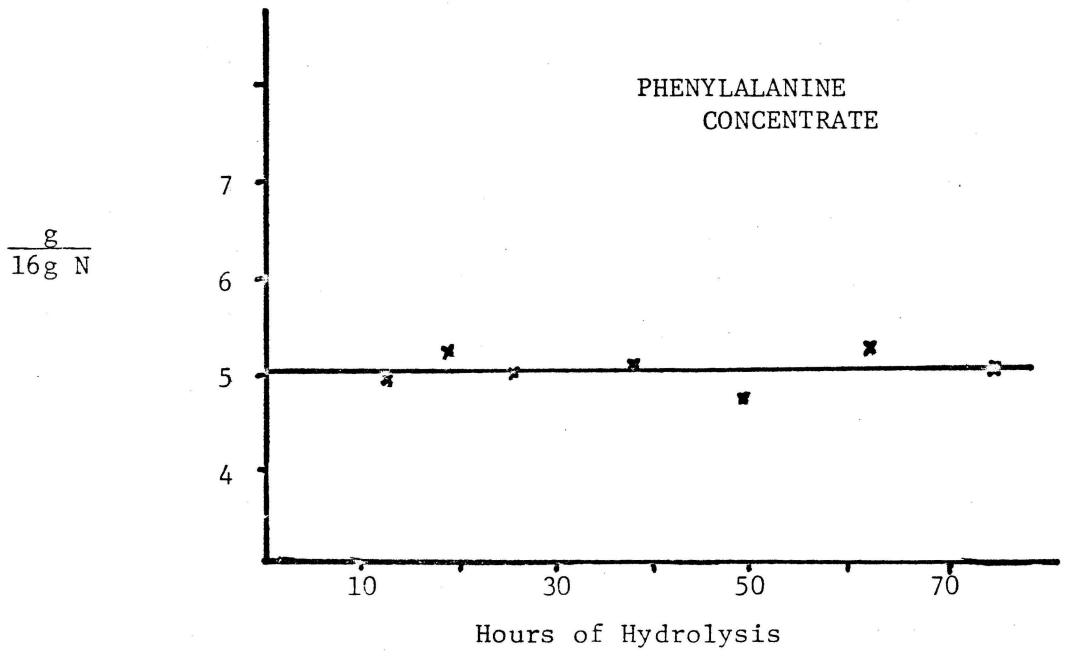


Figure 25. Average measured phenylalanine content of Promosoy 100 as a function of hydrolysis time (X); line calculated by linear regression.

Little change in measured content was observed between 12 and 72 hours of hydrolysis, and linear regression analysis yielded a slope of zero. Behavior of this type was exhibited by valine and lysine in Soya Fluff 200T, and leucine, lysine and phenylalanine in Promosoy 100.

Figure 26 shows a second release pattern in which the measured amino acid content decreased linearly with increased length of hydrolysis time. Methionine and threonine in all soy products were subject to such destruction. In addition, a pattern of this type was also observed for phenylalanine and lysine in Soya Fluff 200W and tyrosine in Soya Fluff 200T and Promosoy 100. Destruction of threonine and tyrosine during acid hydrolysis has been documented in other food products (Davies and Thomas, 1973; Kohler and Palter, 1967). Such release patterns have conventionally been extrapolated to zero time and the intercept value reported. Although arguments may be raised against this procedure, extrapolation was used to determine the values for amino acids having negative linear regression slopes in this study. In most cases the slopes were so small that little error was considered to be involved.

The third pattern of amino acid release found for soy products was that in which the measured amino acid content increased with length of hydrolysis (Figure 27). Patterns of this type were found for isoleucine in all products, leucine in all products except Promosoy 100, and valine in all products except Soya Fluff 200T. Increasing measured amino acid content with increasing length of hydrolysis time was also found for tyrosine in Soya Fluff 200W,

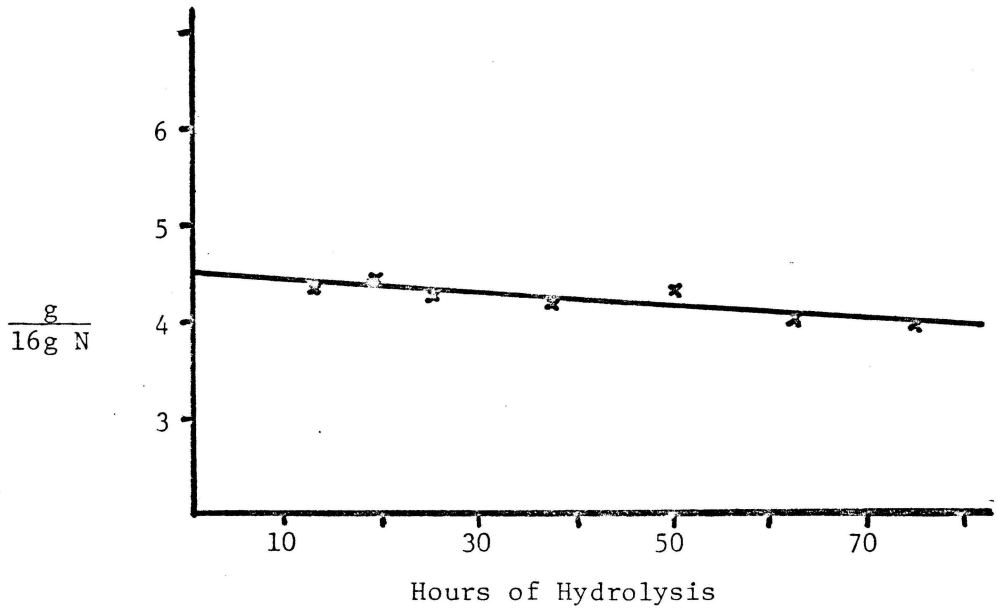


Figure 26. Average measured threonine content of Soya Fluff 200T as a function of hydrolysis time (X); line calculated by linear regression.

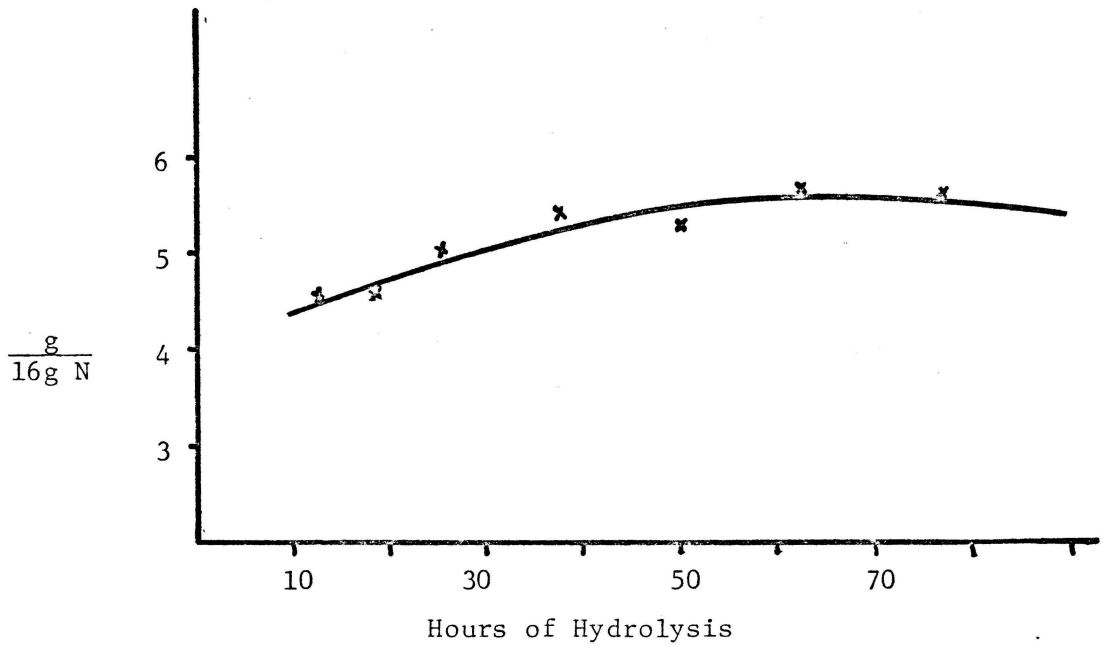


Figure 27. Average measured valine content of Promine D as a function of hydrolysis time (X); curve calculated by curvilinear regression.

Promine D and F, for phenylalanine in Soya Fluff 200T, Promine D and F, and for lysine in Promine D and F. Similar release patterns for valine, isoleucine and leucine have been reported in many protein products (Davies and Thomas, 1973; Kohler and Palter, 1967). Data of this type presents difficulties in selection of the 'best' value for the amino acid content. Frequently the highest average experimental value or 72 hour average has been reported (Tkachuk and Irving, 1969). This method has several apparent inadequacies, including the fact that the time at which the maximum value occurs may not correspond with the measured hydrolysis times. Therefore, data obtained in this research were analyzed by curvilinear regression techniques (Figure 27). For cases which exhibited increasing measured amino acid values with time, predicted maximum values obtained from curvilinear regression analysis were considered to be the 'best' reportable values for the amino acid content of the soy products.

Based on the above three considerations, Table 16 presents the 'best' values for the amino acid composition of the five processed soy products. Also included are values for the non-essential amino acids which were determined by acid hydrolysis from 12 to 72 hours and subjected to curvilinear or linear regression analysis. A 'best' value for methionine is not included because extrapolated values were lower than those determined by performic acid oxidation (Table 9), which is considered the more reliable method for methionine analysis (Blackburn, 1968).

Table 17 presents the essential amino acid content of the five processed soy products as reported by the manufacturer. Extrapolated

Table 16. Extrapolated, linear average or maximum predicted values for amino acid content (g/16g N) of processed soy products.

AMINO ACID	Soya Fluff 200W	Soya Fluff 200T	Promosoy 100	Promine F	Promine D
Thr	4.4[a]	4.1[a]	4.3[a]	4.0[a]	4.1[a]
Val	5.7[c]	4.7[b]	5.2[c]	5.4[c]	5.4[c]
Ile	5.1[c]	5.0[c]	5.0[c]	5.1[c]	5.3[c]
Leu	8.1[c]	7.9[c]	7.9[b]	8.3[c]	8.5[c]
Tyr	3.5[c]	3.9[a]	3.6[a]	3.7[c]	4.2[c]
Phe	5.3[a]	5.4[c]	5.1[b]	5.6[c]	6.2[c]
Lys	6.6[a]	6.6[b]	6.8[b]	6.4[c]	6.9[c]
Asp	13.8[c]	13.0[c]	11.1[b]	13.9[b]	13.3[b]
Ser	5.4[a]	4.5[a]	5.2[a]	5.3[a]	6.1[c]
Glu	18.9[b]	19.4[b]	18.6[b]	19.9[b]	20.6[b]
Gly	4.3[b]	4.1[c]	4.1[a]	4.0[c]	4.2[b]
Ala	4.5[c]	4.9[b]	4.5[b]	3.9[b]	4.5[b]
His	4.2[c]	3.8[b]	3.8[b]	3.6[b]	4.3[b]

[a]Values extrapolated to zero time.

[b]Slopes zero; values represent average of all hydrolysis times.

[c]Maximum predicted value from curvilinear regression.

Table 17. Reported essential amino acid content (g/16g N) of processed soy products.[a]

AMINO ACID	Soya Fluff 200W	Soya Fluff 200T	Promosoy 100	Promine F	Promine D
Thr	4.0	4.0	4.3	3.5	3.6
Val	4.8	4.8	4.8	4.6	4.5
Ile	4.4	4.4	4.6	4.5	4.6
Leu	7.7	7.7	7.9	7.8	7.8
Tyr	3.6	3.6	3.8	3.5	3.6
Phe	5.1	5.1	5.1	5.2	5.2
Lys	6.3	6.3	6.3	6.0	5.7
Try	1.4	1.4	1.5	1.1	1.1

[a]Central Soya, Chicago, Illinois.

or maximum values derived from the time study differed by 10-20% from the manufacturer's reported data in many cases. Notable differences between measured and manufacturer's values occurred for isoleucine in all products and valine in all products except Soya Fluff 200T and Promosoy 100.

Statistical comparison of experimental 24 hour average values, predicted maximum values, predicted 24 hour values, and manufacturer's reported values for release patterns described by curvilinear regression are presented in Table 18. Generally, most of the significant differences occurred in soy isolates.

Nitrogen recovery from amino acid analysis was calculated as illustrated for Promine F in Table 19. Percent nitrogen recovery for all products is given in Table 20.

Table 18. Statistical comparison of predicted maximum predicted 24 hour, experimental 24 hour and manufacturer's reported values for essential amino acid content of processed soy products.[a,b]

Amino Acid	Soya Fluff 200W				Soya Fluff 200T				Promosoy 100				Promine F				Promine D			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Val	ns	***	***	ns	ns	ns	ns	ns	***	ns	ns	ns	***	***	***	*	***	**	***	**
Ile	ns	*	***	ns	ns	ns	**	***	*	*	ns	ns	***	ns	**	ns	***	*	**	ns
Leu	ns	ns	ns	ns	***	*	ns	ns	ns	ns	**	***	***	ns	ns	ns	ns	ns	***	***
Tyr	ns	ns	ns	ns	ns	ns	ns	ns	--	--	--	--	***	ns	ns	*	**	*	***	ns
Phe	--	--	--	--	ns	ns	ns	*	--	--	--	--	***	ns	ns	**	***	ns	***	***
Lys	--	--	--	--	ns	ns	ns	ns	ns	ns	**	***	ns	ns	ns	ns	***	ns	***	***

[a] *** significant at $\alpha = 0.01$.
** significant at $\alpha = 0.05$
* significant at $\alpha = 0.10$

[b] Comparisons include T_1 Predicted maximum value versus predicted 24 hour value.
T_2 Predicted maximum value versus average measured 24 hour value.
T_3 Predicted maximum value versus manufacturer's reported value.
T_4 Average measured 24 hour value versus manufacturer's reported value.

