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Bruce R. Harte

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Charles M Stine

Major professor

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# FUNCTIONAL CHARACTERIZATION AND HEAT INDUCED INTERACTIONS BETWEEN MILK PRODUCTS AND SOY ISOLATE

By

Bruce R. Harte

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ABSTRACT

FUNCTIONAL CHARACTERIZATION AND HEAT INDUCED INTERACTIONS BETWEEN MILK PRODUCTS AND SOY ISOLATE

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The functionality of milk product:soy isolate blends was evaluated. The commercial milk components included: 1) sweet whey powder, 2) electrodialyzed whey powder, 3) whey protein concentrate (WPC), 4) nonfat dry milk (NFDM) and 5) sodium caseinate. A commercial soy isolate was selected based upon ease of dispersibility, soluble protein and flavor. Five functional properties were examined: emulsion capacity, whipping ability, viscosity, solubility and sensory evaluation. Dispersions were prepared at protein levels of 3.2, 5.0 and 8.0% in ratios of 25:75, 50:50 and 75:25. The samples were subjected to various treatments including: heating, pH variation and addition of salts. A second set of treatments was designed after evaluation of the first trials. Additional treatments were selected to further improve functionality. Many of the treatments included variants from the first study in addition to the inclusion of chemical modifiers, gums, emulsifiers and an enzymatic digestion.

Milk products demonstrated the highest solubility, with milk product:soy isolate blends having appreciably

higher solubility than the soy isolate. Specific treatments resulted in widely varying solubility. The viscosities of the milk products, soy isolate and their blends were quite close regardless of the blend ratio. Viscosities rose moderately as the percent protein increased. The majority of treatments had little effect upon viscosities. In general, the soy isolate had less emulsion capacity than the milk products. Blends often had nearly the same emulsion capacity as the milk product. Emulsion capacities were affected by various treatments. Stable foams were produced from NFDM, sodium caseinate, electrodialyzed whey, and their soy isolate blends. WPC whipped into stable foams after subjection to specific treatments. Blends produced foams which had similar specific volumes with slightly less foam stability than the respective milk product. It was possible to substantially improve the whipping properties of the samples by utilization of specific treatments. The milk products, particularly NFDM and sodium caseinate had the highest flavor scores. The flavor characteristics of soy were considered unacceptable. However, when used in combination with milk products the flavor properties were only slightly less than the respective milk product.

Chemical modification, heating, pH variation, enzymatic hydrolysis and change in the ionic environment improved the functionality of many samples. Utilization of different

treatments made it possible to maintain the generally higher functionality associated with the milk products. In many instances, replacement of as much as 50% of the milk protein product by soy isolate was achieved with little loss of functionality.

Milk:soy protein blends were examined for heat-induced interaction. The milk proteins were prepared from sweet whey, acid whey, colloidal casein and sodium caseinate. Samples of these proteins were heated with soy protein. The heat treatments included: unheated-control, 68°C/30 min, 77°C/20 sec, 94°C/10 sec and 121°C/5 sec. After heating, the proteins were fractionated by gel filtration and resolved by gel electrophoresis. Whey protein suffered substantial denaturation at temperatures greater than 77°C/20 sec. Casein was stable to the heat treatments. Soy protein began to dissociate into minor components at 77°C/20 sec. Further heating resulted in the disappearance of the major bands. There did not appear to be any interaction between whey or casein and the major soy proteins. The possibility exists of hydrophobic interaction between casein and soy protein.

An <u>in vitro</u> enzymatic procedure was utilized to examine release of total amino nitrogen from intact proteins as affected by: processing treatments and possible interactions between protein systems. Soy isolate, NFDM and their blend were subjected to 24 different treatments. In general, the

liberation of amino nitrogen was not significantly reduced by use of the process variants. Specific cases (such as the addition of chemical modifiers or extremely high pH) lowered the released amino nitrogen. Protein interaction detrimental to the release of amino nitrogen was minimal.

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# INTRODUCTION

A great deal of effort is being directed toward finding cheaper protein sources for the domestic food industry. Currently, animal derived ingredients and wheat are the major sources for human food, though soybean protein is becoming more popular. Animal protein foods are expensive in terms of land requirements and market price. Vegetable protein foods can supply more protein per acre of land (Childers, 1972). Proteins which are to be used in food products must satisfy three criteria. They must be nutritionally adequate, economically feasible and possess the necessary functional properties. That protein which comes closest to fulfilling these needs will probably have the highest utilization.

Sufficient information is available to formulate a food composition which contains the required amounts of known nutrients, including vitamins, minerals, proteins, fats and carbohydrates in proper balance to supply the calories and chemicals needed by humans (Johnson, 1971). This can be accomplished by combining plant and animal products with synthetic chemicals. The problem is to produce finished products that have the necessary functional and sensory characteristics which make them acceptable to

the consumer. The common protein foods (meat, fish, eggs and dairy products) owe their widespread appeal to the gastronomic pleasure derived from their consumption (Mattil, 1971). The proteins in these foods are structural components that contribute specific functional properties. If vegetable proteins are to be incorporated into the products of today's technology they must possess certain functional characteristics. Acceptable sensory properties are essential in the development of new food products. New protein sources should maintain or improve the quality and acceptability of food products.

The functionality of food proteins denotes any physicochemical characteristic which affects the processing and behavior of the protein in food systems. Functionality is a reflection of complex interactions between the composition, structure and conformation of a protein which may also be influenced by reaction with other food components. In the food research laboratory, many types of protein systems are subjected to measurements in an attempt to quantitate functionality. Typical tests include flavor analysis, dispersibility, gelation, emulsification, foaming, viscosity and water absorption. Burrows et al. (1972) estimated that by 1980 the demand for functional protein in the United States will exceed three billion pounds. This will be approximately 15-20% of the total protein need. The actual demand for new functional proteins will

depend on many factors including the availability and price of animal proteins and the apparent functionality of non-animal proteins. Many vegetable, cereal and seed proteins are being used or investigated for use in roles normally reserved for animal proteins. However, these proteins often lack functionality and nutritional adequacy. Both deficiencies can be improved by combining in one system the proper blend of animal and nonanimal proteins.

The objective of this research was to provide an in depth analysis of the functional (whipping, emulsion capacity, gelation, etc.) properties of milk product:soy isolate blends. As adjuncts to this study two additional projects were undertaken. Protein interaction between different protein systems could have substantial impact upon the functionality of such systems. Milk proteins were combined with soy protein and examined for heat induced interactions. Manipulation of or prevention of such interactions would have appreciable importance in achieving the most functionality from proteins. The functionality of protein blends can be improved by modification with the appropriate treatment. However, if such treatment is detrimental to protein quality the improved functionality will be of little importance. Therefore, the treatments employed in the prior analysis of functionality were assayed regarding their effect on the quality of the proteins used in this research. These three studies were designed to

complement each other. Separate aspects of protein research were brought together to more fully complete the picture.

The commercial milk products used in the study of functionality included: 1) sweet whey powder, 2) electrodialyzed whey powder, 3) whey protein concentrate, 4) nonfat dry milk, and 5) sodium caseinate. A commercial soy isolate was selected, based upon ease of dispersibility, total soluble protein, color and flavor. The materials were blended in liquid systems at various ratios and protein concentrations. The functionality of the blends was evaluated by several different tests as affected by various treatments.

The experimental design to evaluate potential heat induced interactions between the water soluble protein from soy isolate and milk proteins consisted of several heat treatments. These approximated those used in the pasteurization of liquid systems.

*In vitro* enzymatic hydrolysis was used to assay: 1) the effect of processing treatments on the availability of total amino acids and 2) whether or not potential interaction between protein systems would influence release of amino acids. *In vitro* enzymatic digests offer a viable alternative to animal assays as an option for monitoring due to their low cost, speed, and high correlation to animal assays.

The ultimate objective of any study dealing with the functionality of protein systems is to provide the information which may some day make it possible to create any type of protein food from low cost raw materials. This research provides information to those researchers working toward development of dairy:soy protein foods.

# LITERATURE REVIEW

Wolf (1970) reported that soybean proteins are commercially available as flours, grits, concentrates and isolates. The most refined form of soybean proteins are the isolates which contain not less than 90 percent protein and have a minimal of nonprotein components. Soy protein isolate is defined as the major proteinaceous fraction of soybeans prepared from high quality, sound, clean dehulled soybeans (Smith and Circle, 1973). Isolates are almost 100 percent protein and are essentially void of fiber, carbohydrate and other flavor components (Johnson, 1970). Cogan et al. (1967) observed that isolated soy protein has considerable advantages over soy flour from the viewpoint of human consumption. These include blander flavor, whiter color and excellent keeping qualities. In addition, the functional behavior can be modified to satisfy the necessary requirements. Ziemba (1966) stated that soy isolates are generally superior to flours with respect to color, texture, flavor, fiber content, ease and versatility of use. Isolates are the most expensive form of commercial soy protein.

In the past the major interest in soybeans in this country has been to obtain oil for edible purposes, with the meal being primarily used for animal feed. Soy products

have been used for edible purposes for thousands of years but mainly limited to production of edible products in the orient (Johnson, 1970). Wolf (1970) felt that the availability of a greater variety and of more refined forms would (in addition to the increasing cost of animal protein) result in greater interest in soy products.

Smith and Circle (1973) described the general procedure for manufacture of soy isolate. The usual starting material is defatted soy meal or flour of high nitrogen solubility. The flour is extracted in aqueous or mildly alkaline media under certain conditions with respect to temperature, liquid to solids ratio, pH and alkaline reagents. For edible, isolated protein production, aqueous alkaline extraction is preferably carried out at pH below 9 to avoid undue hydrolytic or rheological changes. The extract is separated from the insoluble residue by various screening, centrifuging or filtering devices. A food grade acid is then used to precipitate the protein. The curd-whey mixture is separated by means of centrifugation and several washing steps. The curd can then be either dried as the isoelectric product or neutralized with food grade alkali to form the sodium proteinate. It is usually spray dried.

Nonfat dry milk (NFDM) is the most popular dairy based ingredient (Hugunin, 1977). It imparts excellent flavor, functional properties and nutrition to products. NFDM is

manufactured by separating the fat and nonfat portions of milk by centrifugation. The skimmilk fraction is heat treated to meet the desired standards, pasteurized, evaporated to 45-50% solids and dried.

Caseinates are salts of casein. They are manufactured by isoelectric precipitation from skimmilk. After washing several times an aqueous colloidal suspension is made by the addition of alkali to pH 6.7. The suspension is pasteurized and spray dried (Hugunin, 1977). The caseinate produced is dependent upon the alkali used, although sodium and calcium caseinates are most commonly manufactured.

Whey is that portion of skimmilk remaining after the coagulation and separation of casein. The processing of whey involves centrifugal separation and clarification to remove fine curd and fat, pasteurization, concentration to 45-50% solids and spray drying. Most processed whey in the United States is identified as sweet whey, a byproduct of ripened cheese production (Hugunin, 1977).

The term whey protein concentrate (WPC) identifies all whey products which contain higher than normal concentrations of protein (Hugunin, 1977). The composition of WPC is largely a function of the process used in their preparation. The protein concentration of most WPC is 35-60% (Morr, 1976). Today, such processes as membrane filtration, reverse osmosis, gel filtration, metaphosphate complexing, CMC complexing, electrodialysis and ion exchange are used to

produce WPC. Richert (1975) discussed the principles of gel filtration to produce a WPC. The first step involves removal of insoluble proteins and lipids through a procedure requiring divalent cations. The material then undergoes evaporation and crystallization to remove lactose. The partially delactosed whey concentrate is now subjected to gel filtration to fractionate the high and low molecular weight solutes. Large scale gel filtration may utilize either a centrifugal or column process. After gel filtration the protein fraction is dried.