Table 19. Calculation of percent nitrogen recovery for Promine F.

Amino Acid	MW	%N[a]	$\frac{gAA}{16\ gN}$	$\frac{gN}{16\ gN}$
Thr	119.1	11.76	3.9	.46
Val	117.1	11.96	5.4	.65
Ile	131.2	10.68	5.0	.54
Leu	131.2	10.67	8.3	.89
Tyr	181.2	7.73	3.7	.29
Phe	165.2	8.48	5.6	.47
Lys	146.7	19.16	6.4	1.23
1/2 Cys[b]	120.2	11.66	1.3	.15
Met[b]	149.2	9.39	1.3	.12
Asp	133.1	10.52	13.9	1.46
Ser	105.1	13.33	5.3	.71
Glu	165.1	9.52	19.9	1.89
Gly	75.1	18.66	4.0	.75
Ala	89.1	15.72	3.9	.61
His	209.6	27.08	3.6	.97
Pro[c]	115.1	12.17	5.4	.65
Arg[c]	210.7	32.16	7.8	2.50
Try[c]	204.2	13.72	1.2	.16
TOTAL				14.5
%				90.7

[a]Merck Index (Stecher, 1960).

[b]Determined by performic acid oxidation (Moore, 1963).

[c]Manufacturer literature, Central Soya, Chicago, Illinois.

Table 20. Percentage of recovered nitrogen from amino acid determination of processed soy products.[a]

Product	% N
Soya Fluff 200W	92.6
Soya Fluff 200T	90.0
Promosoy 100	82.6
Promine F	90.7
Promine D	94.0

[a]See Table 19 for an example of calculations.

DISCUSSION

In recent years commercial procedures have been developed to process soybeans into a wide variety of products having unique functional properties and high protein content. Characterization of the effects of processing on the proteins of these products is necessary for further development and improvement of processing procedures and for adequate understanding and enhancement of the nutritional quality of these products.

Numerous experiments have demonstrated that the protein quality of soy products, as measured by rat bioassay, decreases with increased processing (Huge, 1961; Bressani, 1975; Central Soya, 1975). Comparison of the protein content of products analyzed in this study and their PER's reported by their manufacturers (Table 1) indicates that products with higher total protein quantity generally had lower reported PER's. Thus, this series of studies was carried out in order to investigate changes in chemical and molecular properties of the products resulting from processing, and to investigate how these properties relate to reported nutritional quality.

Solubility Studies

Nitrogen solubility studies were conducted in order to characterize changes resulting from processing, to investigate causes of protein insolubility such as hydrophobic or disulfide interactions, and to solubilize maximum protein for further characterization by other methods. Potassium phosphate buffer, pH 7.6, 0.5μ (Briggs and Wolf, 1957), referred to as standard buffer, was used in many

of the studies because of its extensive use in many published studies on soy protein (Wolf, 1972).

Textured soy products were found to have relatively low nitrogen solubility in water (Table 2). This was not unexpected since heat has been reported to reduce the solubility of native soy meal (Belter and Smith, 1952). Cumming et al. (1973) also reported that textured and extruded soy had low protein solubility relative to soy meal. However, although nitrogen solubility of textured soy products was increased by the addition of denaturing agents (sodium dodecyl sulfate) or disulfide bond reducing agents (2-mercaptoethanol) (Table 2), the increase was not as great as had been anticipated. Chiang and Sternberg (1974) reported a 90% increase in nitrogen solubility when spun soy fibers were extracted in phosphate buffer containing mercaptoethanol as compared to extraction in phosphate buffer alone.

Sodium dodecyl sulfate was used as a denaturing agent rather than urea since extracts containing urea required dialysis to remove urea prior to nitrogen determination. SDS, however, may present solubility problems when used with potassium phosphate buffers since potassium dodecyl sulfate is much less soluble than SDS (Boatman, 1976), although no solubility difficulties were observed using SDS in potassium phosphate. In addition, SDS may promote protein hydrophobic interactions and protein insolubilization when a high ionic strength extraction media is used (Boatman, 1976).

The results of this study indicate that processing soy into textured products renders a large amount of the protein insoluble.

Some of this insolubility can be attributed to disulfide formation since extractability was increased by the addition of mercaptoethanol, a disulfide bond cleaving agent, to the extraction solvent. However, at most approximately 20% (based on 60 ml solvent recovery for Ultra Soy extracted in buffer containing SDS and MCE) of the protein in textured soy could be solubilized by these agents, whereas 80% of the proteins in raw soy meal have been reported to be solubilized by the same agents, although not in the presence of SDS (Nash et al., 1974). Thus, processing of textured soy may have caused the formation of very large insoluble polymers having inaccessible disulfide bonds, or may have had some other denaturation affect which caused insolubilization of the proteins.

Unexpected results were also obtained from raw soy meal nitrogen solubility studies (Table 2). Nitrogen extractability was lower in water than has been reported (Nash et al., 1974) and may result from the use of fullfat soybeans in this study as opposed to defatted beans in the study by Nash et al. (1974). The addition of sodium lauryl sulfate (SDS) to standard buffer was expected to increase nitrogen solubility through disruption of hydrophobic interactions. However, standard buffer containing SDS or standard buffer containing both SDS and 2-mercaptoethanol decreased the nitrogen extractability of soy meal compared to extractability by standard buffer alone.

In an attempt to explain this solubility effect, soy meal was extracted in standard buffer without 0.4M NaCl but containing 0.5% SDS. This solvent extracted 1.97 mg N/ml compared with 0.73 mg N/ml extracted with standard buffer containing both SDS and NaCl and 1.90

mg N/ml extracted with standard buffer containing NaCl but without SDS. Thus, the reduction in solubility must be attributed to an effect of SDS in high ionic strength buffer (Boatman, 1976). It is notable that a pronounced salt-SDS effect did not occur in the textured soy products.

Solubility studies were also conducted on autoclaved soy samples in order to compare the effects of heat treatment with the effects of the texturizing process on soy proteins. In addition, the relationship between protein solubility and nutritional value was investigated. Klose et al. (1948) determined the effect of autoclaving on the PER of soy meal (Figure 28). Heat treatment for over 15-20 minutes resulted in decreased nutritional value.

Nitrogen solubility of autoclaved soy meal was found to decrease with increased length of time autoclaved up to 20 minutes (Figure 3). Nitrogen solubility in water and in buffer containing mercaptoethanol extracts were similar. This may indicate the formation of protein aggregates which have inaccessible disulfide bonds or some other type of strong bonding forces similar to those proposed to occur in textured products. Extractability of the autoclaved soy was much less than that of the textured soy, since a maximum of only 5% of the total protein (N x 6.25, assuming 60 ml extract) of soy autoclaved for 15 minutes could be extracted by water as compared with 10-14% extractability of the textured products in water.

Soy meal autoclaved for 10 minutes (properly heated) and for 20 minutes (overheated) had similar nitrogen solubility (Figure 3), and thus nitrogen solubility in these solvents had no apparent

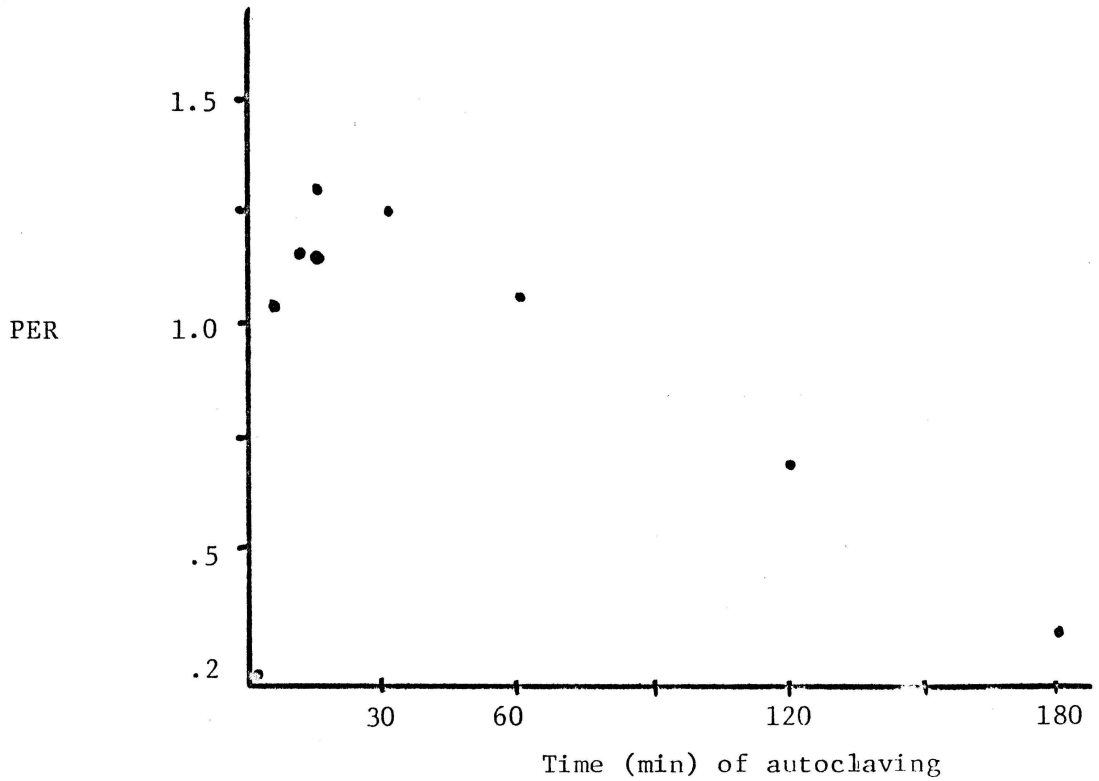


Figure 28. Effect of autoclaving at 15 psi on the protein efficiency ratio of ground, whole soybeans (Kose et al., 1948).

relationship to the nutritional value of soy meal autoclaved up to 20 minutes. Balloun et al. (1953) reported that the nitrogen solubility of autoclaved soy meal decreased until 20 minutes of treatment, and then remained unchanged. They suggested, therefore, that protein solubility be used to indicate overheating of soy meal. Results of this study suggest that such a correlation cannot be made since the solubility of the properly heated and overheated soy samples differed only slightly.

Table 21 presents the nitrogen solubility data of soy flours, concentrate and isolates expressed as a percentage of the total protein content (N x 6.25, assuming 60 ml extract). Soya Fluff 200W changed least in nitrogen extractability with different extraction media and had the greatest extractability in water and standard buffer compared to all other products. This suggests that these proteins were less denatured and contained fewer hydrophobic or disulfide interactions than the other products, all of which had received comparatively greater processing treatment than did Soya Fluff 200W. The increased nitrogen extractability when the product was extracted in standard buffer containing mercaptoethanol is in agreement with the results of Nash et al. (1974) who concluded that some disulfide bonds were naturally present or resulted from the extraction process of soy meal. The lower nitrogen solubility in all solvents of Soya Fluff 200T as compared with 200W can be attributed to the toasting process given to this flour. Part of this insolubility was reversed by the addition of mercaptoethanol to the standard buffer, which suggests the presence of some new disulfide bonds resulting from

Table 21. Percent protein (N x 6.25) extracted from processed soy flours, concentrate and isolates.[a]

PRODUCT	EXTRACTION MEDIA			
	H_2O	SB[b]	SB + 0.1M MCE[b]	SB + 0.1M MCE[b] 8M UREA
Soya Fluff 200W	59.3	59.5	73.1	87.3
Soya Fluff 200T	20.2	28.2	49.5	75.3
Promosoy 100	5.6	5.5	11.0	76.4
Promine F	48.0	18.1	34.5	81.5
Promine D	51.7	28.9	60.4	88.8

[a]Calculated by assuming 60 ml of extract.

[b]SB indicates standard buffer, pH 7.6 potassium phosphate, 0.5μ (Briggs and Wolf, 1957); MCE indicates 2-mercaptoethanol.

processing. When urea and mercaptoethanol were present in the extraction media, the proteins of Soya Fluff 200T were nearly as soluble as those of Soya Fluff 200W. The increased solubility resulting from the addition of urea indicates that the protein insolubility involved hydrophobic interactions as well as disulfide interactions. This is similar to results of solubility studies of textured soy and autoclaved soy meal, in which the heat treatment was proposed to have also caused hydrophobic and disulfide interactions resulting in protein insolubility. The proteins of soy flours, however, were much more soluble than the textured or autoclaved soy meal, regardless of extraction solvent.

The two soy isolates, Promine D and F, had very low protein solubility in standard buffer (Table 21). When both urea and mercaptoethanol were present in the extraction media, the percentage of protein solubilized equalled that of Soya Fluff 200W. Mercaptoethanol alone in the extraction buffer increased solubility over that in standard buffer alone but did not solubilize as much protein as when both urea and mercaptoethanol were present. This indicates that hydrophobic interactions, which could be disrupted by urea, contributed to the insolubility of these processed products. Relative importance of these hydrophobic interactions versus disulfide interactions to the protein solubility of these products cannot be determined from data available. Extraction in standard buffer containing urea alone could help to answer this question.

Nitrogen solubility data for soy isolates are in agreement with the data presented by Kelley and Pressey (1966) in studies on the

extractability of soy fibers in model systems. They found that mild alkaline treatment during processing caused protein unfolding and disulfide-sulfhydryl interchange reactions. Protein unfolding was thought to allow the proteins to form new hydrogen or ionic bonds and thus form aggregates.

The soy concentrate, Promosoy 100, had extremely low protein solubility in all buffers except that containing both urea and mercaptoethanol. Unlike the soy flours and textured soy, the proteins of this product were precipitated by acid at pH 4.5 during the manufacturing process (Alden, 1975). This precipitation step apparently causes the soy proteins to aggregate and form strong hydrophobic and disulfide bonds which can be solubilized only by the addition of urea and mercaptoethanol to the extraction solvent. Hydrophobic bonds appear to be more important than disulfide bonds in this case, since the major solubility increase occurs in the presence of urea. Although the isolates also undergo acid precipitation during processing, the soy proteins are first dissolved in an alkali solution (Alden, 1975). This alkaline treatment apparently prevents extensive protein aggregation during the subsequent precipitation process, possibly through cleavage of the protein molecules or by some change within the protein molecules rendering them less able to aggregate through hydrophobic interactions.

Plots of percent protein extractability versus manufacturer's PER's for processed soy flours, concentrate, isolates and textured soy protein are presented in Figures 29-32. When extracted in water, standard buffer or standard buffer containing mercaptoethanol, a large change in protein solubility occurred without a change in PER for soy

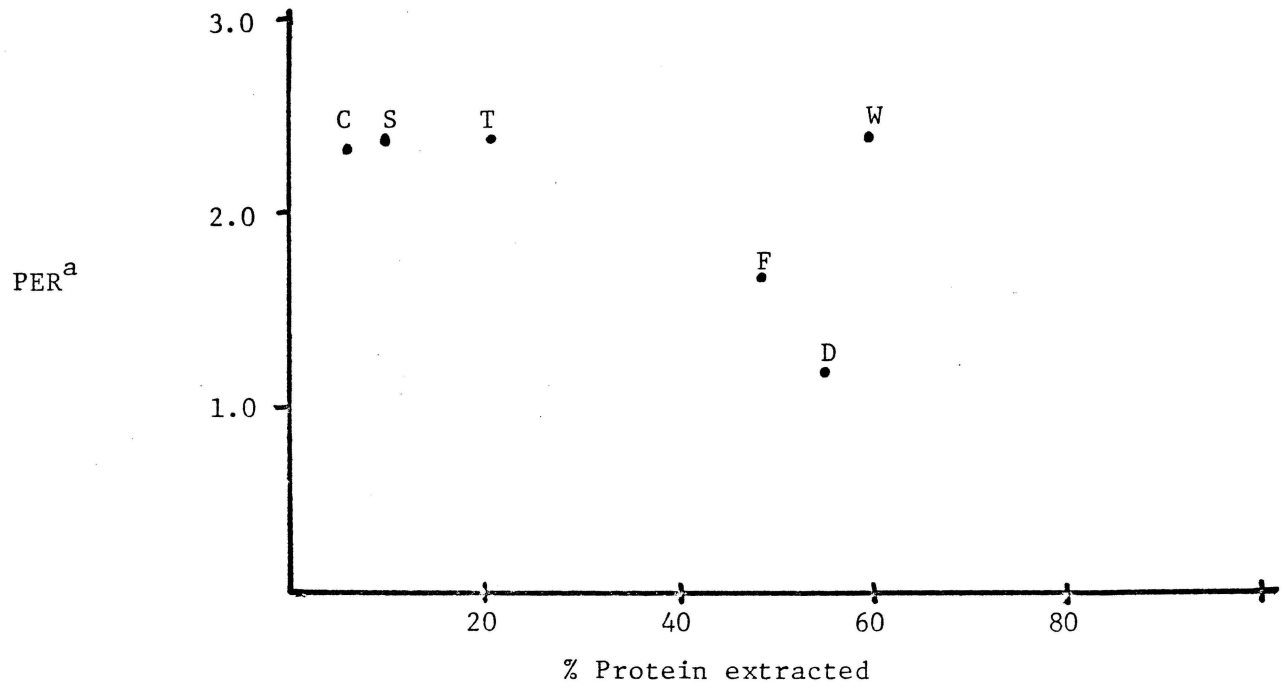


Figure 29. Percent protein solubility and reported PER[a] of processed soy products extracted in water.[b]

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[a]Central Soya, Chicago, Illinois; Wolf and Cowan (1975).

[b]W = Soya Fluff 200W; T = Soya Fluff 200T; C = Promosoy 100; S = Ultra Soy; F = Promine F; and D = Promine D.

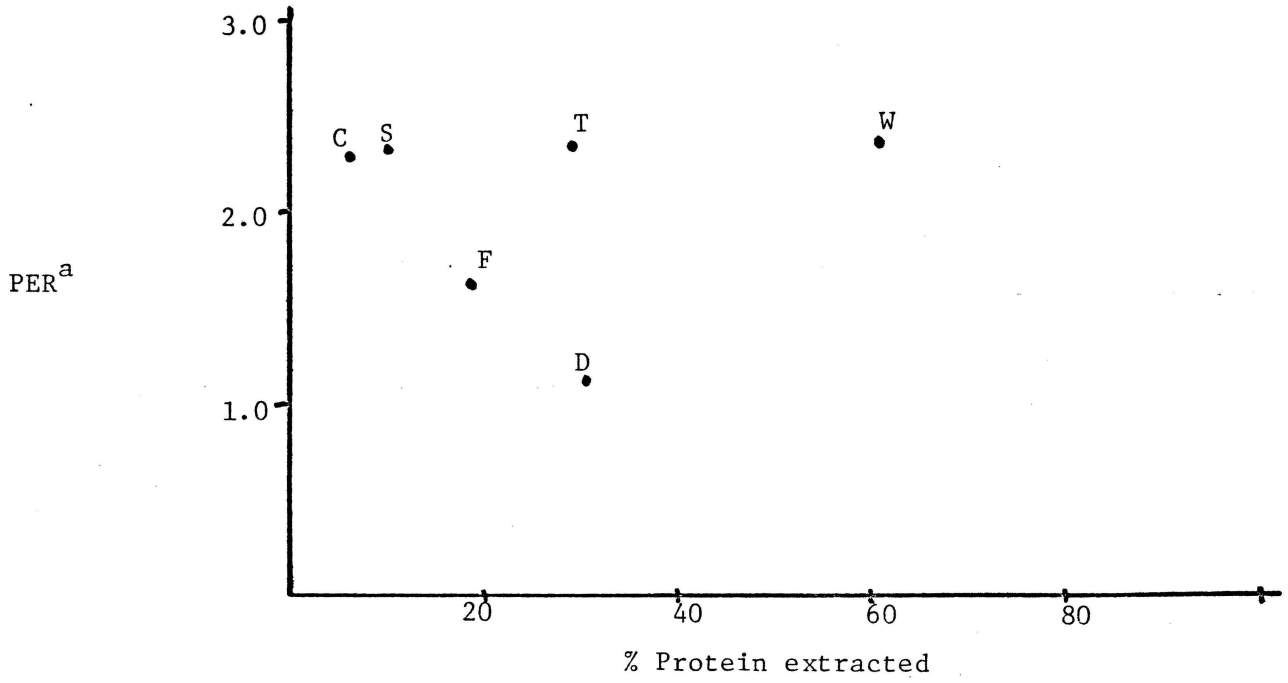


Figure 30. Percent protein solubility and reported PER[a] of processed soy products extracted in pH 7.6 potassium phosphate buffer, 0.5μ.[b]

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[a]Central Soya, Chicago, Illinois; Wolf and Cowan (1975).

[b]W = Soya Fluff 200W; T = Soya Fluff 200T; C = Promosoy 100; S = Ultra Soy; F = Promine F; and D = Promine D.

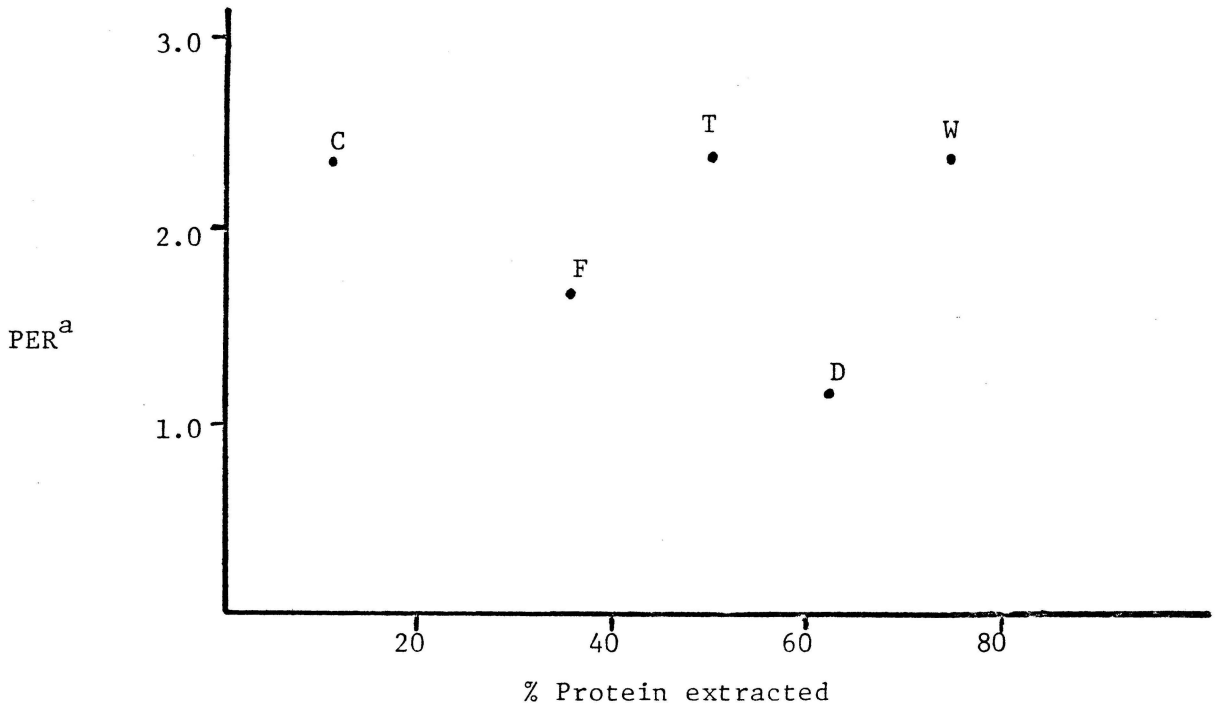


Figure 31. Percent protein solubility and manufacturer's PER's[a] of processed soy products extracted in pH 7.6 potassium phosphate buffer, 0.5μ, containing 0.1M mercaptoethanol.[b]

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[a]Central Soya, Chicago, Illinois.

[b]W = Soya Fluff 200W; T = Soya Fluff 200T; C = Promosoy 100; F = Promine F; and D = Promine D.

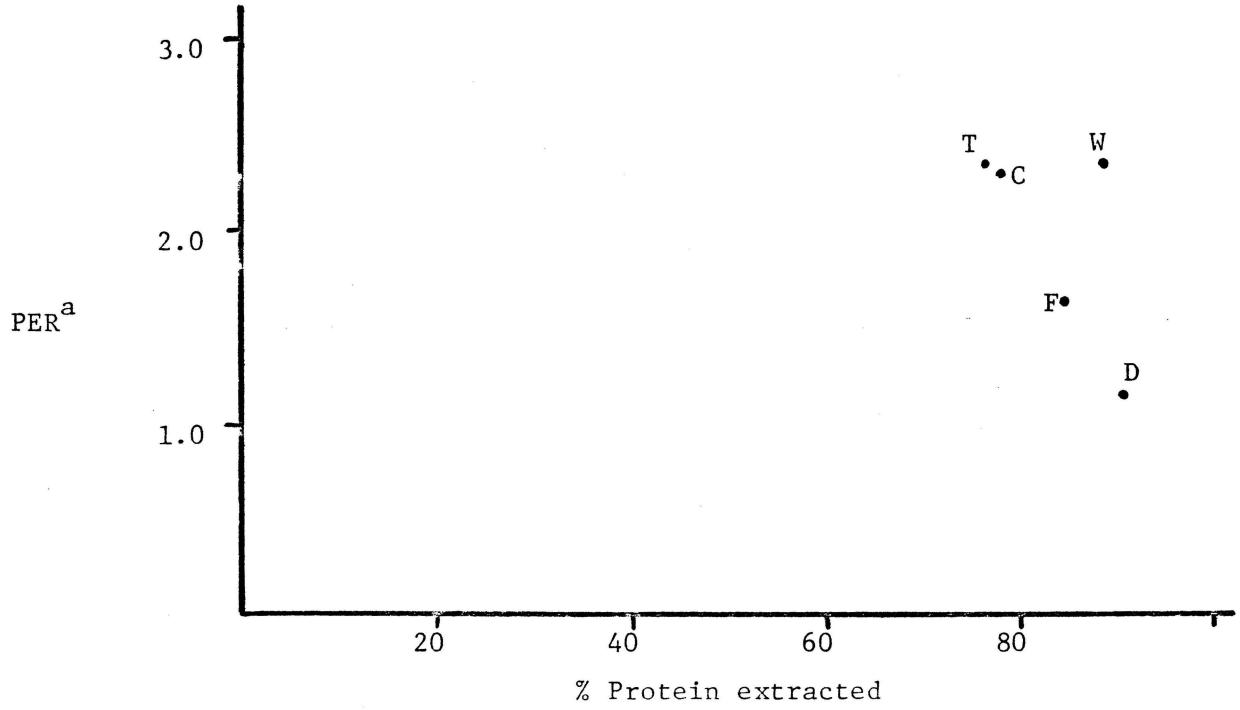


Figure 32. Percent protein solubility and manufacturer's PER[a] of processed soy products extracted in pH 7.6 potassium phosphate buffer, 0.5μ, containing 0.1M mercaptoethanol and 8M urea.[b]

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[a]Central Soya, Chicago, Illinois.