Richert (1975) discussed the principles of electrodialysis to remove salts during the processing of fluid whey. In electrodialysis, ion selective membranes allow passage of ionic species of one charge but not the other. The ions migrate in response to an electrical potential. Commercial operations usually employ repeating pairs of cation and anion membranes which are arranged in stacks of 100 or more cell pairs. Product is circulated through alternating pairs and an electrically conductive brine is circulated between the remaining pairs. The feed material is usually partially delactosed whey concentrate but can be either unconcentrated sweet or acid whey. After removal of charged species, the remainder is dried.

## Functionality

### Solubility

Many of the important functional properties dealing with proteins relate to water-protein interactions, i.e., solubility, viscosity, gelation, foaming and emulsification. The investigation of the functional properties of proteins can be made more efficient if a systematic study is first made of the solubility properties of that protein in a variety of environments (Mattil, 1971). Solubility behavior provides a good index of the potential and limitations of proteins (Kinsella, 1976). The pH-solubility profile is often the first property measured and can provide useful information regarding the optimization of processing procedures. Solubility is affected by a multitude of factors such as protein source and concentration, processing history, heat, ionic strength and interaction with other ingredients. The solubility profile is an excellent index of protein functionality (Kinsella, 1976).

Paulsen *et al*. (1960) determined water dispersible protein (WDP) in soy products. The results were affected by blending time and speed, pH, sample size and blade arrangement. At low pH the soy products had low WDP. It was reported by Yasumatsu *et al*. (1972) that the WDP did not correspond directly to the amount of undenatured, native soy protein. This was because some denatured protein remained soluble. Divalent salts, such as calcium or barium

chloride markedly reduced the WDP of soy protein, even at low levels (Paulsen and Horan, 1965). Wolf (1961) found that the solubility of tne major soybean protein, glycinin, was increased by addition of reducing agents and alkaline pH. Fukushima and Van Buren (1970) demonstrated that disulfide splitters such as mercaptoethanol and cysteine increased the solubility of soy milk products as did alkaline pH treatment. Chelating agents such as EDTA had little effect. The solubility of soybean proteins was at a minimum near pH 4.5 (Wolf, 1970). Solubility increased markedly with alkali treatment and after addition of disulfide reducing agents. Nash and Wolf (1967) studied the solubility of commercial and laboratory prepared soy proteins. Measurements were made in 0.5 ionic strength buffer, pH 7.6, potassium phosphate-sodium chloride buffer with and without mercaptoethanol. Five laboratory soybean globulins prepared by isoelectric precipitation had solubilities of 37-73% in buffer and 66-78% in buffer containing mercaptoethanol. Five commercial samples (isoelectric form) had solubilities of 6.0-59% in buffer and 13-83% in mercaptoethanol buffer. Seven commercial soy proteinates had solubilities ranging from 13-83% and 6.0-81% with and without mercaptoethanol in the buffer. Addition of mercaptoethanol to the buffer increased the solubility of all samples.

The solubility of WPC, soy isolate and sodium caseinate was examined by Hermansson and Akesson (1975). Solubility

was determined on 1% sols in water at 25°C. The dispersions were subjected to heat treatments of 70, 80, 90 and 100°C for 30 min. Unheated samples of soy isolate, caseinate and WPC had solubilities of 52.9, 80.8 and 78.3 respectively. Moderate heating temperatures increased the solubility of the soy isolate but had much less effect upon sodium caseinate and WPC. The solubility of these materials in 0.2 M-1.0 M NaCl was also studied by Hermansson and Akesson (1975). Addition of NaCl decreased the solubility of soy isolate but increased the solubility of sodium caseinate. Heat treated dispersions of WPC which were subjected to addition of NaCl had decreased solubilities, though nonheated samples were only slightly affected. Kelly and Pressey (1966) exposed acid precipitated soy protein to high pH levels by addition of sodium hydroxide. Solubility at pH 12 was markedly reduced. Addition of urea and mercaptoethanol increased solubility. Amiliar et al. (1977) determined the total dispersible protein in reconstituted soy milk. The amount of dispersible protein increased as the pH rose, with addition of sodium bisulfite and homogenization. The amount of soluble soy protein in soy flour was minimal near its isoelectric point but much higher in an alkaline pH range (McWatters and Cherry, 1977). Franzen and Kinsella (1976) reported that the solubility of soy isolate was increased 20, 71 and 80% according to the level of succinylation. As the amount of succinylation increased so did solubility.

Treatment of the soy isolate at alkaline pH also resulted in higher solubility. The nitrogen solubility index of soy protein isolate (as a function of pH and temperature) was determined by Hutton and Campbell (1977). Solubility was minimal at the isoelectric point while increasing rapidly as the pH diverged from that point. At high pH solubility increases were very pronounced though this was temperature dependent. The pH slope was roughly linear in the mid-temperature range and curvilinear at the extremes (4-90$^{o}$C). Bau et al. (1978) examined the solubility of cold, acid and salt precipitated soy protein. The soy fractions (10% w/v) were prepared in dispersions at pH 1.5-9.5. Minimum solubility was at pH 4.4-4.6. As the pH was brought into the alkaline region, solubility increased by 25 to 35%. Heating at 100$^{o}$C for 7 min decreased solubility approximately 50%. Continued heating had no additional effect on the rate of insolubilization.

Howat and Wright (1933) examined the effect of heating temperature upon the solubility of commercial milk powders. The samples were reconstituted in water and subjected to heating. Solubility was determined by protein analysis of the supernatant. There was an increase in solubility as the temperature rose from 20 to 50$^{o}$C. However, as the temperature increased from 50 to 100$^{o}$C solubility decreased. The solubility of caseins near their isoelectric point was investigated by Bingham (1971). As the temperature was

lowered, the solubility of the caseins was greater than at ambient temperature. Aoki and Imamura (1976) determined the solubility of casein after addition of EDTA and heating. The solubility was highest in those samples receiving both heat treatment and 5-15 mM of EDTA. The solubility of unheated casein samples containing EDTA also increased but to a lesser extent. Samples prepared in alkaline pH and heated were much more soluble than those samples receiving only the alkaline treatment. The solubility of soy isolate and milk products dispersed in Koop's buffer was studied by Hoffman (1974). As casein was replaced by soy protein, an almost linear decrease was noted in the amount of soluble protein. There was little decrease in the amount of soluble protein as the percent of soy protein increased in whey protein concentrate-soy isolate blends. Shen (1976) examined the effects of a wide range of experimental conditions on the solubility of three commercial soy isolates and a commercial sodium caseinate. Protein was quantitated by the Biuret method. The solubility of Edi-Pro N (a general purpose soy isolate) did not vary with increase in percent protein (1-10%). The solubility of this isolate was reported to be 54% compared to the 80% solubility of sodium caseinate. The solubility of sodium caseinate did not change as the equilibration temperature rose from 25-62$^{o}$C while the solubility of Edi-Pro N increased 7%. Blending speed had an appreciable effect on protein solubility. Marvopoulous and Kosikowski

(1973) reported on the composition, solubility and stability of whey powders. Eight spray dried powders and three freeze-dried, laboratory samples were analyzed. The solubility of whey powders ranged from 91.4 to 99.3%, with the freeze-dried samples most soluble. The solubility of the 11 powders was lower in 5% NaCl, ranging from 72.4 to 98.2%. Flavor evaluation by a trained panel showed the powders to have good flavor characteristics.

The pasteurization of WPC solutions at temperatures of 78.2°C/15 sec and 62.4°C/30 min resulted in approximately 20% denaturation of the protein (McDonough et al., 1974). Enzymatic hydrolysis with trypsin was used by Jost (1977) to increase the solubility of WPC. Hydrolysis was approximately 8% complete. The functional properties of electrodialyzed whey, Enerpro 50, CMC bound WPC and metaphosphate complexed WPC were examined by Morr et al. (1973). Protein solubility, whipping and emulsion capacity were determined. At pH 4, 6 and 8, electrodialyzed whey had solubilities of 92.2, 91.1 and 93.6 percent respectively. The solubilities of Enerpro 50 at the same pH values were 89.5, 90.2 and 91.2. Hidalgo and Gamper (1977) produced a WPC from sweet whey having a protein content of 88%. This product was examined for thermal stability both in the presence and absence of 0.03 M $CaCl_2$. Solubility decreased rapidly as the samples were heated at temperatures of either 80 or 134°C. When the WPC was heated at high temperatures coagulation occurred,

especially at pH values near the isoelectric point. In the presence of $CaCl_2$ heat stability was reduced over the pH range 2-12. Tryptic hydrolysis of the WPC imprcved thermal stability considerably.

Viscosity and Gelation

Many proteins absorb water and swell, thereby causing changes in hydrodynamic properties that are reflected in thickening and increased viscosity (Kinsella, 1976). Protein gels are composed of three dimensional matrices or networks of intertwined, partially associated, polypeptides in which water is entrapped (Kinsella, 1976). Gels are characterized by a relatively high viscosity, plasticity and elasticity. Viscosity and rheological properties related to flow are usually measured on dispersions, slurries or pastes using the Brookfield viscometer.

Circle et al. (1964) examined the viscosity of soy protein dispersions. Results showed that in the unheated dispersions viscosity rose exponentially with increasing concentration. At pH 6 the viscosity of 10% sols dropped considerably below that at pH 7 or above. At pH 8 and 9 the viscosities rose slightly. A 15% dispersion of isolated soy protein had a low initial viscosity but thickened noticeably when heated one hour (Hafner, 1964). Kelley and Pressey (1966) examined the effect of pH and chemical additives on the viscosity of soy protein dispersions. As alkali was added, viscosity increased rapidly. After a minimum was

reached viscosity slowly decreased, presumably due to protein degradation. Addition of low amounts of bromate and iodate significantly decreased viscosity. Disulfide cleaving agents also reduced viscosity. The viscosity of casein-soy sols dispersed in Koop's buffer was investigated by Hoffman (1974). In general, the viscosity of the suspensions decreased as casein was replaced by soy protein. Whey protein-soy sols were slightly lower in viscosity than casein-soy sols. Replacement of whey protein with soy protein slightly increased the viscosities. Fleming et al. (1974) examined the viscosities of soy isolate and soy concentrate slurries. Viscosities increased as the concentration of protein increased and as the pH was raised. Hutton (1977) studied the effect of pH and heat treatment on the viscosity of soy isolate and concentrate. Apparent viscosities increased dramatically as the temperature was raised to 90$^{o}$C. The flow properties of soy isolate, caseinate and WPC were studied by Hermansson (1975). The viscosity of protein dispersions (4-20%) were determined in a Haake Rotovisco instrument model RVI. The flow properties of soy isolate (Promine D) were characterized by low viscosities below concentrations of 8.0% protein. At 10% protein there was an enormous increase in viscosity. Reducing agents and addition of NaCl decreased the viscosity while heat, oxidation and pH treatments above 7.0 were responsible for substantial increases. The viscosity of sodium caseinate

was very concentration dependent with nearly logarithmic increase over a broad protein range. Addition of NaCl caused an increase in viscosity as did pH adjustment to pH 9.8-10. At higher pH values the viscosity rapidly declined. WPC had the lowest viscosities of all the materials studied. However, at high protein concentrations (18-20%) the material became pseudoplastic which resulted in tremendous viscosity measurements. Factors such as pH and ionic strength had little effect on viscosity. The viscosity of untreated WPC dispersions did not change appreciably until a solids level of 45% was reached. At concentrations of 10-20% the viscosities were 10 cp or less. Neither succinylation nor heat treatment significantly altered the viscosities of soy isolate at pH 7.0. Only slight changes in viscosity were observed upon addition of $CaCl_2$ (0.2-5.0%) to soy isolate at pH 7.0 and 10.0.