[b]W = Soya Fluff 200W; T = Soya Fluff 200T; C = Promosoy 100; F = Promine F; and D = Promine D.

flours, textured soy and soy concentrate. With the exception of the concentrate, the processing of these products mainly involves heat treatment. Protein solubility apparently has no relationship to the protein nutritional value (PER's, Table 1) of this series of products. In contrast, the PER decreased linearly with increased solubility of processed soy concentrate and islates. As stated previously, these products had undergone more extensive processing involving acid or alkali treatment. This data supports the observation of Bressani (1975) that PER increased with increased insolubility of the proteins.

The nitrogen solubility of the five processed soy products did not vary greatly when products were extracted in standard buffer containing mercaptoethanol and urea (Figures 32).

Protein Molecular Weight Characterization

Column Chromatography

Gel filtration using Sephadex G-200 was used to separate and characterize the water extractable proteins from raw soy meal and from three textured soy products. Sephadex G-200 fractionates proteins in the 600,000-5,000 molecular weight range, allowing them to be eluted in decreasing order of molecular weight (Pharmacia, 1975). Although extractable proteins from the three textured products could not be differentiated by this technique, differences between raw soy meal and textured soy were apparent (Figures 4 and 5). However, since only 5-10% of the textured soy protein could be extracted by water, these results may or may not be indicative of changes in the proteins as a whole.

Peak B of raw soy meal apparently represents the majority of the soy proteins as determined by the Lowry protein test (Figure 8). Fractions from textured soy eluted at the same time as Peak B from soy meal apparently contained little protein, however (Figure 7). Highest Lowery protein absorbance occurred in peaks C, D and E in textured soy (Figure 7). Peaks C and D of textured soy were much less clearly separated than the corresponding peaks from raw soy meal. These results suggest that processing of textured soy has broken some of the proteins into lower molecular weight (more slowly eluted) proteins. These observations are supported by UV maximum absorbance data for peak fractions (Table 4). Tyrosine and tryptophan absorb light strongly at 280 nm while nucleic acids have maximum absorbance at 260 nm (Lehninger, 1970). Peak B of soy meal had maximum absorbance at 275 nm whereas later eluting peaks absorbed at 264-268 nm. Thus Peak B apparently contained protein whereas later eluting peaks probably contained large amounts of nucleic acids or other non-protein substances which absorb at 260 nm. In textured soy, peak E had absorbance maximum at 273-280 nm, which suggests that it contained large amounts of protein.

Obara and Kimura (1967) reported that peak B of water extracted soy proteins consisted mainly of 11S proteins as well as some 7S and 15S proteins. The S designation refers to the Svedberg unit, 1 x 10^{-13} sec, which is a measure of the ultracentrifugal sedimentation rate (Lehninger, 1970). The disappearance of peak B in the textured products can be partially explained by the known properties of the 7, 11 and 15S proteins. Heat and pH extremes have been reported to

cause disruption of the quaternary structure of these proteins and cause cleavage into numerous subunits (Wolf, 1972). Many of these changes are believed to involve disulfide bond formation and destruction. Wolf and Tamura (1969) heated 11S protein at 100°C and found that it resulted in 3-4S fractions. It is expected that numerous additional reactions would occur when 11S protein is heated in the presence of other water-extractable soy proteins (Wolf, 1972). Thus, results of this study indicating the conversion of some higher molecular weight proteins to lower molecular weights during processing of textured soy protein are quite understandable.

Gel Electrophoresis

Gel electrophoresis of extractable proteins of processed soy products was carried out in order to further characterize changes at the molecular level and to identify protein components involved in processing changes. Initial studies were conducted using a Tris-HCl separation system (Appendix 3) since preliminary studies indicated this system to give sensitive separation of soy meal proteins in a short time (less than one hour). However, neither water nor buffer extracts of three different textured soy proteins could be differentiated by the gel patterns they produced. Water and buffer extracts of textured soy products were clearly different from unprocessed soy, however (Figures 7 and 10). It appeared that proteins of textured soy underwent some degradation, resulting in the formation of extractable proteins of different molecular weight than those of raw soy meal. Further evidence of the presence of disulfide bonding in textured products

was provided by the extracts in Tris-glycine containing 0.1M mercaptoethanol, since these extracts separated into more protein peaks than did water extracts (Figure 12). Autoclaved soy meal extracts also separated into fewer distinct peaks in the faster moving regions than did native soy meal. As with textured extracts, autoclaved extracts in mercaptoethanol yielded more protein peaks than did water extracts. These results support conclusions from solubility data that disulfide bonding occurred during heat treatment of soy meal.

Sodium dodecyl sulfate (SDS) electrophoresis was also used to separate extractable proteins in an attempt to differentiate textured soy products and to provide a molecular weight estimation of proteins extracted. It was apparent from results that the texturization process caused changes in extractable protein molecular weight, especially aggregation to higher molecular weight (unextractable proteins) and disintegration to lower molecular weight (27,000-8,500) proteins (Figure 17). The increased extractability of textured products in buffer containing mercaptoethanol was reflected in the greater number of protein peaks separated (Figure 20). Results were very similar to those reported by Cumming et al. (1973) for water extracted textured soy proteins.

Several reports have been published estimating the molecular weight of the basic subunit of the 11S soybean protein. Catsimpoolas et al. (1967) claimed the subunit to have a molecular weight of 26,000. Okabo et al. (1969) claimed the 11S subunit to be 36,000 while Cumming et al. (1973) reported it to be 28,000. Separation of extractable

textured soy proteins in this study resulted in a major peak at 27,000 MW.

Attempts to differentiate extractable proteins from the processed soy flours, isolates and concentrate using an SDS discontinuous buffer electrophoresis system were unsuccessful since no difference between extracts was apparent (Figure 24). It is possible that the electrophoresis system was unable to detect differences in the extracts. However, it is also possible that the extraction medium (standard buffer containing 8M urea and 0.1M mercaptoethanol), was the least likely medium to detect differences in the protein patterns. Results of the solubility studies (Table 21) indicate that the various products differed least in percent protein solubility when extracted with standard buffer containing urea and mercaptoethanol as compared with extractability in water, standard buffer or standard buffer containing MCE. It is probable that differences would be detected in these proteins if extraction were carried out in water or standard buffer.

Sulfhydryl and Disulfide Interactions

Since results of solubility and electrophoretic analysis of textured soy products strongly suggested the involvement of disulfide bonding during processing, characterization of sulfhydryl and disulfide content of extracts of textured soy and soy meal was attempted.

Analysis of sulfhydryl (SH) and disulfide (SS) content was made immediately following protein extraction (day 1) and again 24 hours following extraction (day 2) in order to determine effects of time

after extraction on measured SS and SH. Results of the immediate analysis (day 1) will be considered first.

Ultra Soy extracted in water had significantly lower day 1 SH content than did Burger Bonus or Plus Meat (Table 5). Ultra Soy also had a significantly lower SH content than Plus Meat when extracted in buffer containing SDS. Ultra Soy probably received comparatively greater heat treatment during processing than did the other textured products (Marable, 1975). Most striking was the case of soy meal, for which no SH groups were detectable in extracts made in buffer containing SDS. As already noted in the solubility studies, buffer containing SDS extracted almost 40% less nitrogen than buffer without SDS. This effect may be due to a promotion of hydrophobic interactions and protein aggregation by the combination of high ionic strength buffer and SDS (Boatman, 1976). Since this insolubilization apparently involved the SH containing proteins, it is probable that aggregation was partially caused by SH-SS exchange reactions resulting in disulfide bond formation. Similar SH-SS exchange reactions resulting in SS bond formation have been proposed to occur in several soy protein systems (Fukushima and Van Buren, 1970a,b; Circle et al., 1964). Wolf and Briggs (1956) reported that decreased extractability of soy meal in high ionic strength solutions primarily resulted from a decreased extractability of the 11S and 15S proteins. These proteins have been reported to be especially prone to form SS polymers (Wolf, 1972).

As noted previously, the SDS-ionic strength combination had little effect on the nitrogen solubility of the textured products. Buffer

containing SDS extracted as much or more nitrogen from textured soy as did buffer alone. However, buffer containing SDS extracted less nitrogen from raw soy meal than did buffer alone. This evidence also indicates differences in the proteins of textured soy and soy meal, and suggests that the texturizing process has caused a major change in the sulfhydryl containing proteins, 11S and 7S. This is in agreement with results of column chromatographic separation of water-extractable textured proteins, which indicated a decrease in protein eluted at the 11S and 15S position.

Results for total SH (SH + 2SS) content of extractable proteins were especially interesting (Table 7). The results for textured soy products are similar to each other regardless of extraction media. However, when extracted in water, buffer or buffer plus SDS, total SH content of the textured extracts was significantly less than that of soy. When the extraction media contained mercaptoethanol, however, Plus Meat and Ultra Soy were not significantly different from soy in total SH content. Burger Bonus contained slightly fewer total SH's than did soy meal (Table 7). These results indicate that the decreased protein extractability in textured soy (with the possible exception of Burger Bonus) compared to soy involved the SH and SS-containing proteins, and probably resulted from formation of SS bonds during processing. Since the total SH level of extracted textured proteins was statistically the same as or approached that of soy when mercaptoethanol was present, it is probable that little destruction or oxidation of SH- or SS-containing proteins occurred during processing. This conclusion is based on the assumption that the extractable

proteins reflect the properties of the proteins in general. This assumption may or may not be correct. Attempts to measure SH and SS in total protein of defatted soy meal were unsuccessful due to difficulties in maintaining a suspension and resulting high turbidity.

Several problems were encountered with the SH-SS determination of extracts. The age of the soy meal was found to affect the extractability, and thus SH-SS content determined for soy meal must not be considered absolute. Such a decrease with age was also noted by Nash et al. (1964). The extraction process itself was also found to be the least reporducible step. Furthermore, time after extraction was observed to affect the determinations, and therefore a study of effect of time on extraction was undertaken.

SH content of all products extracted in all buffers, with the exception of soy in buffer containing SDS, decreased from day 1 to day 2(Tables 5, 8). This decrease could result from air oxidation of the SH's to SS's. Such an oxidation would be expected to increase the SS content by one-half the SH decrease. However, in many cases the increase in SS is not sufficient to account for the entire SH decrease (Table 6). It is probable that some SH's were oxidized to other nonreactive forms. In some cases the total SH content increased from day 1 to day 2(Table 7). This probably resulted from some further protein denaturation exposing SS or SH groups which were inaccessible on day 1. These results stress the importance of prompt measurement of SH and SS levels of extracts since many misleading and unrelated reactions may occur with time.

Sulfur Amino Acid Oxidation

Methionine content of processed soy products was measured by performic acid oxidation to methionine sulfone (Moore, 1963) and by direct determination of methionine itself following acid hydrolysis. Methionine content measured directly was found to decrease with increased time of acid hydrolysis (Tables 11-15). Average 24 hour hydrolysis values in the various products as determined the direct method ranged from 14 to 33% lower than the corresponding methionine values measured by performic acid oxidation (Table 9).

Methionine was reported to degrade into five ninhydrin-positive peaks during acid hydrolysis (Lunder, 1972) Jennings and Lewis (1968) reported that 30 to 59% of the methionine content of plant materials was lost during acid hydrolysis. Although methionine sulfone has been found to be stable to acid hydrolysis (Blackburn, 1968), methionine sulfoxide is partially converted to methionine (Floyd et al., 1963). Thus measurement of methionine following acid hydrolysis may underestimate methionine due to methionine degradation, or overestimate methionine if methionine sulfoxide is present and undergoes degradation. Results from this study indicate that direct determination of methionine probably underestimates the total methionine content of processed soy proteins, considering all oxidation states, since methionine sulfoxide was only present in one soy isolate.

The performic acid oxidation method does not distinguish between methionine, methionine sulfoxide and methionine sulfone or between cystine and cysteic acid (Blackburn, 1968). Reported values of 'methionine' or 'cystine' measured by this method thus actually

reflect the sums of the oxidized and unoxidized forms. Therefore, determination of methionine and cystine in processed proteins by performic acid oxidation probably results in overestimation of the actual values. Performic acid oxidation is commonly utilized for measurement of 'cystine', while methionine is generally measured by direct determination following acid hydrolysis (Central Soya, 1975).

The total sulfur amino acid content, as determined by performic acid oxidation, of processed soy products indicated only minor differences between products (Table 9). The 'methionine' and 'cystine' contents of the processed soy isolates were slightly lower than those of other products. This finding was not unexpected since numerous reports have documented some sulfur amino acid destruction during isolation of soy proteins (Rios Iriarte and Barnes, 1966).

Measured total sulfur amino acid content of the processed soy products did not correlate with reported PER's of the products. Promine D and F had identical cystine and methionine values, but have PER's of 1.1 and 1.6 respectively (Table 1). This is not surprising since although sulfur amino acids are most limiting in soy protein, many studies have failed to find correlation between total sulfur amino acid content and nutritional value (Liener, 1976). For this reason, the possibility of some of the sulfur amino acids existing in oxidized and thus possibly unavailable forms was investigated.

All processed soy products were found to contain cysteic acid (Table 9). The greatest amount of cysteic acid (14.3 and 23.2% of the total 1/2 cystine content) was found in the two soy isolates.

These two products underwent relatively more processing treatment than the other products, and unlike the other products, these isolates were subjected to alkaline treatment during processing (Alden, 1975; Central Soya, 1975). Andersson (1970) proposed a mechanism by which disulfide groups are cleaved by mild alkali to form an unstable sulfenic acid group (-SOH) which may then undergo atmospheric oxidation to sulfinic (SO_2H) or sulfonic (SO_3H) acid (Figure 33). Andersson (1970) cautioned that this reaction could occur in chemically modified or denatured proteins in which the stable, native structure had been destroyed. Solubility studies discussed previously indicated denaturation and modification of the isolated soy proteins, and thus the formation of cysteic acid by the above mechanism is possible. In addition, cleaving of disulfide bonds during the formation of cysteic acid by such a mechanism would be expected to result in increased protein solubility. Solubility studies demonstrated increased protein solubility in the processed soy isolates as compared with the concentrate and the most highly processed flour (Table 21).

Strong alkaline treatment of cystine has been found to result in the formation of dehydroalanine, and the proposed mechanism also involves the formation of an unstable sulfenic acid group (Cecil and McPhee, 1959). In the presence of lysine, the dehydroalanine formed can undergo further reactions to form lysinoalanine (Bohak, 1974). DeGroot and Slump (1969) detected lysinoalanine in alkali-treated soy proteins. In light of these studies, therefore, the presence of cysteic acid in processed soy isolates is not surprising

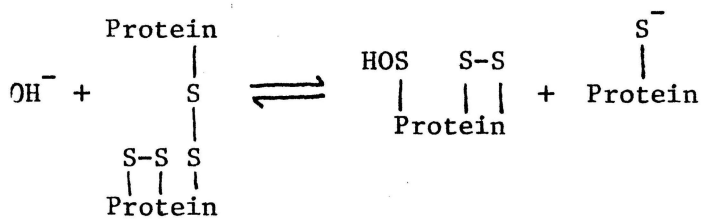


Figure 33. Proposed mechanism for hydrolytic splitting of disulfide bonds and the formation of sulfenic acid (Andersson, 1970).

since the first step leading to both lysinoalanine and cysteic acid involves the formation of an unstable sulfenic acid group. It should be noted that cysteic acid has been shown to be completely unavailable to rats and chicks (Anderson et al., 1976; Kuzmicky et al., 1974), and since the sulfur amino acids are most limiting in soy products, the formation of even small amounts of cysteic acid from cystine could be of nutritional significance.

None of the processed soy products were found to contain methionine sulfone. Cuq et al. (1973) did not detect methionine sulfone in H_2O_2-treated casein. More severe oxidizing conditions must be needed to produce methionine sulfone.

Difficulties were encountered with the determination of methionine sulfoxide in soy products. Two methods, barium hydroxide hydrolysis and sodium hydroxide hydrolysis, were investigated. Treatment with barium hydroxide was found to result in low and variable recovery of methionine sulfoxide and aspartic acid. Problems with low recovery of methionine sulfoxide after alkaline hydrolysis were suggested by Neumann (1967). Low tryptophan recovery has been noted in some alkaline-hydrolyzed samples (Miller, 1967; Hugli and Moore , 1972). Miller (1967) reported that, although precipitation of barium with either carbon dioxide or sulfuric acid resulted in low tryptophan recovery, prior neutralization of the solution with hydrochloric acid prevented these losses. The low recoveries of tryptophan were attributed to amino acid binding by the precipitated barium carbonate; and it is probable that methionine sulfoxide was similarly lost.

Methionine sulfoxide recoveries using a sodium hydroxide hydrolysis procedure were also low, but were very reproducible. Therefore, determination of 19-84 μmoles methionine sulfoxide per gram protein in Promine D obviously must not be considered an absolute content. It is a demonstration of the presence of a significant quantity of methionine sulfoxide in this sample. Because of the low recovery of free methionine sulfoxide, discussed in Results, it is probable that the measured content of this amino acid in protein samples, calculated on the basis of 100% recoveries, is an underestimation. Furthermore, although no methionine sulfoxide was measured in any of the other processed soy products, it is possible that some does exist but at a level undetectable by the analytical procedure used in this study. Although the ability of humans to utilize methionine sulfoxide is still subject to question, it is agreed that this oxidized form of methionine is less well utilized than methionine in other species (Anderson et al., 1976). Therefore, further improvement of the methodology for the determination of methionine sulfoxide in proteins as well as further efforts to analyze for this amino acid in processed soy proteins seems justified.

Assuming that both methionine sulfoxide and cysteic acid are completely unavailable to humans as sulfur amino acid sources, Table 22 presents the 'available' and the total sulfur amino acid content of each of the processed soy products. Lowest sulfur availability, 71%, occurred in Promine D, an isolate which also had the lowest PER relative to the other products. Methionine sulfoxide content of this product was assumed to be 31 μmoles per gram protein for this

Table 22. Total[a] and available[b] sulfur amino acid content (μmoles/g protein) and percent available sulfur amino acids of processed soy products.

	Total	Available	% Available
Soya Fluff 200W	227	221	97.4
Soya Fluff 200T	210	205	97.6
Promosoy 100	219	215	98.2
Promine F	195	180	92.3
Promine D	195	139	71.3
Burger Bonus	220	214	97.3
Ultra Soy	227	220	96.9
Plus Meat	214	207	96.7

[a]Determined by performic acid oxidation.

[b]Performic acid oxidation content less cysteic acid and methionine sulfoxide.

calculation. Textured products, flours and isolates had only slightly decreased availability. These results support the conclusion drawn from the sulfhydryl and disulfide analysis of extracted textured soy proteins, which indicated that little or no sulfur oxidation to cysteic acid in extractable proteins had occurred during processing.

Chemical scores of five processed products were calculated and are presented in Table 23. Scores were based on methionine + half-cystine content since the ratio of these amino acids to total essential amino acid content of soy products as compared to that of egg protein was lower than the corresponding ratio of any other essential amino acids. When based on manufacturer's reported sulfur amino acid content or that determined by performic acid oxidation, ratios of all products were similar, despite their very different PER's. When chemical scores were based on sulfur amino acids corrected for cysteic acid and methionine sulfoxide, products having different PER's also had clearly different chemical scores. Correlation coefficients for PER values and chemical scores calculated from this study were 0.995, 0.831 and 0.934 for scores based on manufacturer's data, performic acid analysis, and 'available' sulfur, respectively. Although PER's and chemical scores based on data reported by the manufacturer correlated well, these values do not correlate with those reported for other food proteins. Maize has a reported PER of 1.1 and chemical score of 41; wheat has a PER of 1.5 and chemical score of 43 (Hegsted, 1974). Thus, chemical scores based on data reported by the manufacturer as well as those based on measured methionine and cystine by performic acid oxidation, apparently overestimate the

Table 23. Manufacturer's PER's[a] and calculated chemical scores[b] of processed soy products calculated from manufacturer's data, performic acid determination, and from chemically determined available sulfur amino acid content.

Source of Sulfur Amino Acid Content	Soya Fluff 200W	Soya Fluff 200T	Promosoy 100	Promine F	Promine D
Manufacturer[c]	64	64	63	59	56
Performic Acid[d]	58	61	63	58	54
Available[e]	67	63	60	57	43
PER[a]	2.3	2.3	2.27	1.6	1.1-1.2

[a]Central Soya, Chicago, Illinois.

[b]Chemical store based on sulfur amino acids and calculated as described by Kofranyi, 1972.

[c]Manufacturer data, Central Soya, Chicago, Illinois.

[d]Total sulfur amino acid content determined by performic acid oxidation (Moore, 1963).

[e]'Available' sulfur = total sulfur from performic oxidation less cysteic acid and methionine sulfoxide.

nutritive value of these products. Chemical scores calculated from measured chemically 'available' sulfur amino acids do correlate well (0.934) with reported product PER as well as PER and chemical score values determined in other food protein products.

Correlation of 'available sulfur' chemical score values (calculated from this study) and product PER's further emphasize the importance of oxidized sulfur amino acids to the nutritional value of processed soy products. Reported failures to correlate sulfur amino acid content of processed products with their nutritional values may be attributed to the presence of such oxidized forms. In addition, the results of reported studies which replaced methionine lost during processing but failed to restore PER's to preprocessing levels (Rios Iriarte and Barnes, 1966) may also be partly attributed to the presence of unavailable sulfur amino acids.

Rate of Amino Acid Hydrolysis

Length of acid hydrolysis was found to affect the measured quantity of each essential amino acid in five processed soy products (Table 11-15). Few similarities in hydrolysis behavior other than conformity to the three previously described types of release patterns were observed among the various soy protein products. The effect of length of hydrolysis on several amino acids of Promine D is shown in Figure 34. Differences in the release rates of the various amino acids indicate that no single hydrolysis time is suitable for the accurate measurement of the amino acid composition of this product. Similar differences in optimum hydrolysis time for individual

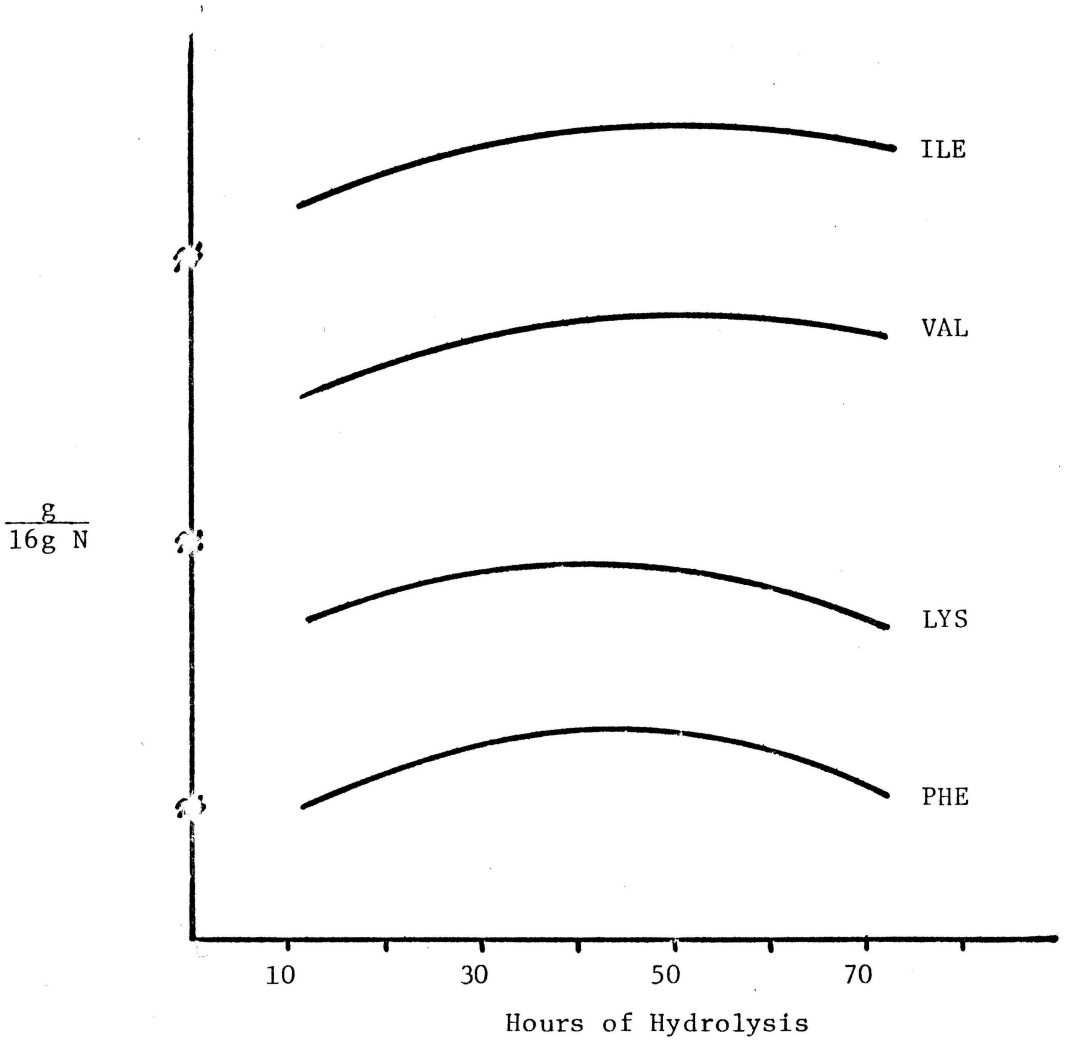


Figure 34. Curvilinear regression curves for lysine, phenylalanine, valine and isoleucine content of Promine D as a function of hydrolysis time.

amino acids were observed for each of the other products. Therefore, results indicate that no single hydrolysis time is adequate for accurate measurement of the amino acid content of processed soy proteins.

Ratios of extrapolated, maximum or average values to the average experimental 24 hour values for each product are given in Table 24. Valine and isoleucine values were most often affected by length of hydrolysis since correction factors were usually large for these amino acids. This finding is in agreement with published hydrolysis time studies on soy meal (Tkachuk and Irvine, 1969). However, experimental ratios reported in Table 24, which may be considered 24 hour hydrolysis correction factors, do not always agree with the correction factors reported by Tkachuk and Irvine (1969) for soy meal, nor with standard correction factors published in the Handbook of Biochemistry (Sober, 1970, Table 25). Published results are generally based on 72/24 hour ratios or on highest measured content for the time periods studied. Published ratios are similar to the ratios of maximum to average 24 hour values for Soya Fluff 200T, a toasted flour. Ratios for valine and leucine for all other products are higher.