Guy *et al.* (1969) prepared a whey-soy flour mixture suitable for beverages. The product reconstituted in water to yield a milk with a cereal flavor. The viscosities of the soy flour-whey mixtures were relatively insensitive to heat treatment. When the total solids were greater than 45%, significant changes were associated with high temperature treatment. Homogenization reduced the viscosity and solubility index. The viscosity and thickening of several proteins were correlated with the water binding properties of model meat systems by Hermansson and Akesson (1975). WPC,

soy isolate and sodium caseinate were examined in unheated and heated dispersions. Heat (70-100°C/30 min) caused gelation of 10% dispersions of soy isolate and WPC but did not cause gelation of caseinate. Of the three proteins, soy isolate had the highest viscosity, swelling ability and formed the strongest gels. Caseinate had greater swelling ability and higher viscosity than WPC. WPC had the lowest viscosity and swelling ability of the three proteins but did form firm gels upon heating. In a further examination, Hermansson and Akesson (1975) investigated the effect of salt on dispersions of soy isolate, sodium caseinate and WPC. As the amount of NaCl increased (0.2 M to 1.0 M) there was a marked decrease in the gelation of soy isolate. The viscosity of the caseinate sols increased as the concentration of NaCl increased. Swelling and gelation were not affected. Nonheated WPC dispersions were little affected by addition of salt. However, when heated to 80°C gel strength increased rapidly as NaCl was added.

Sosulski et al. (1976) compared the functional properties of rapeseed and soybean protein products. Oil emulsification, whippability, viscosity and gelation were examined. Viscosity increased with increase in protein concentration for soy isolate. When soy isolate was subjected to heat treatment at 100°C, smooth, firm gels were formed. The oil emulsification properties of the soy isolate were much less than that of the corresponding rapeseed product. Most of

the soy isolates did not exhibit good foam properties. The swelling ability of several protein systems was studied by Hermansson (1972). The soy isolate had excellent swelling properties while sodium caseinate demonstrated much less and WPC had vitrually none. The viscosity of both soy isolate and sodium caseinate was much higher when examined over a broad concentration range than WPC. The swelling ability of soy isolate decreased as the ionic strength increased. WPC was essentially unaffected by increase in ionic strength. The swelling ability of caseinate increased rapidly as the pH went up or down.

Ehninger and Pratt (1974) studied the effect of sugars, NaCl, and freeze thaw cycles on the gelation and stability of soy protein dispersions. Gelation was also studied at low protein levels and at pH ranges feasible in a food system. Gelation was measured with a Brookfield RVD viscometer. Viscosity tended to increase exponentially as the concentration of protein increased, though it was pH dependent. Viscosity was much higher at elevated pH. Addition of 5 or 10% sucrose to the dispersions decreased the viscosity of the gels while addition of dextrose at these same levels resulted in slight increase. Dispersions containing 0.2 M NaCl at pH 6.5 had decreased viscosities. Modler and Emmons (1977) prepared a soluble WPC by adjusting the pH of sweet whey to 2.5-3.5 prior to heating at 90$^{o}$C for 15 min. Concentration of the whey prior to heating or addition of

iron increased protein recoveries but decreased solubilities. The viscosities of the reconstituted samples (33% total solids) ranged from 4,000 to 36,800 cp. At protein concentrations of 2, 4, 6 and 8%, gelation occurred when this WPC was heated at 95°C for 20 min. The samples retained excellent color and flavor. Kalab et al. (1971a) reported on gels produced from NFDM. The gels were prepared by slurrying the material (50% NFDM) into sausage casings and heating for 10 min in boiling water. A penetrometer was used to measure firmness. This study was continued by Kalab et al. (1971b) when he examined various physical factors influencing the firmness of heat induced milk gels. Aqueous dispersions of 40-60% NFDM were heated at temperatures of 80-100°C for 10 to 30 min. Temperatures below 80°C did not induce gelation of 50% NFDM dispersions. Gel firmness was maximum at 100°C and high milk solids concentration. Kalab and Emmons (1972) investigated the effect of chemical additives upon the firmness of heat coagulated milk gels. Several additives (0.25 M) were screened for effectiveness. Divalent cations, especially $Ca^{++}$ promoted gelation as did the addition of oxidizing and crosslinking agents. Addition of reducing agents and sulfhydryl blockers to the skimmilk resulted in soft, sticky gels. Neutral salts such as NaCl and KCl had no effect on gel firmness. Catsimpoolas and Meyer (1970) examined the gelation phenomenon of soybean globulins. Aqueous dispersions of the proteins (8-10%) were

prepared and heated at specified temperatures. A Brookfield viscometer equipped with a helipath stand was used to measure apparent viscosity. The minimum protein concentration necessary to obtain a self supporting gel structure was 8 percent. Highest viscosity values were obtained at neutral or mildly alkaline pH. Both high and low pH decreased gel strength. Gel firmness was greatest when the samples (pH 7.0) were heated at 80°C for approximately 30 min. Continued heating at this temperature had no detrimental effect though excess heating at higher temperatures substantially reduced gelation. The viscosity of globulin dispersions in NaCl decreased as the amount of NaCl increased. Gels were prepared by Catsimpoolas and Meyer (1971a) from dispersions of soybean globulins in water-alcohol and water-glycol mixtures. The viscosities of the heated slurries were determined after cooling with a viscometer. Rheological data demonstrated that these mixtures formed gels of higher viscosity than water dispersions. Apparent viscosities increased as the length of the aliphatic chain increased. Solvents such as acetone, dioxan and dimethyl formamide also markedly enhanced gel viscosity. The role of lipids in the gelation of soybean globulins was examined by Catsimpoolas and Meyer (1971b). Protein-lipid dispersions were prepared by addition of protein to the water-lipid mixture and heating. Gels produced from mixtures containing saturated fats had higher viscosities than those produced with unsaturated fat. As the length

of the fatty acid chain length decreased, viscosities increased. The role of sulfhydryl groups on the physical characteristics of tofu like gel products was investigated by Saio et al. (1971). The texture of the tofu became softer and more adhesive when sulfhydryl blocking agents were used. Saio (1973) found that the strength of soy protein gels was increased by the addition of calcium salts. Gels made from the 11S protein were firmer than those made from 7S protein. Heat coagulated gels from soy protein isolate were hard and elastic (Yasumatsu et al., 1972a). Denatured soy isolates were more desirable because partial denaturation was believed to be conducive to preparation of firm gels. Soy isolates with a nitrogen solubility index (NSI) of 50 had better gel formation qualities than one with a NSI of 80 (Yasumatsu et al., 1972). Circle et al. (1964) reported that soy protein dispersions readily gelled when heated. At protein concentrations of 10% gelation was best when the dispersions were heated at 100°C for 45 min. Addition of sodium sulfite and free cysteine substantially reduced gelation while salts had much less effect. At protein concentration greater than 10% heating could be reduced without loss of gelation. The effect of heat, pH, ionic strength and protein fraction on the expansion characteristics of soy gels were examined by Saio et al. (1974). Expansion increased as the temperature of heating rose from 100 to 132°C. Calcium gels had greater expansion and were

more elastic than acid gels. Fleming and Sosulski (1975) evaluated the gelling ability of aqueous dispersions of 10% protein from soybean flours, concentrates and isolates. Gelation was induced by heating the slurries in sealed containers at 90°C for 45 min in a water bath. After cooling in an ice bath, viscosities were determined. Uniform firm gels were produced from the soy isolate. A pH cycling treatment, 7 to 12 to 7, increased gelation strength of the gels.

The viscosities of soy proteins prepared by cold, acid and salt precipitation were compared by Bau et al. (1978). Viscosities were determined on 10% protein slurries. Moist heating at 100°C increased the viscosity of all fractions. Prolonged heating at this temperature resulted in sharply decreased values. Viscosities were at a minimum near the pH of their isoelectric point (4.5). As the pH diverged from this point in either direction, viscosities increased.

McDonough et al. (1974) formed firm, resilent gels from 10% WPC solutions which did not leak after standing for several hours. Heat denaturation resulted in what the author described as classical gel structure. Schmidt et al. (1978) studied the heat induced gelation of peanut/whey protein blends. Heating at 70°C or less resulted in no gel formation. Heating temperatures of 100°C-45 min were necessary to obtain satisfactory gelation. Qualitative techniques showed no difference in gel strength as the pH was raised from 7 to 10. At pH 11 the gels were very soft with

measurable browning. Gels formed satisfactorily when 25% of the protein was from peanut flour. Sodium chloride (up to 0.5 M) increased the gel strength of the whey protein gels. Addition of $CaCl_2$ at levels of 10 and 30 mM resulted in increased gel firmness.

Emulsion Capacity

The emulsifying properties of food proteins are a primary functional characteristic. Three different procedures, i.e. emulsifying capacity, emulsion stability and emulsifying activity are used in the investigation of this property (Kinsella, 1976). Emulsion or emulsifying capacity (EC) is the method most commonly used, and is defined as the volume of oil (ml) that can be emulsified by protein (gm) before phase inversion occurs.

Swift et al. (1961) developed a method to determine EC. This procedure, known as the viscosity drop method, utilized a high speed mixer and melted lard to prepare the emulsions. The mixer speed, rate of fat addition and temperature affected EC. Carpenter and Saffle (1964) developed a method to determine EC subjectively. An "Osterizer" was modified to accompany an inverted ball jar. Formation and collapse of the emulsion was viewed through the glass jar. Blender speed, mix temperature, protein aliquot and rate of oil addition affected the final volume. A method (using sausages) was described by Inklaar (1969) as being capable of

accurately evaluating protein emulsion capacity and stability. Using this procedure, soya isolate had better EC than sodium caseinate. Both products had better EC at higher pH. Becher (1966) suggested electrical conductivity as a method for determining types of emulsions. Electrical resistance was used by Webb et al. (1970) to measure EC of protein. Corn oil was delivered in close proximity to the propeller blades and mixed with the protein extract at high speed. Emulsion formation and collapse were monitored by a ohm recorder measuring resistance. Precise control of blender speed, rate of oil delivery, and location of delivery tube were critical in obtaining repeatability. The validity of using electrical a-c impedance and d-c resistance methods in the evaluation of EC was investigated by Haq et al. (1973). For dilute, aqueous systems undergoing agitation, Webb's et al. (1970) procedure was shown to be satisfactory. In highly viscous systems, a-c impedance measurements had greater accuracy. Marshall et al. (1975) used a red colored dye (oil-red O) to determine the EC of protein slurries, including soy isolate. The author claimed greater accuracy for this method due to the increased visibility of the inversion point.

Morr et al. (1973) investigated the EC of several different WPC and found similiar results for all samples tested. The EC ranged from 32-40 g corn oil for a 0.1% solution except for CMC complexed WPC which was double that of the

other WPC. The effect of enzymatic hydrolysis on the EC of whey protein was studied by Kuehler and Stine (1974). EC was not significantly affected by proteolysis with prolase. Emulsion capacities were approximately 3.0, 1.9 and 2.2 g/ oil/mg protein for dried whey, whole casein and nonfat dry milk.