The differences in ratios of extrapolated or maximum predicted values to 24 hour values between the five processed soy products suggests that processing has affected the rate of acid release of the amino acids. Large differences in measured content compared to the manufacturer and 24 hour values occurred in Promine D. This

Table 24. Ratios of maximum or extrapolated values to average experimental values for the essential amino acids in processed soy products.

	RATIOS				
	Maximum or Extrapolated/24-Hour Average				
	Soya Fluff 200T	Soya Fluff 200W	Promosoy 100	Promine D	Promine F
Threonine	1.03	1.05	1.04	1.07	1.00
Valine	1.04	1.20	1.07	1.10	1.10
Isoleucine	1.00	1.08	1.11	1.10	1.06
Leucine	1.05	1.06	1.00	1.02	1.02
Tyrosine	1.01	1.05	1.02	1.09	1.00
Phenylalanine	1.01	1.05	1.00	1.02	1.00
Lysine	1.01	1.04	0.99	1.05	1.05

Table 25. Published correction factors for 24 hour amino acid hydrolysis times.

AMINO ACID	Handbook of Biochemistry[a]	Tkachuk and Irvine(1969)
Threonine	1.02	1.03
Valine	1.12	1.10
Isoleucine	1.11	1.09

[a]Sober, 1970.

product, a soy isolate having the lowest reported PER of all products studied, was most affected by length of hydrolysis.

Since the measured content of many of the essential amino acids was higher than that reported by the manufacturer, nitrogen recovery based on measured amino acid content was calculated (Table 20). Percent nitrogen recovery based on data supplied by the manufacturer was also calculated and is presented in Table 26. Recovery was based on amino acid molecular weights.

Percent nitrogen recovery based on results of this study were higher than those based on manufacturer's data for all products except Promosoy 100 (Tables 20, 26). Calculated nitrogen recoveries probably underestimate true values at least in some products since glutamine + glutamic acid were calculated as glutamic acid and asparagine + aspartic acid were calculated as aspartic acid. True values for these amino acids could not be determined by methods employed by this study. Calculation of amide nitrogen content from ammonia recovery would overestimate the glutamine and asparagine contents since the ammonia peak also contains ammonia from urea and other non-amino acid nitrogen containing compounds as well as nitrogen resulting from serine and threonine degradation (Smith and Stockell, 1957).

Nitrogen recovery data from this experiment indicates that a maximum of 6-17% of the nitrogen content of processed soy proteins is non-protein nitrogen (NPN). The maximum NPN content based on manufacturer's data was 10-17%. The values corrected for protein amide nitrogen could be lower as discussed above. Becker et al. (1946)

Table 26. Nitrogen recovery of processed soy products calculated from data supplied by the manufacturer.[a,b]

Product	% Nitrogen Recovered MW
Soya Fluff 200W	87.1
Promosoy 100	89.6
Promine F	87.6
Promine D	83.4

[a] Central Soya, Chicago, Illinois.

[b] Complete amino acid content of Soya Fluff 200T not available.

reported that raw soybeans contain 3-8% NPN. The NPN content of processed soy proteins could be higher or lower than that of raw soybeans. Because of extraction and precipitation steps in processing, it is probable that the concentrates and the isolates have lower NPN than raw soy. Therefore the nitrogen recovery data based on amino acid content determined in this study seem more reasonable than the data calculated from manufacturer's values. This, of course, suggests that the amino acid data from this study is also more reliable than manufacturer's data.

Several nutritional implications can be suggested from results of this study on effect of length of acid hydrolysis on measured amino acid content on processed soy proteins. Promine D and F had nearly identical amino acid content as reported by the manufacturer (Table 19) despite their widely differing reported PER values of 1.1 and 1.6 respectively (Table 1). However, amino acid content of these two proteins clearly differed when measured by the methods used in this study (Table 16). These differences are reflected in the patterns of ratios of each essential amino acid to total essential amino acid content for these two isolates (Table 27). Although the nutritional effects of disproportionate amounts of amino acids have been reported (Harper, 1974), the relationship between the patterns of essential amino acids and nutritional value is still unclear.

An additional nutritional implication which can be drawn from this study involves the effect of processing on the rate of amino acid release *in vivo*. Since processing has clearly affected the rate

Table 27. Ratio of each essential amino acid to total essential amino acid content (mg/g) of processed soy products.

AMINO ACID	Soya Fluff 200W	Soya Fluff 200T	Promosoy 100	Promine F	Promine D	Egg[a]
Threonine	102	98	102	92	91	99
Valine	132	113	123	128	120	141
Isoleucine	118	120	118	120	118	129
Leucine	188	189	187	197	193	172
Tyrosine	81	94	85	88	93	81
Phenylalanine	123	130	121	132	138	114
Total aromatic	204	224	201	220	230	195
Lysine	153	158	161	152	153	125
Tryptophan[b]	32	34	36	28	36	31
1/2 cystine[c]	30	34	36	31	29	46
methionine[c]	32	31	31	31	29	61
Total sulfur	62	65	67	62	58	107

[a]Liener, 1972.

[b]Central Soya, Chicago, Illinois, 1975.

[c]Performic acid determination.

of amino acid release by acid hydrolysis, it is possible that the release rate by enzymatic hydrolysis *in vivo* has also been altered, and this in turn could result in a change in protein quality (Taira et al., 1969).

SUMMARY AND CONCLUSION

Numerous high protein soy products have become commercially available during the last 10 years (Alden, 1975). In general, the protein nutritional value of these products decreases with increased processing and total protein content (Bressani, 1975). Although amino acid destruction during processing has been cited as partially responsible for loss of nutritional quality, supplementation with amino acids destroyed frequently does not completely restore the nutritional value to the preprocessing level (DeGroot and Slump, 1969). Additional factors must be responsible for nutritional changes. Therefore, this study was undertaken in order to characterize protein molecular and chemical changes resulting from processing, and to attempt to relate these changes to manufacturer's reported protein efficiency ratios.

Eight commercially processed soy products and raw and autoclaved soy meal were studied. Processed products included three textured soy products, two soy flours, one soy concentrate and two soy isolates. Manufacturer's reported PER's ranged from 1.1 to 2.3 and were generally inversely related to the crude protein quantity (N x 6.25), which ranged from 33.1% to 86.7%.

Nitrogen solubility of the three textured products was similar regardless of extraction solvent. Textured soy products were less soluble than raw soy meal. Part of the decreased solubility of the

textured products was attributed to disulfide and hydrophobic interactions since protein solubility could be increased by the addition of mercaptoethanol and SDS to the extraction media. However, a large proportion of the protein could not be solubilized, suggesting processing-induced formation of large polymers having inaccessible disulfide bonds or some other type of bonding.

Soy meal autoclaved for more than 10 minutes was less soluble than textured soy, and was affected little by the addition of mercaptoethanol to the extraction media. Autoclaved soy apparently also contained large insoluble polymers similar to those proposed to exist in textured soy.

Hydrophobic and disulfide interactions were also found to play an important part in the proteins of soy flours, isolates and concentrate. Soy concentrate had lower nitrogen solubility than the flours or isolates. The decreased solubility was attributed to an effect of acid precipitation during processing.

The nitrogen solubility of soy autoclaved for 10, 15 or 20 minutes differed only slightly. These times correspond with reported 'underheated,' 'properly heated' and 'overheated' autoclaving times for soy meal (Klose et al., 1948). Likewise the solubility of textured soy, toasted flour and untoasted flour decreased with increased heat treatment during processing although all products had nearly identical PER's. Protein solubility of products which were processed using acid or alkali did correlate with PER's. Protein solubility of soy concentrate and isolates increased linearly with decreased product PER. This relationship may reflect the nutritionally available

cystine of the products. Isolates, which receive alkaline treatment during processing, were found to contain a significant amount of cysteic acid. Alkali treatment of disulfide bonds is believed to cause bond reduction followed by the formation of an unstable sulfenic acid group (Andersson, 1970). This reaction of disulfide bonds would be expected to result in increased protein solubility. Thus, such a mechanism could account for the increased protein solubility occurring simultaneously with decreased nutritional value of the soy isolates.

Chromatographic separation of proteins of textured soy indicated that soluble proteins were primarily of smaller molecular weight than the soluble proteins of native soy. Electrophoretic analysis indicated that the major soluble protein of textured soy was at 27,000 molecular weight. This protein corresponds roughly with the reported size of the major 11S protein subunit (Catsimpoolas et al., 1967), and thus also suggests a breakdown of some 11S protein during the formation of textured soy. Gel patterns for textured soy closely resembled those from soy meal autoclaved for 20 minutes.

Major differences between raw soy protein and textured soy protein were also apparent from disulfide and sulfhydryl analysis. No SH's were found in soy meal extracted in buffer containing SDS and NaCl, whereas textured soy extracts had 58-85 μmoles SH/gN when extracted in the same medium. The combination of SDS in high ionic strength buffer was found to reduce greatly the solubility of raw soy meal, but not of textured soy. These results are interpreted as further indication that the texturizing process of soy had changed the

native structure of the proteins and probably affected the sulfur-containing 11S proteins. Since total sulfhydryl content of textured soy and soy extracted in buffer containing SDS and mercaptoethanol was similar, it was concluded that little or no sulfur oxidation had occurred during processing provided that extractable proteins reflected the total protein content. This conclusion was supported by the measured presence of only a small amount of cysteic acid in these protein products.

Analysis of the total sulfur amino acid content indicated only minor destruction during processing. There was little correlation between total sulfur amino acid content of the products and reported PER. However, a significant amount of cysteic acid was found in several of the products, with highest content occurring in the highly processed soy isolates. One soy isolate was also found to contain a significant amount of methionine sulfoxide.

None of the other processed products were found to contain methionine sulfoxide, however, and no products were found to contain methionine sulfone. Chemical scores of products correlated with reported PER's when calculated based on "available" sulfur amino acids.

Processing also affected the rate of amino acid release by acid hydrolysis. Standard 18 or 24 hour hydrolysis times were found to be inadequate for the accurate determination of the essential amino acid content of soy products. Methionine and threonine content decreased with length of hydrolysis in all cases. Other essential amino acids either decreased, increased or remained constant between 12 and 72 hours of hydrolysis. When the measured amino acid content

increased with time of hydrolysis, the data were usually better described by curvilinear regression than by linear regression analysis. In addition, curvilinear analysis yielded a predicted maximum value based on all data points, whether or not the time for the predicted maximum was an experimental time.

Patterns of release were found to vary between amino acids and between the different soy products. Values for soy isolates were found to be most often affected by length of hydrolysis time. Thus, it was apparent that processing had affected the protein in such a way as to alter the rate of release of essential amino acids. It is possible that in vivo hydrolysis may also be affected by processing (DeGroot and Slump, 1969).

In conclusion, this study indicated that processing of soy products results in disulfide and hydrophobic interactions among the proteins, as well as in other heat-induced reactions resulting in insolubility of many of the proteins. The texturizing process caused protein degradation similar to that which occurs in overheated soy meal and probably involves the 11S protein fraction.

Although the nitrogen solubility of heat-processed soy products (textured soy, flours) did not correlate with product PER's, nitrogen solubility of products processed using acid and alkali increased linearly with decreased PER. Finally, processing, especially that involving alkali, was also found to result in the formation of oxidized forms of sulfur amino acids and the formation of molecular structures or peptide bonds which cause differing rates of release of amino acids during acid hydrolysis.

LITERATURE CITED

Alden, D. 1975. Soy processing: From beans to ingredients. J. Am. Oil Chem. Soc. 52:244A.

Anderson, G. H., Ashley, V. M. and Jones, J. D. 1976. Utilization of L-methionine sulfoxide, L-methionine sulfone and cysteic acid by the weanling rat. J. Nutr. 106:1108.

Andersson, L. 1970. Hydrolysis of disulfide bonds in weakly alkaline media. II. Bovine serium albumin dimer. Biochem. Biophys. Acta 200:363.

Anonymous. 1973. Nutritional implications of sulfur amino acid oxidation. Nutrition Reviews 31:220.

Badenhop, A. F. and Hackler, L. R. 1971. Protein quality of dry roasted soybeans: Amino acid composition and protein efficiency ratio. J. Food Sci. 36:1.

Badenhop, A. F. and Hackler, L. R. 1973. Methionine supplementation of soy milk to correct cystine loss resulting from an alkaline soaking procedure. J. Food Sci. 39:471.

Balloun, S. L., Johnson, E. L. and Arnold, L. K. 1953. Laboratory estimation of the nutritive value of soybean oil meals. Poultry Science 33:517.

Becker, J. C., Milner, R. T. and Nagel, R. A. 1946. A new method for determination of nonprotein nitrogen in soybean meal. Cereal Chem. 17:447.

Belter, P. A., and Smith, A. K. 1952. Protein denaturation in soybean meal during processing. J. Am. Oil Chem. Soc. 29: 170-174.

Beveridge, T., Toma, S. J. and Nakai, S. 1974. Determination of SH- and SS- groups in some food proteins using Ellman's reagent. J. Food Sci. 39:49.

Blackburn, S. 1968. In "Amino Acid Determination," Marcel Dekker, Inc., New York.