Pearson et al. (1965) compared the EC of soy sodium proteinate, potassium caseinate and NFDM by the viscosity drop procedure. The EC of soy was greatest at pH 10.7 and $\mu$=0.05. At higher ionic strength (0.3) EC decreased slightly. At pH 5.1 EC was much lower. Potassium caseinate had satisfactory emulsifying properties in water at pH 6.9. Emulsion capacity was highest at pH 10.4 and ionic strength 0.05 while lowest at pH 5.4. NFDM had slightly less EC than potassium caseinate at high pH. The EC of NFDM was greatest at pH 5.6. At low concentration NFDM had good EC while at high concentration EC decreased. Several protein additives were assayed by Smith et al. (1973), including soy isolates, flours, concentrates and NFDM. EC and stability were evaluated in frankfurter emulsions. In this system animal proteins had better EC than the soy products. Hoffman (1974) reported that the EC of casein-soy blends decreased as the amount of soy protein increased. In addition, the ability of WPC to emulsify oil decreased significantly as soy protein was substituted for whey protein. Crenweldge et al. (1974) investigated the EC of NFDM, soybean concentrate, cottonseed

flour and bovine hemoglobin. A viscosity drop procedure was utilized in determining EC. Both soy and NFDM had emulsion capacities resembling pH solubility profiles. At pH values near the isoelectric point NFDM and soy exhibited minimal EC. As the pH increased to 7.0 and above, EC increased rapidly. NFDM had greater EC than the soy concentrate. Various proteins were compared by Lauck (1975) as fat binders in sausage products. The proteins included Enerpro 50 (WPC), dried sweet whey, NFDM and promine D (a soy isolate). Binders were added either as dry ingredients or predispersed in water. Following laboratory testing, pilot plant production of sausage products containing the binders was initiated with subsequent evaluation. The laboratory tests indicated that, as a class, protein containing binders might be better than meat proteins in binding fat. The pilot plant evaluation revealed that the frankfurters produced with the various binders were of good quality but did differ in several aspects. The whey product sausages had thin emulsions while the soy isolate frankfurters had a definite off-flavor. Yasumatsu et al. (1972) examined the emulsion activity of soy products. Systems containing both skim milk and soy flour had the highest emulsion activity. Soy protein extract had more emulsifying activity than soy protein isolate in a sodium chloride brine. High correlation coefficients were observed between dispersible nitrogen and emulsifying properties.

Succinylation enhanced the emulsifying capacity of soy isolate dispersion (Franzen and Kinsella, 1976). When succinic anhydride was added on a g to g basis with protein EC doubled. McWatters and Cherry (1977) reported on the EC of defatted soybean flour. Suspensions, prepared in distilled water were evaluated at several pH values. Maximum emulsification was at pH 6.5. The emulsion properties of a soy concentrate and isolate were examined by Hutton and Campbell (1977). The interdependent influence of pH and temperature on EC was reviewed. Soy dispersions were studied at pH 5.0, 6.0 and 7.0 with temperature treatments of $4^{o}C$ and $90^{o}C$. Emulsification was significantly affected by such treatment. At low pH emulsification was substantially less, probably due to the loss of solubility.

Whipping Ability

Foaming or whipping, i.e. the capacity to form stable foams with air, is an important functional property of many foams (Kinsella, 1976). Foaming properties include whippability or foamability, both of which are used interchangeably. These properties are measured by foam capacity, foam expansion or overrun, all of which refer to the volume increase of a protein dispersion following incorporation of air by agitation or whipping. Foam stability refers to the ability of foam to retain its volume over time.

Webb (1941) examined the foaming capacity of reconstituted skimmilk by whipping at 1000 rpm's. Foam stabilities

and specific volumes were determined for samples containing 10-30 percent solids. As the concentration of solids increased to 20%, specific volumes decreased but stability times increased. Whipping properties were reduced when severe heat treatments were employed. Peter and Bell (1930) investigated the foaming characteristics of normal and modified whey protein solutions. Untreated whey had poor foaming ability, though heating to 60°C resulted in some improvement. Addition of $Ca(OH)_2$ had a pronounced effect, significantly improving the whipping quality of the whey protein. When the solution was neutralized, the improvement in whipping was still evident. Addition of NaOH or calcium salts failed to have the same effect. However, when calcium salts were added in an alkaline medium an excellent foam was produced. The addition of sodium sulfite considerable improved whipping characteristics.

Tamsma et al. (1969) described the increased foaming from skimmilk, homogenized prior to drying. NFDM produced in this manner was whipped to form a stable foam after reconstitution in water. The percent overrun and the stability of the resultant foams was dependent upon homogenization pressure and percent total solids. Foam stability was optimal at 30% total solids.

The whipping properties of unheated WPC were studied by Morr et al. (1973). Metaphosphate complexed WPC failed to form stable foams while CMC complexed WPC had the most

stable foams of any of the materials tested. None of the WPC had as high an overrun as sodium caseinate. The whipping properties of spray-dried whey protein/CMC complexes were examined by Hansen and Black (1972). The powder was resuspended in water and whipped to foam resembling egg white. A 4% protein sol whipped for 15 min proved optimal. Heat treatment and homogenization decreased both specific volume and foam stability. Foam development increased rapidly as the pH went from 5 to 9. Addition of $Ca(OH)_2$ resulted in the greatest improvement in whipping quality. Addition of $H_2O_2$ increased foaming with the best results achieved at a concentration of 0.1%. At higher levels of $H_2O_2$ foam development deteriorated. Addition of 3% sucrose after whipping improved foam stability. Foam stability was reduced when soy isolate was added to the sols. Jelen (1973) conducted whipping studies with delactosed cheese whey. The concentrated product was whipped for 10 min. The unheated whey protein had poor whippability, poor foam stability and low overrun. Heating to 90°C improved all whipping properties, especially after the heat denaturable, acid precipitable proteins were removed. Overruns greater than 2000% were achieved with the acid supernatant at 34% total solids. Addition of 5-40 g soluble starch per 100 g of the whey concentrate increased the foam stability but resulted in lower overruns. Devilbiss *et al.* (1974) examined WPC as a possible replacement for egg white in angel food cakes.

Commercial samples of WPC were obtained and dispersed in water at a concentration of 30%. The samples were whipped for 10 min prior to baking. Samples heated at 55°C for 60 min had increased foam stabilities which did not collapse when heated. The whipping properties of WPC were studied by McDonough et al. (1974). Untreated solutions did not form stable foams unless the sols contained at least 25% protein. Heat treatment significantly improved the whipping characteristics, apparently due to partial denaturation of the protein. Adjustment of the pH upward with $Ca(OH)_2$ resulted in excellent foams which were not duplicated by adjustment with NaOH. Samples subjected to both heat and alkaline pH treatment had excellent whips with stability times of several hours. The effect of enzymatic hydrolysis on the whipping properties of whey protein was studied by Kuehler and Stine (1974). Heat treatment was a necessary prerequisite to insure foam production and stability. Specific volumes (SV) ranged from 10-15 ml/g with ½ stability times, up to a temperature of 85°C. The data indicated that the greater the net charge on the protein, the greater was the tendency of the sols to foam. Thus, at pH 9 and 2, foams were produced with the largest SV, though acid foams had low stability times. Stability increased with alkalinity above pH 6.0. Addition of 0.1% CMC and heating to 85°C resulted in doubled stability times. Addition of 0.5% CMC increased stability times additionally. Four percent casein sols had

foams of poor stability. Enzymatic hydrolysis (prolase) for one hour increased SV. Additional digestion slightly lowered SV. Stability times decreased through the first hour of digestion and then leveled off. A limited amount of hydrolysis was desirable to increase foaming properties, though excessive digestion decreased SV and stability of the foams. The foaming capacity (FC) of 10% WPC sols was determined by bubbling $N_2$ through the solution at a constant rate and noting the expansion in a given time (Cooney, 1976). Foam stability (FS) was measured by recording the time required for ½ the liquid volume to drain from the foam. FC was enhanced by removing triglycerides via ultracentrifugation. Instantaneous heating to 60°C overcame the inhibitory effect triglycerides had on FC. FS was inversely dependent on the ionic strength of the WPC. Calcium and barium ions decreased FC more than monovalent cations at pH 4.3. Phosphates and tripolyphosphates improved foaming at pH 4.3 but not at pH 7.0. Foam overrun was optimum at pH 8.0. Foaming was improved by addition of 0.50 mM SDS but not by Tween 20 or Triton X-100. Trypsin hydrolysis of WPC increased the overrun but slightly decreased the FS. Min and Thomas (1977) investigated several variables which affected the physical properties of dairy whipped toppings. These included: stabilizer and emulsifier concentration, milk protein fraction, homogenization pressure and interaction among selected ingredients. Stabilizer level markedly affected overrun and

firmness. Toppings made with a stabilizer concentration of 0.3% had maximum overrun. Emulsifier concentration and type had a positive influence on overrun and firmness. The milk protein fraction (skim milk colloid, whey protein, CMC precipitated milk protein, sodium caseinate and NFDM) had a very significant effect on the foaming capacity. Toppings made with sodium caseinate and whey protein were unsatisfactory. Calcium (50 mg/ml) was added prior to whipping of some topping formulations. Increased overrun and firmness were noted. Tripling the $Ca^{++}$ concentration substantially increased overrun. Hoffman (1974) examined the whipping properties of soy isolate and milk products at low protein levels. Replacement of casein with soy isolate had little effect on SV but drastically reduced FS. Whey and soy mixtures maintained their SV but were so unstable that drip could not be measured.

Watts (1937) investigated the whipping ability of commercial soy flours and a solvent extracted laboratory prepared soy flour. All commercial products tested had little or no whipping ability. Petroleum ether, dry heat and pressure were used to produce the soy flour. This product, reconstituted in water, whipped to a stiff white foam resembling egg white. The material whipped best at concentrations of 7-8 percent and at pH values far removed from its isoelectric point. Salt (NaCl) at concentrations up to 2 percent increased the whipping ability. The extraction of a

substance responsible for the whipping ability of soy flour was reported by Watts and Ulrich (1939). It was apparent that neither the glycinin nor the legumelin were the active substance. Stable foams were produced by water dispersing 3-10% purified soy protein. The dispersions were heated prior to whipping. Stable foams were produced at all pH's except 3-6. Franzen and Kinsella (1976) found that succinylation markedly enhanced the FC and FS of soy isolate dispersions. The values were approximately double that of the unmodified isolate. Addition of NaCl also increased the FC of native soy isolate while addition of sucrose decreased FS. Soy isolate was subjected to an alcohol wash prior to whipping (Eldrige et al., 1963). The samples were heated for 15 min in a boiling water bath prior to whipping. The low density foams had stability times of approximately 200 min. The alcohol washed protein had improved color and flavor. Stable foams were produced below pH 3.0 and above 6.5. Minimum foam stability (in addition to the lowest volume foams) occurred in the isoelectric area of protein. Heating the protein enhanced foam expansion. Addition of 5.0% NaCl resulted in foams of low stability. The addition of tripoly and hexametaphosphate had little effect on the whips. Yasumatsu et al. (1972) measured foam expansion and stability in model systems containing soybean proteins. Native soy flour had high foam expansion and stability. Denatured forms of soy flour had poor whipping

characteristics. The highest whipping values were from systems containing both soy flour and skimmilk. The whipping properties of soy flour were maximum at pH 6.5 (McWatters and Cherry, 1977).

Sensory Evaluation

Flavor is perhaps the most important property in determining food acceptability and where flavor is the less dominant trait, mouth feel or texture assumes greater importance (Kinsella, 1976). Color, odor, flavor and texture are key attributes in deciding whether or not a new protein will be used in a food product. Proteins affect texture in sols, gels, foams, emulsions and extruded foods. The flavor of a food product can be affected by proteins due to browning reactions, sulfide elimination, proteolysis and by entrapment and binding. Color can be significantly affected by browning reactions. Altering a protein source may result in flavor changes in the food. These flavors may be contaminants of the protein per se, or they may be generated during processing and storage.