Boatman, S. 1976. Private communication. Dept. of Chemistry, Hollins College, Roanoke, Virginia.

Bohak, Z. 1964. N^{ε}-(DL-2 amino-2-carboxyethyl)-L-lysine, a new amino acid formed in alkaline treatment of proteins. J. Biol. Chem. 239:2878.

Bressani, Ricardo. 1975. Nutritional contribution of soy protein to food systems. J. Am. Oil Chem. Soc. 52:254A.

Briggs, D. R. and Wolf, W. J. 1957. Studies on the cold-insoluble fraction of the water-extractable soybean proteins. I. Polymerization of the 11S component through reactions of sulfhydryl groups to form disulfide bonds. Arch. Biochem. Biophys. 72:127.

Catsimpoolas, N., Rogers, D. A., Circle, S. S. and Meyer, E. W. 1967. Purification and structural studies of the 11S component of soybean proteins. Cereal Chem. 44(6):631.

Cecil, R. and McPhee, J. R. 1959. Sulfur chemistry of proteins. Adv. Prot. Chem. 14:255.

Central Soya. 1975. Product information sheets. Chicago, Illinois.

Chiang, J. P. C. and Sternberg, M. 1974. Physical and chemical changes in spun soy protein fibers during storage. Cereal Chem. 51:465.

Circle, S. J., Meyer, E. W. and Whitney, R. W. 1964. Rheology of soy protein dispersions. Effect of heat and other factors on gelation. Cereal Chem. 41:157.

Circle, S. J. and Smith, A. K. 1972. Processing soy flours, protein concentrates, and protein isolates. In "Soybeans: Chemistry and Technology." AVI Publishing Company, Westport, Conn.

Cogan, Uri, Yaron, Anina, Berk, Z. and Zimmermann, G. 1968. Effect of processing conditions on the nutritive value of isolated soybean proteins. J. Agr. Food Chem. 16:196.

Cumming, D. B., Stanley, D. W. and DeMann, J. M. 1973. Fate of water soluble soy protein during thermoplastic extrusion. J. Food Sci. 38:320.

Cuq, J. L., Provansal, M., Guilleux, F. and Cheftel, C. 1973. Oxidation of methionine residues of casein by hydrogen perioxide. Effects on in vitro digestibility. J. Food Sci. 38:11.

Davies, M. G. and Thomas, A. J. 1973. An investigation of hydrolytic techniques for the amino acid analysis of foodstuffs. J. Sci. Fd. Agric. 24:1525.

DeGroot, A. P. and Slump, P. 1969. Effects of severe alkali treatment of proteins on amino acid composition and nutritive value. J. Nutr. 98:45.

Ellinger, G. M. and Palmer, R. 1969. The biological availability of methionine sulphoxide. Proc. Nut. Soc. 28:42A.

Evans, R. J., and Butts, H. A. 1949. Studies on the heat inactivation of methionine in soybean oil meal. J. Biol. Chem. 178:543.

Evans, R. J., Groschke, A. C., and Butts, H. A. 1951. Studies on the heat inactivation of cystine in soybean oil meal. Arch. Biochem. 30:414.

Evans, R. J., and McGinnis, J. 1948. Cystine and methionine metabolism by chicks receiving raw or autoclaved soybean meal. J. Nutr. 31:449.

Floyd, N. F., Cammaroti, M. S. and Savine, T. F. 1963. The decomposition of DL-methionine sulfoxide in 6N hydrochloric acid. Arch. Biochem. Biophys. 102:343.

Fukushima, D. 1959. Studies on soybean proteins. I. Water dispersibility of protein of defatted soybean flour as a criterion for degree of denaturation. Bull. Agr. Chem. Soc. Japan. 23:7.

Fukushima, D. and Van Buren, J. 1970a. Effect of physical and chemical processing factors on the redispersibility of dried soy milk proteins. Cereal Chem. 47:571.

Fukushima, D. and Van Buren, J. 1970b. Mechanisms of protein insolubilization during the drying of soy milk. Role of disulfide and hydrophobic bonds. Cereal Chem. 47:687.

Hackler, L. R. and Stillings, B. R. 1967. Amino acid composition of heat-processed soymilk and its correlation with nutritive value. Cereal Chem. 44:70.

Hackler, L. R., Stillings, B. R. and Polimeni, R. J., Jr. 1967. Correlation of amino acid indexes with nutritional quality of several soybean fractions. Cereal Chem. 44:638.

Harper, A. E. 1974. Effects of disproportionate amounts of amino acids. In "Improvement of Protein Nutriture," National Academy of Sciences, Washington, D. C.

Hegsted, D. M. 1974. Assessment of protein quality. In "Improvement of Protein Nutriture," National Academy of Sciences, Washington, D. C.

Hill, R. L. 1965. Hydrolysis of proteins. Adv. Prot. Chem. 20:37.

Horan, F. E. 1966. Defatted and full-fat soy flours by conventional processes. Intern. Conf. Soybean Protein Foods, USDA, Peoria, Illinois.

Huge, W. E. 1961. Present and potential uses of soybean flour, grits and protein concentrates in foods. Proc. Conf. Soybean Products for Protein in Human Foods, USDA, Peoria, Ill.

Hugli,T. E. and Moore, S. 1972. Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysates. J. Biol. Chem. 247:2828.

Ishino, K. and Okamoto, S. 1975. Molecular interaction in alkali denatured soybean proteins. Cereal Chem. 52:9.

Jennings, D. and Lewis, O. A. M. 1968. Methionine loss during protein hydrolysis of plant material. J. Agr. Food Chem. 17:668.

Kelley, J. J., and Pressey, R. 1965. Studies with soybean protein and fiber formation. Cereal Chem 43:195.

Klose, A. A., Hill, B. and Fevold, H. L. 1948. Food value of soybean protein as related to processing. Food Technol. 2:201.

Kofranyi, E. 1972. Protein and amino acid requirements. In "Protein and Amino Acid Functions," Bigwood, E.J., ed. Pergamon Press, Elmsford, N.Y.

Kohler, G. O. and Palter, R. 1967. Studies on methods for amino acid analysis of wheat products. Cereal Chem. 44:512.

Kuzmicky, D. D., Kohler, G. O., Walker, H. G., Jr., and Mackey, B. E. 1974. Availability of oxidized sulfur amino acids for the growing chick. Poultry Sci. 53:1945.

Lehninger, A. L. 1970. "Biochemistry." North Publishers, Inc. New York, N. Y.

Liener, I. E. 1953. Toxic factors in edible legumes and their elimination. Am. J. Clin. Nutr. 11:281.

Liener, I. E. 1972. Nutritional value of food protein products. In "Soybeans: Chemistry and Technology," Smith, A. K. and Circle, S. J., eds., AVI Publishing Company, Westport, Conn.

Liener, I. E. 1976. Legume toxins in relation to protein digestibility--A review. J. Food Sci. 41:1086.

Lipton, S. H. and Bodwell, C. E. 1976. Specific oxidation of methionine to methionine sulfoxide by dimethyl sulfoxide. J. Agr. Food Chem. 24:26.

Longenecker, J. B., Martin, W. H. and Sarrett, H. P. 1964. Improvement in the protein efficiency of soybean concentrates and isolates by heat treatment. J. Agr. Food Chem. 12:411.

Lowry, K. L., Caton, J. E. and Foard, D. E. 1974. Electrophoretic methods for detecting differences in seed proteins of soybean varieties and induced mutants. J. Agr. Food Chem., 22:1043.

Lowry, O. H., Roseborough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.

Lunder, T. L. 1972. Determination of methionine sulfoxide content of intact protein based on rearrangement of sulfoxide with acetic anhydride. Anal. Biochem. 49:585.

Mahoward, T. A., Noltmann, E. A. and Kuby, S. A. 1962. Studies on adenosine triphosphate-adenosine 5'-phosphate transphosphorylase (myokinase). J. Biol. Chem. 237:1138.

Mann, R. L. and Briggs, D. R. 1950. Effect of solvent and heat treatments on soybean proteins as evidenced by electrophoretic analysis. Cereal Chem. 27:258.

Marable, N. L. 1976. Private communication. Dept. Human Nutrition and Foods, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Miller, D. S. and Carpenter, K. J. 1964. Availability of sulfur amino acids in protein foods. I. Total sulfur amino acid content in relation to sulfur and nitrogen balance studies with the rat. J. Sci. Fd. Agric. 15:810.

Miller, D. S. 1967. Determination of the tryptophan content of feedings stuffs with particular reference to cereals. J. Sci. Fd. Agric. 18:381.

Miller, D. S. and Samuel, P. D. 1968. Methionine sparing compounds. Proc. Nutr. Soc. 27:21A.

Miller, D. S. and Samuel, P. D. 1970. Effects of the addition of sulphur compounds to the diet on utilization of protein in young growing rats. J. Sci. Fd. Agric., 21:616.

Miller, D. S., Carpenter, K. J., Morgan, C. B., and Boyne, A. W. 1964. Availability of sulphur amino acids in protein foods. 2. Assessment of available methionine by chick and microbiological assays. Br. J. Nutr. 19:249.

Miller, D. S., Carpenter, K. J., and Milner, C. K. 1965. Availability of sulphur amino acids in protein foods. 3. Chemical and nutritional changes in heated cod muscle. Br. J. Nutr. 19:347.

Miller, S. A., Tannenbaum, S. R. and Seitz, A. W. 1970. Utilization of L-methionine sulfoxide by the rat. J. Nutr. 100:909.

Moore, Stanford. 1963. On the determination of cystine as cysteic acid. J. Biol. Chem. 238:235.

Nash, A. M., Kwolek, W. F. and Wolf, W. J. 1974. Extraction of soybean proteins with aqueous 2-mercaptoethanol. Cereal Chem. 51:220.

Nash, A. M. and Wolf, W. J. 1967. Solubility and ultracentrifugal studies on soybean globulins. Cereal Chem. 44:183.

Neumann, N. P. 1967. Analysis for methionine sulfoxides. Methods in Enzymol. 11:487.

Neville, D. M. 1971. Molecular weight determination of protein-dodecyl sulfate complexes in a discontinous buffer system. J. Biol. Chem. 246:6328.

Njaa, L. R. 1962. Some problems related to detection of methionine sulphoxide in protein hydrolysates. Acta Chem. Scand. 16:1359.

Noltmann, E. A., Mahowald, T. W. and Kuby, S. A. 1962. Studies on adenosine triphosphate transphosphorylases. II. Amino acid composition of adenosine triphosphate-creatine transphosphorylase. J. Biol. Chem. 237:1147.

Obara, T., and Kimura, M. 1967. Gel filtration fractionation of the whole water-extractable soybean proteins. J. Food Sci. 32: 531-534.

Okabo, K., Asano, M., Kimura, Y., and Shibasaki, K. 1969. On the basic subunits dissociated from C(11S) component of soybean proteins with urea. Agr. Biol. Chem. (Tokyo) 33:463.

Osborn, T. B. and Mendel, L. B. 1917. The use of soybean as food. J. Biol. Chem. 32:369.

Pieniazek, D., Rakowska, M., and Kunachowicz, H. 1975a. The participation of methionine and cystine in the formation of bonds resistant to the action of proteolytic enzymes in heated casein. Br. J. Nutr. 34:103.

Pieniazek, D., Rakowska, M., Szkilladziowa, W., and Grabarek, Z., 1975b. Estimation of available methionine and cysteine in proteins of food products by in vivo and in vitro methods. Br. J. Nutr. 34:175.

Rackis, J. J. 1966. Soybean trypsin inhibitors: Their inactivation during meal processing. Food Technol. 20:1483.

Rice, E. E. 1970. Nutritive value of oilseed proteins. J. Am. Oil Chem. Soc. 47:408.

Rios Iriarte, B. J. and Barnes, R. H. 1966. The effect of over-heating on certain nutritional properties of the protein of soybeans. Food Technol. 20:131.

Saio, K., Kajikawak, M. and Watanabe, T. 1971. Food processing characteristics of soybean proteins. Part II. Effect of sulfhydryl groups on physical properties of tofu-gel. Agr. Biol. Chem. 35.

Saio, K., Terashima, M. and Watanabe, T. 1975a. Food use of soybean 7S and 11S proteins. Heat denaturation of soybean proteins at high temperature. J. Food Sci. 40:537.

Saio, K., Terashima, M. and Watanabe, T. 1975b. Food use of soybean 7S and 11S proteins. Changes in basic groups of soybean proteins by high temperature heating. J. Food Sci. 40:541.

Scales, F. M. and Harrison, A. P. 1920. Boric acid modification of the Kjeldahl method for crop and soil analysis. J. Ind. Eng. Chem. 12:350.

Slump, P. and Schreuder, H. A. W. 1973. Oxidation of methionine and cystine in foods treated with hydrogen peroxide. J. Sci. Fd. Agric. 24:657.

Smith, A. K. and Circle, S. J. 1972. Processing soy flours, protein concentrates, and protein isolates. In "Soybeans: Chemistry and Technology," AVI Publishing Company, Westport, Conn.

Smith, E. L. and Stockell, A. 1957. Amino acid composition of crystalline carboxypeptidase. J. Biol. Chem. 228:501.

Snedecor, George W., and Cochran, William G. 1967. "Statistical Methods." Iowa State University Press, Ames, Iowa.

Sober, Herbert A., ed. 1970. "Handbook of Biochemistry," 2nd ed., Chemical Rubber Company, Cleveland, Ohio.

Stecher, P. G., ed. 1960. "Merck Index of Chemicals and Drugs," 7th ed., Rahway, N.J.