The astringency in milk products was thought to be caused primarily by heat altered whey proteins and milk salts (Josephson, 1967). Both of these groups were associated, by adsorption or by interaction, with the casein micelles. Lang et al. (1976) examined the influence of compositional variations and processing on the sensory

properties of skimmilk. Pasteurization at 79.4°C and 85.0°C produced a heated flavor noticeable to trained panelists. Variations in homogenization pressure and fat content did not result in detectable flavor or mouthfeel differences. Judges detected differences between samples with and without 200-300 ppm of stabilizer when the samples were heated at 85°C. The panelists were not able to detect 400 ppm of emulsifier unless fat contents were below 0.5%. The addition of stabilizers increased viscosity while addition of emulsifiers did not.

Sensory evaluation of casein-soy sols revealed a significant decrease in flavor scores as casein was replaced with soy protein (Hoffman, 1974). The odor of soy isolate was described as beany. No color change was noted as soy isolate replaced WPC in sols though the blends were described as having a beany odor. Maga and Lorenz (1973) conducted a sensory and analytical flavor evaluation of NFDM, sodium caseinate, isolated soy proteinate and various other protein supplements. Odor intensities and flavor evaluations were repeated randomly by 20 taste panelists using a scale of 1 to 10. A product with a completely bland character received a score of 10 while a strong characteristic was scored 1. Of the 12 products examined, NFDM had the most bland odor and flavor when tested in a rehydrated system. As a group, the milk protein supplements were the most bland products. The soy products had lower flavor and odor

scores. In another study Maga and Lorenz (1972) examined the flavor and odor intensities of milk, marine and vegetable protein supplements. Sensory panel scores were recorded for odor and flavor properties. Milk products included NFDM, sodium caseinate, whey powder and demineralized whey powder. Soy products included soy isolate, soy concentrate and soy flour. There were no statistical differences between odor intensities of any of the milk products. Many of the vegetable products had less bland odor properties. Reconstituted NFDM was judged to have the most bland flavor. The flavor of soy isolate was judged to be statistically inferior. Demineralized whey powder was considered to be more bland than standard whey powder. As a group, the milk products had a definite flavor advantage over the vegetable products.

Kalbrener et al. (1971) reported on taste panel studies on commercial soy flours, concentrates and isolates. Samples were evaluated in 2% dispersions in water. A raw defatted flour prepared in the laboratory received an odor score of 5.8, a flavor score of 4.1 and was described as beany and bitter. Commercial flours generally were rated higher, ranging from 4.2 to 6.7 for flavor. Concentrates had flavor scores ranging from 5.6 to 7.0. Isolates scored from 5.9 to 6.4. It was generally agreed that beany and bitter flavors persisted in isolates, though at low levels. An inverse relationship was found between flavor score and solubility. Raw flour had the highest solubility but lowest

flavor scores. Maga (1970) compared the flavor profile of commercial isolate and raw soybeans of the same source. Overall intensity scores revealed that the isolate had the blandest flavor and odor properties. Processing decreased the green and bitter flavors, probably due to the sweet like flavor which appeared. Repeated washings with water and solvents removed some but not all of the objectionable soy flavor.

Bitterness was found in a partial proteolytic hydrolyzate of soybean protein (Yamashita et al., 1969). Arai et al. (1970) pointed out that enzymatic proteolysis of soy resulted in the formation of bitter compounds. This bitterness was caused by peptides containing leucine at the C-terminal. In an earlier study Fujumaki et al. (1968) found that the beany flavor generally decreased during the early stages of enzymatic digestion, though the bitter flavor increased. Bitterness was found in soybean digests after partial hydrolysis with pepsin (Fujumaki et al., 1970). Johnson (1975) reported that enzymatically modified soy proteins could be used as whipping agents, though there might be problems with flavor due to bitterness.

Mattick and Hand (1969) investigated the raw, green bean like flavor of soybeans which they described as the major sensory defect of soybean products. A compound was isolated and identified as ethyl vinyl ketone which had a green bean like flavor. Pelissier (1976) compared the bitter

taste of enzymic hydrolysates from cow, ewe and goat caseins. Caseins from bovine milk were generally more bitter than the other caseins. The total hydrophobicity of the protein and the nature of natural proteases were important in the development of bitterness.

Sensory evaluation by a trained panel showed that alkali pretreated soy had better flavor properties than the water soaked control (Badenhop and Hackler, 1975). However, this treatment did result in partial destruction of the amino acid cystine. The use of alkali treatment to improve the flavor of soymilk was explored by Bourne et al. (1976). Addition of NaOH caused a rapid increase in pH while addition of $Na_2CO_3$ or $NaHCO_3$ caused much less. An experienced taste panel demonstrated a greater acceptability for soymilk adjusted to pH 7-7.5 with NaOH but noted a soapy flavor with the other compounds. Soymilks were then prepared with sodium salts at the same sodium ion concentration that was used in the original tests with NaOH. These samples were given approximately the same scores by the panel as the NaOH treated group, even though the pH was not in the same range. This led the authors to conclude that the sodium ion concentration rather than the pH was the effective mechanism.

The effect of chemical modification on flavor was investigated by Franzen and Kinsella (1976) who examined the role of acetylation and succinylation on the sensory properties of soy protein. The color of the sols was lightened

and no flavor problems were reported by these treatments.

Cowan et al. (1973) reviewed the flavor components of soybean products. Steam treatment in combination with alcohol extraction produced soybean flakes with flavor scores of 7.0 (1-10 scale, 10 most desirable). Beany and bitter flavors were still detectable even at low concentrations. The effect of hexane:ethanol azeotropic extraction on the organoleptic qualities of defatted soy isolates was investigated by Honig et al. (1976). Flavor and odor scores were based on a ten point system (1 strong, 10 bland). Following azeotropic extraction the isolate had a flavor score of 7.2 which was an improvement over the original score of 6.2. Odor intensities were lower and were judged to be comparable to sodium caseinate. Eldridge et al. (1977) examined alcohol treatment as a mechanism for reducing the beany flavor of soybean protein products. Raw soybeans and soybean products were steeped or wet milled with ethyl alcohol to inactivate enzymes in situ or with disruption of cellular disruption. When aqueous alcohol was used enzymatic activities were reduced and flavor properties were improved. Flavor scores improved from 6.1 (control) to 7.7 for a soy isolate.

Yasumatsu et al. (1972) observed that almost all types of soy bean products have some undesirable flavor characteristics. These defects have proven to be the greatest barriers to increased use of soybean products in food.

Principle component analysis was used to analyze the flavor profile of soy isolate. Even though soy isolate was the most purified form of soy protein, it still had flavor problems. Johnson (1970) made the observation that the use of soy products in human food has largely been limited due to flavor problems.

## Heat Stability

### Soy Protein

The nomenclature system based on sedimentation coefficients has been used extensively for soybean proteins (Smith and Circle, 1973). Four main fractions have been isolated with sedimentation coefficients of 2S, 7S, 11S and 15S. The 2S fraction is a multiprotein component with molecular weights ranging from 8,000-24,000. The 7S globulin has a molecular weight of approximately 180,000 and is composed of many subunits. The 11S fraction has a molecular weight of approximately 350,000 and is also composed of many subunits. Together the 7S and 11S comprise between 65 and 70% of the total protein. The 15S component is a large, molecular weight species having a molecular weight of approximately ½ million.

Mann and Briggs (1955) examined the effect of heat on soybean proteins using electrophoretic analysis with a Tiselius apparatus. Protein sols were heated by immersing in an oil bath for the desired time and temperature.

Temperatures ranged from 45°C to 90°C with heating times of 2 to 30 hr. Electrophoretic patterns varied widely with heat treatment with some apparent aggregation. The quantity of protein precipitated increased with increased temperature and length of heating. Watanbe and Nakayama (1962) described the formation of soluble aggregates during heating of water extracts from defatted soy meal. Ultracentrifugal analyses indicated that the 11S, 7S and 15S fractions partially disappeared after 10 min at 80°C or higher temperatures.

The major reserve protein of soybeans, glycinin was heated by Catsimpoolas et al. (1969) at temperatures of 35-90°C for 1 hr. No significant changes were observed to occur from 35-50°C and only slight change was noted between 50-70°C. When the protein was heated at temperatures above 70°C a sudden alteration occurred. Examination by disc electrophoresis revealed significant dissociation of the protein into subunits. The protein was not completely dissociated as some undissociated glycine could still be detected. Some precipitation of the protein occurred at 90°C. Wolf (1970) reported that heating dilute solutions of 11S globulin caused about one half of the protein to precipitate while the remainder was converted into a 3-4S form that remained soluble.

Wolf and Tamura (1969) heated 0.5% solutions of 11S fraction at 100°C in a pH 7.6, $\mu$=0.5 buffer. The solution

became turbid and precipitation occurred. The 11S protein disappeared in less than 5 min and a soluble aggregate of 80-100S appeared. With continued heating the aggregate grew and precipitation occurred in 7 min. Disappearance of the 11S was accompanied by appearance of a 3-4S component. This fraction reached its maximum in 5-7 min and was stable to heat for more than 30 min. Saio et al. (1971) investigated the effect of heating on the 7S and 11S fractions of soybean protein. The proteins were heated at temperatures of 0-100°C for 1 min. After heating at the higher temperatures (70°C and above) several fast moving bands appeared on polyacrylamide gels. The less mobile 7S and 11S bands disappeared. The appearance of the fast moving bands seemed to coincide with an increase in sulfhydryl groups.

The antigenic changes involved in the thermal denaturation of glycinin, the major reserve protein of soybeans were reported by Catsimpoolas et al. (1971). The protein, consisting of multiple subunits was dissociated by heating into both soluble and insoluble complexes. Glycinin was heated at temperatures of 30-90°C for 30 min. Disc electrophoresis was used to demonstrate the changes occurring to the native protein. The gel patterns from samples heated to 70-75°C were almost identical to the unheated sample. As the temperature rose to 80°C and above, the intensity of the glycinin band gradually declined and disappeared entirely at 90°C. Concurrent with the disappearance of

glycinin, several faster migrating components appeared in the gels. In addition, the amount of glycinin not entering the gels increased.

The fate of water soluble soy protein during thermoplastic extrusion was examined by Cumming et al. (1973). The extruded material consisted of commercial defatted soybean meal which had been cooked to temperatures of 93, 121, 149, 177 and 204°C. After processing, a portion was extracted with distilled water and subjected to PAGE in an alkaline system on 7% gels. As the cooking temperature rose the concentration of those components close to the origin slowly disappeared and bands with faster migration rates appeared. Six major fractions were found in these gels, though differing in intensity. Fractions A and B decreased with increasing temperature while fractions D and E increased. In the unprocessed meal, dense bands were identified as 7S, 11S and 15S fractions. At elevated cooking temperatures there was a reduction in all three fractions, accompanied by a noticeable increase in breakdown products.

Saio et al. (1975) induced soy 7S and 11S proteins to gel by heat treatment at 100-170°C. After heating the samples, the material was solubilized with a solution containing 0.075 M sodium dodecyl sulphate (SDS) and 0.025 M mercaptoethanol (ME). After dialysis, a portion was submitted to both disc and SDS polyacrylamide gel electrophoresis (PAGE). Disc PAGE was difficult to interpret because

of the large number of minor components which resulted in substantial diffusion. With SDS electrophoresis, the unheated, cold insoluble fraction (CIF) resolved into two principal bands and several minor ones. Similar patterns were obtained by heating to temperatures of 130°C. No bands were observed at heating temperatures above 150°C. The effect of heat on the PAGE patterns of crude 7S gel was similar to that observed for the CIF proteins. In a further study, Saio et al. (1975) investigated the breakdown of protein subjected to heat treatments greater than 150°C. Sodium dodecyl sulfate and disc electrophoresis with o-phthalaldehyde as the stain were employed. The authors concluded that the gross structure of soybean subunits degraded to form lower molecular weight substances by heating at temperatures above 150°C.

Aldrick (1977) examined selected milk and soy fractions for specific heat induced interaction. Soy 7S and 11S proteins were prepared from whole soybeans. Sodium caseinate and β lactoglobulin were prepared from fresh whole milk. Soy-milk protein combinations were heated in a 1:1 ratio at three temperatures; 63, 74 and 121°C. After cooling and centrifugation, aliquots of the protein were electrophoresed in the appropriate gel system. The gel bands corresponding to the 7S fraction diminished in intensity at 63°C/30 min and vanished at higher temperature. The soy 7S fraction was found to move independently of either sodium caseinate or

β lactoglobulin. The soy 11S fraction was more stable to heat treatment at 74°C than the 7S fraction. The 11S fraction also moved independent of either β lactoglobulin or sodium caseinate.

Milk Proteins

Caseins are those phosphoproteins precipitated from raw skimmilk by acidification to pH 4.6 at 20°C (Whitney et al., 1976). Caseins can be divided into four main groups based upon their electrophoretic mobilities: $\alpha_s$ caseins, β caseins, k-caseins and γ-caseins. The $\alpha_s$-caseins consist of one major and several minor components. Approximately 45-55% of the skimmilk protein is in this fraction. The molecular weight of $\alpha_s$-casein is about 23,000 (Rose et al., 1970). The milk protein present in the second largest amount is β casein which accounts for 25-35% of the total skimmilk protein and has a molecular weight of 19,000. The remaining two fractions k and γ represent 8-15 and 3-7% of the total skimmilk protein.

The whey proteins are those remaining in the serum after precipitation of casein by addition of acid (Whitney et al., 1976). The major whey protein, β lactoglobulin is responsible for 7-12% of the total skimmilk protein and has a molecular weight of 18,000. The whey protein present in the second greatest amount is α lactalbumin which has a molecular weight of about 14,000 and represents 2-5% of the total skimmilk protein. The immunoglobulins are a

heterogeneous group of proteins with molecular weights ranging from 160,000 to 1,000,000. They represent 1.9-3.3% of the total skimmilk protein. Bovine serum albumin accounts for 0.7-1.3% of the skimmilk protein and has a molecular weight of approximately 66,000. The proteose peptone fraction is a multicomponent group with molecular weights ranging from 4,000 to 41,000. They represent approximately 2-6% of the total skimmilk protein.

Pasteurization at 67°C/30 min produced no appreciable change in the nitrogen distribution of whole milk (Shahani and Sommer, 1951). Pasteurization at 73°C/30 min resulted in a decrease in the globular nitrogen and an increase in the NPN. Sullivan et al. (1957) examined the changes occurring in the centrifugable nitrogen fraction of skimmilk as a function of time following several heat treatments. Portions of skimmilk were heated for one min at 75, 104, and 132°C, cooled and centrifuged. All samples had a marked decrease in protein content after heat treatment. The decrease in nitrogen was roughly proportional to the increase in heat treatment.

Josephson et al. (1967) heated selected milk systems at various temperatures to determine astingency flavor response. Sephadex gel filtration, PAGE, electron microscopy and chemical analyses were used to determine changes in the size, shape and composition of protein-salt particles. The astringent components in rennet whey, heated at 63-90°C

for 30 and 10 min were quite large as evidenced by their sedimentation patterns. The proteins in heated rennet whey (90°C/10 min) were present as aggregates of sufficient size to prevent their migration into the gel (PAGE). Heating for 2 hr was required to produce distinct astringency in caseinate ultrafiltrate systems.

Morr (1965) investigated the effect of heat upon the composition of the protein aggregates in normal and concentrated skimmilk systems. A portion of unheated skimmilk was used as the control with the remaining milk heated to 88°C for 10 min. After heating and cooling aliquots were fractionated by ultracentrifugation. The centrifugal supernatant and sediment fractions were then examined for compositional makeup. Heating skimmilk at 88°C for 10 min had no significant effect upon the protein particle size distribution or chemical composition of the protein-sediment fractions.

In another study, Morr (1969) investigated the extent of protein aggregation-disaggregation produced by heating skimmilk at 90°C for 10 and 30 min. An additional series of high temperature-short time treatments (UHT) extending to 149°C for up to 16 sec were also examined. Heating at 90°C caused formation of $\geq 75 \leq 500S$ protein particles whereas UHT treatment favored formation of larger size particles. It appeared that UHT treatments in excess of 127°C for 16 sec favored formation of nonsedimenting

nitrogen compounds, presumably by disaggregating casein micelles. Similar amounts of whey protein disappeared from the 90°C heated skimmilk ultracentrifugate as from comprable pH 4.6 whey systems. Whey proteins heated at 90°C for 10 and 30 min showed extensive denaturation and subsequent aggregation when subjected to zonal electrophoresis. Progressively higher UHT treatments produced gradual reduction in the quantity of all whey proteins. Heat treatment at 90°C for 10 and 30 min did not substantially alter the zonal electropherograms of casein. Heating milk to 90°C caused substantial aggregation of the whey proteins but only minor changes in the physical dimensions of the casein micelles (Hostettler et al., 1965). Ultra-high temperature heating of whey protein systems was responsible for only small amounts of denaturation compared to conventional sterilization. Conventional and UHT sterilization processes produced gross aggregation of casein micelles. However, with continued heating at high temperatures the casein micelles began to break down into soluble casein.

Hansen and Melo (1977) examined the effect of ultra-high temperature steam injection upon the constituents of skimmilk. Alterations occurring to the native protein systems were determined by two techniques: 1. isolation procedures for specific proteins and 2. electrophoretic and densitometric methods. Processing skimmilk at temperatures of 138 to 149°C for 8-10 sec denatured many of the milk

proteins. Whey proteins were most susceptible to denaturation while caseins were least affected. β lactoglobulin and α lactalbumin decreased substantially.

Wilson (1971) examined the large protein particles formed during sterilization and storage of concentrated skimmilk. Concentrated skimmilk was sterilized in a heat exchanger at 155°C with no holding time and canned aseptically. Protein particles from the sterilized concentrate were fractionated in a centrifuge and subjected to gel electrophoresis and Kjeldahl nitrogen determinations. After heating and storage the percent of sedimenting material had substantially increased. Particles with a diameter greater than 0.75 μ were primarily composed of casein.

The objective of research conducted by Fox et al. (1966) was to ascertain the factors responsible for the presence of nonsedimentable nitrogen (NSN) in sterile milk concentrates. Milk samples were heated in a thermostated bath at temperatures of 70-117°C for 30 min. The NSN decreased as the heating temperature increased to 90°C. The NSN began to increase as the temperature rose to 110°C and above. Disc electrophoretic techniques were used to establish the identity of the nonsedimenting species. Below 103°C the NSN consisted principally of whey proteins while at temperatures of 110°C and above it was predominantly casein.

Rowland (1937) examined the heat denaturation of the albumin and globulin fractions in milk heated at temperatures of 75-100°C. Denaturation of albumin and globulin took place rapidly in samples of milk heated at temperatures of 75°C and above and was complete in approximately 60, 30, 10-15, and 5-10 min at 80, 90, 95 and 100°C, respectively. There was no change in the nonprotein nitrogen (NPN) content of milk heated at temperatures up to 100°C. With continued heating at 95 and 100°C small amounts of NPN developed. At 115 and 120°C, appreciable hydrolysis of protein followed denaturation of the protein. The heat stability of whey proteins separated from skimmilk by treatment with sodium chloride and hydrochloric acid was studied by Harland and Ashworth (1945). Treatment at 80°C for 45 min or at higher temperatures for shorter times resulted in the denaturation of 93-95 percent of the whey proteins. Hetrick (1950) reported the effect of high temperature short time heat treatment on the denaturation of albumin and globulin in milk. Heat treatments of 81°C to 152°C with holding times of 0.03 to 64 sec were used. Heat treatment sufficient to cause the first noticeable cooked flavor resulted in the denaturation of approximately 58% of the protein.

Milk was subjected to heat treatments of 65-96°C for 30 min by Larson and Rolleri (1955). Heat treatment of 70°C for 30 min denatured 29% of the total serum proteins

due to the specific denaturation of 89% of the immune globulins, 32% of the β lactoglobulin, 52% of the serum albumin and 6% of the α lactalbumin. The denaturation curves indicated that the immune globulins were the least and α lactalbumin the most heat resistant. Harland et al. (1955) reported on the quantitative changes which occurred during heat treatment of skimmilk at temperatures ranging from 80-141°C. A high temperature tubular heater was used in these experiments. At 141°C a heating time of 6 sec was sufficient to denature 67% of the serum proteins. Ninety seven seconds at 100°C denatured 84% of the serum proteins. As the heating temperature increased, a secreased holding time was necessary to denature approximately the same amount of protein. The role of colloidal phosphate and pH on the heat stability of milk was examined by Rose (1962). Heat stability was determined after treatment at 140°C over a wide range of pH values. The denaturation of β lactoglobulin was highly correlated to the heat stability of the milk. The heat denaturation of the milk proteins β lactoglobulins A and B were compared by Gough and Jenness (1962). Five ml aliquots of 1% solutions were heated at 73°C for 7-97 min. After heating for 30 min there was a 46.3% decrease in β lactoglobulin A and 71.4% decline in α lactoglobulin B.

Melachouris and Tuckey (1966) investigated the denaturation of the whey proteins when milk was heated to high

temperatures for short times. Whole milk was divided into separate lots which received heat treatments of 61.7°C for 30 min, 93.3, 110, 126.7 and 143.3°C for 2.08 sec. The denaturation markedly increased as the heat treatment increased. The β lactoglobulin fraction was very sensitive to heat (70% denatured at 143°C). Albumin also exhibited an appreciable degree of denaturation when the milk was heated at 110°C or higher. The proteose-peptone fraction decreased as temperature of heating rose. The globulins were rapidly denatured even at the lower temperatures. The serum protein denaturation in skimmilk which resulted from direct steam injection was assessed by Dill et al. (1964). Seven temperatures, ranging from 75 to 141°C and four holding times of 8 to 190 sec were employed. The amount of denaturation varied from 10% for the least severe treatment to greater than 80% for the most severe. As the holding time increased, the percent of denatured protein increased. Kenkare et al. (1964) examined the heat induced aggregation of skimmilk serum proteins. Samples were heated in Erlenmeyer flasks by immersing in a boiling water bath or in a steam autoclave to temperatures of 120°C. Heating destabilized the serum proteins in acid-prepared serum to a greater extent than protein from ultracentrifugal serum. The 90.5°C treatment denatured 65 and 40% of the protein, respectively. Gel filtration with sephadex G-100 indicated that heating acid-prepared serum protein caused aggregation

and destabilization of each of the protein fractions.