Taira, H., Kayanagi, T., Takanohashi, T., and Oikawa, K. 1969. Studies on amino acid contents of processed soybean. Part XI. Evaluation of nutritional losses of overheated defatted soybean flour. Agr. Biol. Chem. 33:1387.

Tkachuk, R. and Irvine, G. N. 1969. Amino acid compositions of cereals and oilseed meals. Cereal Chem. 46:206.

Walker, H. G., Kohler, G. O., Kuzmicky, D. D., and Witt, S. C. 1974. Problems in analysis for sulfur amino acids in feeds and foods. Presented at 168th National American Chemical Soceity Annual Meeting, Atlantic City, N.J.

Weber, K. and Osborn, M. 1969. Reliability of molecular weight determination by dodecyl sulfate polyacrylamide electrophoresis. J. Biol. Chem. 244(16):4406.

Webb, Kenneth E., Jr. 1975. Unpublished data. Dept. of Animal Science, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Wilcox, P. E., Conen, E. and Tan, Wen. 1957. Amino acid composition of α-chymotrypsinogen, including estimation of asparagine and glutamine. J. Biol. Chem. 228:999.

Wolf, W. J. 1972. Purification and properties of the proteins. In "Soybeans: Chemistry and Technology," Smith, A. K. and Circle, S. J., eds., AVI Publishing Company, Westport, Conn.

Wolf, W. J., and Briggs, D. R. 1959. Purification and characterization of the 11S component of soybean proteins. Arch. Biochem. Biophys. 85:186.

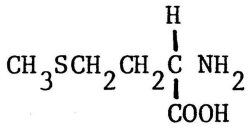
Wolf, W. J. and Cowan, J. C. 1975. "Soybeans as a Food Source," 2nd ed., CRC Press Inc., Cleveland, Ohio.

Wolf, W. J. and Tamura, T. 1969. Heat denaturation of soybean 11S protein. Cereal Chem. 46:331.

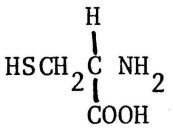
APPENDIX 1

Structure of sulfur amino acids and their derivatives.

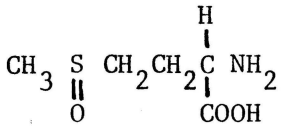
Methionine



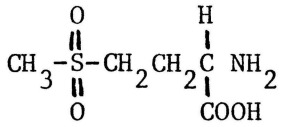
Cysteine



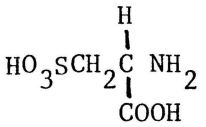
Methionine Sulfoxide



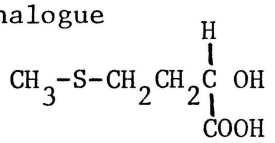
Methionine Sulfone



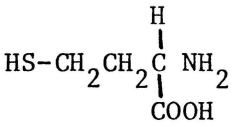
Cysteic Acid



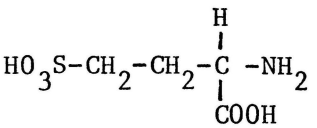
Methionine Hydroxy Analogue



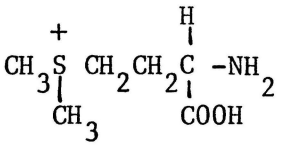
Homocysteine



Homocysteic Acid

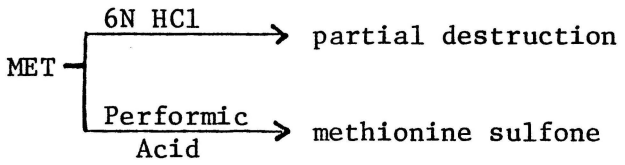


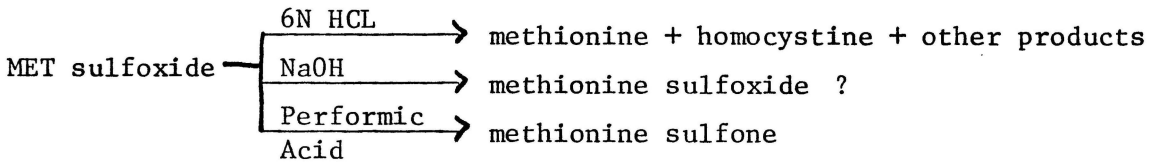
Methionine Methyl Sulfonium Salt

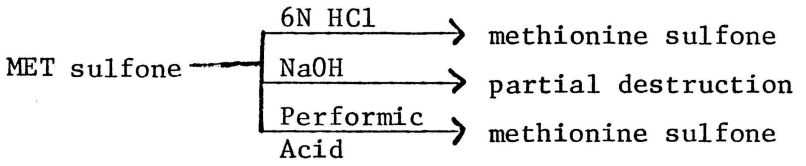


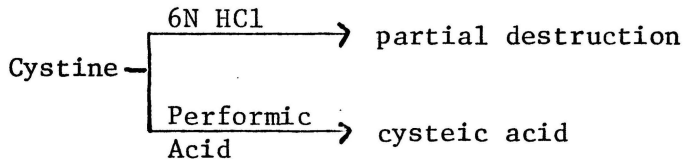
APPENDIX 2

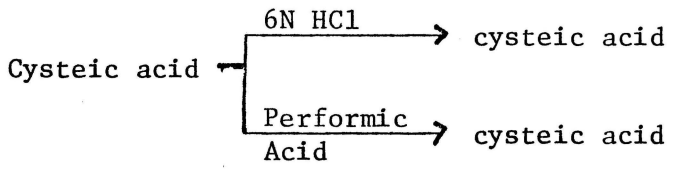
Selected reactions of sulfur amino acids and their derivatives.











APPENDIX 3

Electrophoretic reagents for SDS sodium phosphate and Tris-HCl gel Electrophoresis.

Acrylamide-Bis Solution

22.2 g Acrylamide
0.6 g Bisacrylamide
per 100 ml solution

Na Phosphate Buffer pH 7.9

68.4 g Na_2HPO_4
3.6 g NaH_2PO_4
4.8 g Sodium Dodecyl Sulfate
4.8 g mercaptoethanol
2400 ml H_2O

Tris-HCl Buffer pH 8.6

6.0 g Tris per liter
titrated to pH 8.6 with HCl

Gel Solution:

18.5 ml buffer (Na Phosphate or Tris-HCl)
9.5 ml Acrylamide-Bis solution
1.4 ml sodium persulfate (1.5% solution, freshly prepared)
0.06 ml TEMED

Running Buffer: Upper and lower electrophoresis chambers were filled with buffer diluted 1:2 with water.

APPENDIX 4

Electrophoretic reagents for SDS gels in a discontinuous buffer system.[2]

Upper Gel Buffer pH 6.1

0.0267 M H_2SO_4

0.054 M Tris

Lower Gel Buffer pH 9.18

0.0308 N HCl

0.4244 M Tris

Dialysis Buffer

Upper gel buffer containing 0.1% SDS and 0.1% mercaptoethanol.

Upper Buffer pH 8.64

0.04 M Boric Aicd

0.041 M Tris

0.1% Sodium Dodecyl Sulfate

Acrylamide-Bisacrylamide Solutions

Upper Gel	0.4 g	Bisacrylamide
	6.0 g	Acrylamide
	per 100 ml	solution
Lower Gel	0.2 g	Bisacrylamide
	22.0 g	Acrylamide
	per 100 ml	solution

Upper Gel

0.25 ml persulfate (1.5% solution, freshly prepared)

0.1 ml 10% TEMED

3.8 ml Upper Acryl-bis solution

3.5 ml Upper gel buffer

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[2]Adapted from Neville (1971).

Lower Gel

0.45 ml sodium persulfate (1.5% solution, freshly prepared)

0.17 ml 10% TEMED

7.5 ml Lower Acryl-bis solution

7.0 ml Lower Gel Buffer

Running Buffers

Upper chamber:

Upper buffer undiluted

Lower chamber:

Lower gel buffer diluted 14:30

APPENDIX 5

Calculation of sulfhydryl, disulfide and total sulfhydryl content of extracted textured and soymeal proteins.[2]

$$\frac{\mu MSH}{gN} = \frac{73.53\ A_{412} \cdot D}{C}$$

A_{412} = corrected absorbance at 412 nm where corrected absorbance = measured sample absorbance - Ellman's blank - protein blank

C = sample nitrogen concentration, in mg N/ml

D = dilution factor

73.53 = $\frac{10^6}{1.36 \times 10^4}$ where 1.36×10^4 is the molar absorptivity (Ellman, 1959).

SS = $\frac{\text{total SH} - \text{SH}}{2}$

[2] Beveridge et al. (1974).

APPENDIX 6

Conditions for separation of oxidized sulfur amino acids using a Technicon NC-1P Amino Acid Analyzer.

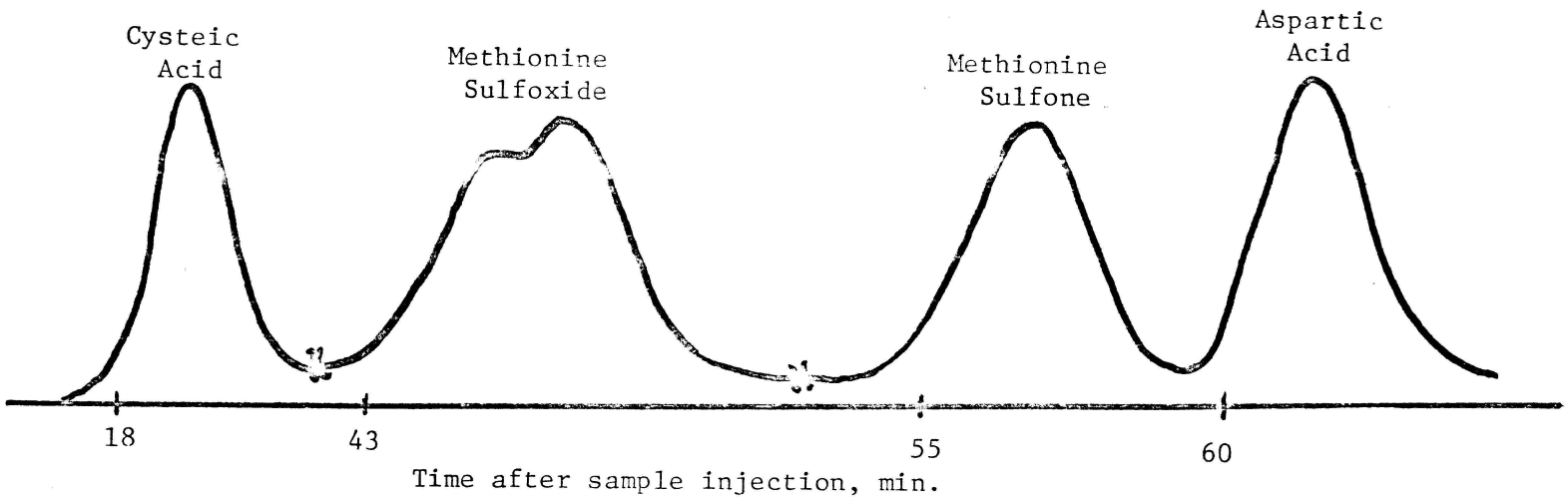
Flow Rate - 0.3 ml/minute

Buffer 1[a]	pH 2.60	20 minutes
Buffer 2[a]	pH 3.05	22 minutes

[a]Buffers prepared from directions described by Technicon Corporation for pH 3.25 buffer, except titrated to lower pH with 6N HCl.

APPENDIX 7

Separation pattern of oxidized sulfur amino acids and aspartic acid on a Technicon NC-1P Amino Acid Analyzer.[a]



[a]Conditions for separation described in Appendix 6.

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CHEMICAL AND MOLECULAR PROPERTIES OF PROCESSED SOY PROTEINS

by

Noelle Link Kehrberg

(ABSTRACT)

Selected chemical and molecular properties of two soy flours, two soy isolates, three textured soy products, one soy concentrate and raw and autoclaved soy meal were determined. Product protein content ranged from 33.1% to 86.7% and reported PER's ranged from 1.1 to 2.3.

Hydrophobic and disulfide interactions were found to occur in the proteins during processing, as well as other interactions causing loss of protein solubility. Solubility decreased greatly with increased heat treatment for untoasted flour, toasted flour and textured soy, although the reported PER's of the products were identical. Solubility increased linearly with decreased PER of acid or alkali processed soy concentrates and isolates.

Extractable textured soy proteins were found to be of smaller molecular weight than those of raw soy meal when analyzed by gel filtration and electrophoretic techniques. Textured soy had a major protein at 27,000 molecular weight, and gel patterns were similar to those of soy meal which was autoclaved for 20 minutes. Total sulfhydryl content (sulfhydryl plus reduced disulfides) of textured soy equalled that of soy meal, suggesting little destruction or oxidation of sulfur amino acids during hydrolysis. Time after extrac-

tion was found to affect the measured sulfhydryl and disulfide content.

All processed soy products were found to contain cysteic acid. Soy isolates contained the highest amount of cysteic acid, and one isolate also contained methionine sulfoxide. None of the products were found to contain methionine sulfone.

Length of time of acid hydrolysis was found to affect the measured essential amino acid content of processed soy flours, concentrate and isolates. Methionine and threonine values decreased with time in all products. Release patterns for other essential amino acids varied with each soy product. Amino acids which increased in measured value with increased length of hydrolysis time were analyzed by curvilinear regression. Differences between maximum measured value and 24 hour hydrolysis time value most often occurred in soy isolates. Standard 18 or 24 hour hydrolysis times were found inadequate for accurate measurement of essential amino acids in processed soy proteins.