Josephson et al. (1967) examined several milk systems for heat induced changes in the protein-salt balance. Sephadex gel filtration, PAGE, electron microscopy, chemical analyses and flavor evaluation were employed. Gel filtration elution patterns from heated milk revealed measurable losses of whey protein. Similarly, results from PAGE indicated that the characteristic whey protein bands were absent from the heated ($90^{o}$C-10 min) skimmilk supernatant. Patterns of heated rennet whey demonstrated that the proteins were present as aggregates of sufficient size to prevent their migration into the gel. The nature and extent of protein aggregation in heated whey systems were studied by Morr and Josephson (1968). Heating at $90^{o}$C for 10 min caused drastic reduction in the amount and resolution of $\beta$ lactoglobulin, $\alpha$ lactalbumin, serum albumin and globulin fractions. Gel filtration patterns indicated appreciable aggregation of the proteins. The calcium content and thiol-disulfide reactions were of major importance in these interactions. Approximately 80% of the cottage cheese whey proteins were denatured when heated at $91^{o}$C for 30 min (Guy et al., 1967). Denaturation increased dramatically in the temperature range of 85-$90^{o}$C. Nielson et al. (1973) used response surface experimental design to evaluate the role of four factors, e.g. temperature (60-$90^{o}$C), heating period (1-30 min), pH (4.5-8.5) and total solids

(6-60%) on the denaturation of cottage and colby cheese whey proteins. The most important of the four different parameters was shown to by heating temperature. Minimum protein denaturation occurred in the intermediate pH region (6-7). The whey proteins were least susceptible to denaturation at 20% total solids. PAGE patterns revealed denaturation of the proteins when the whey systems were heated at 85.6°C for 17.2 min. This heat treatment resulted in aggregation of the denatured whey protein. Schafer and Olson (1975) heated milk at ultrahigh temperatures (UHT) prior to manufacture into Mozzarella cheese. Raw skimmilk was heated in a tubular heat exchanger at temperatures of 80, 90, 100, 110, 120 and 130°C for 2 sec. There was a linear increase in the percentage of denatured whey protein as the heating temperature rose to 120°C. Approximately 27% of the whey protein was denatured at this temperature while at 80°C only 2-3% was denatured.

Whey was heated in a helically coiled tube heat exchanger at temperatures of 65.6-148.9°C (Senter et al., 1973). The heating, holding and cooling times were 4.8, 0.18 and 1.9 sec, respectively. Proteins and disulfide and sulfhydryl groups were quantified fluorometrically and electrophoretically. The α lactalbumin component was the most heat resistant. None of the heat treatments (65.6-148.9°C) decreased the electrophoretic area of this protein. β lactoglobulin was sensitive to temperatures of 87.8-148.9°C

with denaturation increasing with length of holding time.

Calcium caseinate was found to be very stable to heat (White and Davies, 1958). Temperatures greater than 130°C for 30 min were required to precipitate the complexes. Kresheck et al. (1964) studied the behavior and properties of the major milk caseins at temperatures of 30, 50, 70 and 90°C. Light scattering techniques were used to examine the heated samples for changes in particle size. Heating times of 30 min to 2.5 hr were employed. The summation of values from the light scattering experiments revealed little change in molecular weight or radius of gyration for whole casein at the temperatures studied. Turbidity increases were slight even when heated at the higher temperatures for 2.5 hr. Heated casein sols were examined for protein degradation by Alais et al. (1967). Casein dispersions were heated to 120°C for 10-80 min and analyzed for peptide content by electrophoresis, peptide mapping and NPN. The amount of NPN increased from 0.45% for unheated whole casein to 2.25% for casein heated 80 min. Electrophoresis of heated samples showed PAGE patterns to be only slightly altered. Fractions were not as distinct with slightly more material failing to enter the gel. Peptide mapping revealed the presence of high molecular weight peptides in the heated casein samples. Cheeseman and Knight (1974) examined the nature of casein aggregates in heated milk. Gel filtration on sepharose 6B was used to demonstrate the size distribution

of casein aggregates in fresh, heated and UHT treated milks. The elution profile of the nonheated samples were separated into five areas. The first peak eluted at the void volume and was primarily composed of large casein aggregates. Heat treatment at 100°C for 40 min or 143°C for 3 sec resulted in slight changes in the size distribution of casein micelles.

The changes which occurred to casein after sterilization were reported by Aoki and Imamura (1974a). Samples of milk were heated at 135°C for 45 sec prior to storage. This heat treatment resulted in an increase in the amount of nonsedimenting, soluble casein. This was further demonstrated by Aoki et al. (1974b). The amount of soluble casein increased when whey protein free milk (WPFM) was heated at temperatures of 135-140°C for 15 sec. Soluble nitrogen was determined on the ultracentrifugal supernatant from the superheated WPFM. Soluble casein began to increase at 105-110°C. Soluble casein further increased as the heating temperature rose. The amount of soluble casein was greatest in concentrated samples. The cold disaggregation of casein micelles in heated, concentrated WPF milks was studied by Aoki and Imamura (1975). The amount of soluble casein was much greater in the heated product compared to the unheated sample. Heating at temperatures above 105°C may have caused formation of soluble casein by disruption of hydrogen and hydrophobic bonds. Aoki et al. (1977)

heated WPF milk at 135-140°C for 15 sec to further examine changes occuring to casein micelles. By means of electron microscopy and differential centrifugation it was confirmed that casein micelles aggregated when concentrated WPF milk was heated at these temperatures. The amount of micelles which sedimented at 3000 x g nearly doubled after heating.

El-Negoumy (1978) described the changes occurring in sodium caseinate model systems heated to 100°C for 30 min in the presence of various ionic species. The sols, containing 3% sodium caseinate were dispersed in deionized water with one of the following species: 1) 0.02 M $CaCl_2$, 2) 5% lactose monohydrate, 3) 0.011 M $KH_2PO_4$, 4) 0.01 M $Na_3$Citrate, 5) 0.039 M $NaCl_2$, 6) 0.02 M $CaCl_2$ + 5% lactose + 0.011 M $KH_2PO_4$ + 0.01 M $Na_3$ Citrate + 0.039 M NaCl and 7) milk dialyzate. All model systems (except an unheated control) were heated to 100°C for 30 min prior to dialysis and freeze drying. A portion of each sample was chromatographed on ion-exchange diethylaminoethylcellulose. Electrophoretic analysis of chromatographic fractions was done on starch gels. Sodium caseinate sols heated in the presence of these ionic species underwent change in composition for most of the model systems. Formation of aggregates varied with the composition of the dispersing medium.

## In Vitro Estimation of Protein Quality

### Assay Procedures

Block and Mitchell (1946) demonstrated a workable system for the quantitative evaluation of a protein's nutritive value. The amino acid composition of a protein was determined by chemical means. Using whole egg protein as the standard, the nutritive value of protein was expressed as a chemical score equal to the greatest deficit in an essential amino acid in the test protein.

An *in vitro* technique was used by Melnick *et al.* (1946) to explain various factors affecting the nutritive value of soybean products. Following enzymatic digestion, the amount of hydrolysis was measured by a modified formal procedure. *In vitro* digestibility increased for soy products subjected to autoclaving when compared to the raw material. This data was supported by animal bioassays. Sheffner *et al.* (1956) examined the relationship between the amino acid profile released by digestive enzymes and the biological value (BV) of food proteins. The ultimate purpose of their work was to develop an *in vitro* procedure which could adequately estimate the nutritional value of proteins. Pepsin digests were prepared by incubating 1 g of protein with 25 mg of pepsin and incubating at 37°C for 24 hr. Amino acid analyses were performed by microbiological procedures. After analysis of the digest and residue material, a pepsin-digest-residue (PDRI) index was

calculated by comparing the ratio of 11 amino acids in a test protein to that in a reference protein (egg). This index incorporated both the essential amino acid pattern released by *in vitro* pepsin digestion and the amino acid pattern of the residue. The PDRI had high correlation with the net protein utilization (NPU) value for a variety of proteins. Division of the PDRI by the coefficient of digestibility yielded values which accurately predicted the biological values of the proteins studied. *In vitro* methods of protein evaluation were useful in screening new protein foods and processing methods because of their rapidity (Akeson and Stahman, 1964). These researchers devised a rapid, accurate *in vitro* procedure using a pepsin-pancreatin digest. An index of protein quality was calculated from the amino acids released during digestion with pepsin and pancreatin. Using whole egg as a standard, excellent correlation was observed between the pepsin-pancreatin index for 12 proteins and their biological values determined from feeding trials.

Van Buren *et al.* (1964) examined several indices used in the determination of protein quality. The methods investigated included soluble nitrogen, urease inactivation, available lysine, free amino groups and the Hunter "L" color procedure. There was no significant correlation between soluble nitrogen and protein efficiency ratio, but the free amino groups, Hunter "L" values and available lysine had

satisfactory correlation with PER at the 99% level. The protein damage which resulted from overheating of soymilk was measured with the greatest accuracy through determination of the available lysine.

*In vitro* enzymatic studies have demonstrated that amino acid availability and amino acid content of foods may differ markedly (Morrison and Rao, 1966). Acid hydrolysis procedures release all amino acids regardless of their availability. *In vitro* enzymatic procedures only release those amino acids "available" thus providing much meaningful information on the inactivation of amino acids due to processing effects. A comparison was made of several *in vivo* and *in vitro* methods by Buchanan (1969). Several leaf protein concentrates were employed as sample material. Papain solubility and true digestibility were highly correlated. Pepsin-pancreatin solubility was less well correlated. Microbiological estimations of available amino acids, involving predigestion with pepsin did not show good correlation with true digestibility.

Pepsin and trypsin *in vitro* enzymatic digestions were used by Yamashita *et al.* (1970) to estimate the digestibility of soy plastein. Pepsin and trypsin *in vitro* digestibilities were 84.8 and 76.5%, respectively. These values were almost identical to those of the undenatured soy protein. Evaluating protein digestibility by *in vitro* methods are important because of their rapidity and sensitivity

(Saunders *et al.*, 1973). This researcher used several different *in vitro* techniques in arriving at the protein digestibility of alfalfa protein concentrates. The enzymatic procedures included hydrolysis with papain, pepsin-pancreatin and pepsin-trypsin. After digestion, the residue protein was determined with subsequent calculation of percent digestible protein. This was in contrast to Akeson and Stahmann (1964) who measured free amino acids following enzymatic hydrolysis. The values obtained from the systems containing pepsin had a high correlation with values from rat feeding studies. Poor correlation was found with the papain digestion. Maga *et al.* (1973) measured the initial rate of proteolysis with trypsin as a simple *in vitro* means of gastronomic acceptability. The *in vitro* enzyme hydrolysis was a modification of Sheffner's (1963) procedure. The products tested included: commercial sodium caseinate, peanut flour, cottonseed flour, fish protein concentrate and soy isolate. There were significant differences between rates of proteolysis for the different products. Sodium caseinate was by far the most easily digestible protein source. Soy isolate was the least digested material. Steaming resulted in faster hydrolysis for all samples tested.

Osner and Johnson (1975) used several methods to predict the nutritional and chemical changes occurring to heated casein. The relative nutritive value (RNV),

available amino acids, net protein utilization (NPU) and pepsin *in vitro* digestibility were determined. The NPU and BV of casein processed at 120°C for 8 hr was lower than the control. The availability of seven amino acids fell uniformily and correlated with RNV. The pepsin-pancreatin digest index, pepsin digest residue index and NPU were used to monitor the effect of heat processing on casein (Stahmann and Woldegiorgis, 1975). It was observed that all three methods predicted similar values for protein quality. An enzyme score, which was calculated like the chemical score, compared the essential amino acids released from the test protein (heat processed casein) and those released from egg protein. The chemical score and enzyme score compared well with the enzyme indexes.

Several procedures possibly having importance in rapidly estimating protein quality were reviewed by Satterlee (1977). The model incorporated the essential amino acid (EAA) profiles of a sample and reference protein with their *in vitro* digestibilities. An *in vitro* PER could be determined with this procedure in 72 hr. Another method which was reviewed included the use of the Protozoan, Tetrahymena pyriformis W, to measure food protein quality. Using an enzyme predigestion the subsequent Tetrahymena growth was highly correlated to the PER of the protein. This method was inexpensive and could predict a calculated PER within 72-96 hr.

Hsu et al. (1977) developed a multienzyme technique for estimating protein digestibility. This procedure was sufficiently sensitive to detect the effects of processing and protease inhibitors on the enzyme hydrolysis of proteins. The multienzyme system consisted of trypsin, chymotrypsin and peptidase. It was found that the pH of a protein suspension after 10 min of digestion was significantly correlated with in vivo apparent digestibility. The correlation coefficient was 0.09 with a standard error of estimation (of in vitro or in vivo) of ± 1.72. The effect of processing and heat treatment on protein digestibility were satisfactorily explained using this technique. The presence of fat in the samples had no apparent effect on the in vitro digestibilities. It was suggested that substances containing strong buffering capacities might affect the results though the food systems and protein sources investigated had no effect on the in vitro protein digestibilities.

## Evaluation of Processing Effects by In Vitro Enzymatic Procedures

Valaris and Harper (1973a) investigated the effect of carboxymethylcellulose (CMC) on the rate of peptic digestion of $\alpha_s$ casein. Pepsin was immobilized on glass beads. At concentrations of 0.02% and 0.03% CMC inhibited proteolysis of $\alpha_s$ casein by immobilized pepsin. The inhibitory mechanism could not be satisfactorily explained. The effect

of CMC on the proteolysis of $\alpha_s$ casein by immobilized trypsin was also studied by Valaris and Harper (1973b). CMC inhibited activity on intact $\alpha_s$ casein. However, CMC failed to inhibit tryptic degradation of $\alpha_s$ casein partially hydrolyzed by pepsin. They suggested that CMC would have no significant effect on the tryptic digestion of casein and that any nutritional significance would relate only to its effect on pepsin digestion.

McCune (1977) determined protein quality using an in vitro enzymatic digestion and a rat bioassay (PER). The model systems were prepared from a casein-glucose mixture and a casein-safflower blend. Thermal and chemical treatments included: 1) incubation of the casein-safflower oil mix at 55°C to promote oxidation of the oil, 2) autoclaving of casein and 3) autoclaving of a casein-glucose mixture. Protein quality was most accurately predicted by use of an in vitro pepsin-pancreatin sequential digestion to release amino acids. The enzyme index was calculated by computing the geometric means of the ratios of the released essential amino acids of the test protein to a reference protein. This index correlated best with the bioassay results. Casein exposed to lipid oxidation had substantially reduced PER. Casein and casein-glucose mixtures subjected to autoclaving had slightly lower PER.

Evans and Butts (1949) investigated the heat inactivation of methionine in soybean meal. No inactivation occurred

when soybean protein was autoclaved by itself, but 46 to 97% was inactivated when glucose was added to the samples. An enzymatic *in vitro* digestion was used.

Because biological experiments are expensive and time consuming a faster method was sought by Menden and Creamer (1966) to evaluate protein quality. Casein was used as the model system and was subjected to severe heat treatment both in the presence and absence of glucose. Enzymatic hydrolysis with pancreatin led to the conclusion that heat treatment of casein did not decrease the availability of essential amino acids. Heat treatment in the presence of glucose significantly diminished the availability of amino acids.

Rao and Rao (1972) examined the effect of non-enzymatic browning on the nutritive value of casein-sugar complexes. The autoclaving of casein with arabinose, glucose or lactose resulted in a reduction of amino nitrogen, partial destruction of lysine, arginine, methionine and leucine. *In vitro* digestibility of the protein also decreased. Loss of available lysine, due to the binding of the ε amino groups with the aldehyde group of the sugars occurred most rapidly with arabinose, glucose and lactose. Acid and enzymatic hydrolysis was used by Hankes *et al*. (1948) to examine the effect of autoclaving on the liberation of amino acids from casein. Pepsin, whole pancreatin and erepsin were selected as the enzymes to be used in the *in vitro* digestions. Amino

acids were quantitated by microbial procedures. Samples were prepared by autoclaving casein for 4 min and for 20 hr at 15 psi. Autoclaving casein for 4 min had no effect on the amino acid composition while autoclaving for 20 hr slightly reduced cystine. The release of amino acids by pepsin, pancreatin or erepsin digests was higher in the casein autoclaved for 4 min than in unheated casein. Pancreatin digestion for 2 hr released 19% of the α amino nitrogen. An additional 2 hr digestion with erepsin released 27% of the total. Continued digestion for 5 days released 50% of the α amino nitrogen. An investigation into the liberation of essential amino acids from raw, properly heated and overheated soybean oil meal was made by Riesen et al. (1947). Acid, alkaline and pancreatic hydrolyses were performed on each sample. The results indicated that the essential amino acids liberated by acid hydrolysis were unaffected by heat treatment, with the exception of lysine, arginine and tryptophan. Prolonged autoclaving (4 hr) decreased the liberation of these amino acids. Liberation of the essential amino acids was increased by proper heating when subjected to pancreatic digestion. Excessive heating decreased liberation of essential amino acids. Cystine destruction and cystine inactivation of autoclaved soybean oil meal were studied by Evans et al (1951). Cystine destruction was determined by acid hydrolyzing a sample and quantitating the amino acids. Cystine

inactivation was estimated by an *in vitro* enzymatic hydrolysis. Autoclaving soybean oil meal for 4 hr at 15 psi destroyed 31-36% of the cystine. An *in vitro* enzymatic hydrolysis with trypsin and erepsin revealed that 82-87% of the cystine had been inactivated. No cystine remained available after cystine was autoclaved in the presence of glucose.

The nature and extent of amino acid inactivation in milk processed by four widely used methods was studied by Mauron *et al*. (1955). Destruction and inactivation of the amino acids, tryptophan, tyrosine, methionine and lysine were determined in fresh and boiled milk, in roller and spray dried milk powders, and in evaporated and sweetened condensed milk. The enzymatic liberation of amino acids was followed by a dynamic *in vitro* digestion procedure using pepsin and pancreatin. No differences were detected between the amino acid compositions of fresh and boiled milk. Tryptophan, tyrosine and methionine were not affected by any of the processing treatments. Destruction of amino groups was noted in a slightly scorched milk powder, with 13% inactivated as measured by *in vitro* digestion. Lysine suffered the most damage of any of the amino acids. Slight destruction took place in spray dried milk (4%) but rose to 26% in slightly scorched roller dried milk. Ford and Salter (1966) used both static and dynamic digestions to estimate protein quality. Portions of freeze-dried fish

were subjected to different heat treatments prior to *in vitro* digestion with pronase or successively with pepsin, pancreatin and erepsin or sequentially with pepsin and papain. The digests were analyzed for soluble protein, peptide content and free amino acids. Broadly similiar results were achieved with the different enzyme digestions and correlated satisfactorily with rat feeding studies. As the severity of heating increased, the proportion of certain amino acids in the digests diminished. Severe heating retarded the *in vitro* enzymic release of several amino acids which was consistent with animal growth studies.

Dimler (1975) examined the protein quality of milk proteins using an *in vitro* enzymatic approach. Three milk proteins were studied: whole casein, $\alpha_s$ casein and β lactoglobulin. Enzymatic hydrolysis with pepsin for 20 hr was followed by a second digestion with pancreatin for 6.5 hr. After digestion, TCA was added to precipitate the unhydrolyzed protein. The clear supernatant was analyzed for total nitrogen, alpha amino nitrogen and free amino acids. The milk proteins were subjected to four different treatments prior to analysis; these included 1) untreated, 2) autoclaved for 30 min at 15 psi, 3) autoclaved with glucose and 4) autoclaved with 5x the amount of glucose. After digestion of the treated samples the total free amino acids remained unchanged. Several essential amino acids suffered some losses. Both $\alpha_s$ casein and β lactoglobulin

digest fractions had progressive reduction in several amino acids.

# EXPERIMENTAL PROCEDURES

## Functional Studies

Mixtures of milk products and soy isolate were combined to determine which functional characteristics could be demonstrated. The materials utilized in this study were:

1. Nonfat dry milk (NFDM) was obtained from Land O' Lakes, Inc., Minneapolis, Minnesota.
2. Edi-ProN, a soy isolate, was obtained from Ralston Purina, Inc., St. Louis, Missouri.
3. Sodium caseinate was obtained from Milk Proteins, Inc., Troy, Michigan.
4. Enerpro 50, a dehydrated whey protein concentrate (WPC) was obtained from Stauffer Chemical, Inc., Westport, Connecticut.
5. Spray dried sweet whey was obtained from Land O' Lakes, Inc., Minneapolis, Minnesota.
6. Electrodialyzed whey powder was obtained from Foremost Foods, Inc., Appleton, Wisconsin.
7. Promine D, a soy isolate, was obtained from Central Soya, Inc., Chicago, Illinois.

### Preparation of Samples

In order to properly assess the functional value of the blends, dispersions were prepared at protein levels of 3.2, 5.0 and 8.0% (w/w) in ratios of 0:100, 25:75, 50:50, 75:25 and 100:0 (milk product to soya isolate). The blends

were prepared in distilled water and mixed for 10 minutes at 2500 rpm by a Fisher variable speed mixer. The dispersions were subjected to the following treatments:

1. control
2. heating 68°C/30 min
3. heating 77°C/no hold
4. heating 77°C/30 min
5. heating 94°C/4 sec
6. heating 121°C/4 sec
7. adjustment to pH 8.5
8. adjustment to pH 4.5
9. addition of 0.1% $Na_2HPO_4$
10. addition of 0.2% "
11. addition of 0.3% "
12. addition of 0.1% $Na_3$ Citrate
13. addition of 0.2% "
14. addition of 0.3% "
15. addition of 0.1M $CaCl_2$
16. homogenization 2250 psi

Six tests were chosen as indicators of the functional value of the samples. These were emulsion capacity, whipping ability, viscosity, solubility, solubility index and sensory evaluation. After the first series of functional tests was completed and evaluated an additional set was designed to further improve functionality. Eleven protein systems were chosen as representative of the samples studied in part I. A protein concentration of 3.2% was used in these samples. The blends included: NFDM-100, NFDM 50:50 soy isolate, electrodialyzed whey-100, electrodialyzed whey 75:25 soy isolate, WPC-100, WPC-75:25 soy isolate and soy isolate-100. These samples were subjected to the following conditions:

1. 0.1M $CaCl_2$ with 0.1% $Na_3$Citrate
2. 0.1M $CaCl_2$ with 0.1% $Na_2HPO_4$
3. 0.1M $CaCl_2$ with 0.1% EDTA
4. 0.1M $CaCl_2$ and heating 68°C/30 min
5. 0.1M $CaCl_2$ and 0.1M EDTA
6. 0.1M $CaCl_2$ and 1.0% EDTA
7. addition of 0.1% EDTA
8. addition of 0.1% $Na_2HPO_4$ and heating 68°C/30 min
9. addition of 0.1% $Na_3$Citrate and heating 68°C/30 min
10. adjusting the pH to 8.5 and heating 68°C/30 min

11. addition of 0.06% $H_2O_2$ and catalase treatment
12. addition of 0.06% $H_2O_2$-68°C/30 min and catalase
13. addition of 0.01% L cysteine
14. addition of 0.01% L cysteine-0.06% $H_2O_2$ and catalase
15. addition of 0.01% mercaptoethanol
16. addition of 0.5% sodium hexametaphosphate (SHMP)
17. addition of 123 ppm sodium dodecyl sulfate (SDS)
18. addition of succinic anhydride one g per g protein
19. addition of maleic anhydride one g per g protein
20. addition of 5% sucrose
21. addition of 5% sodium chloride
22. addition of 0.1% carboxymethylcellulose (CMC)
23. addition of 0.1% CMC and heating 68°C/30 min
24. addition of 0.5% CMC
25. addition of 0.2% monoglycerides
26. addition of glycan and homogenization at 2250 psi

The final experimental treatment consisted of an enzymatic hydrolysis with a protease (prolase). The enzyme was added to the samples which were then adjusted to pH 7.5 and incubated for one hour at 50°C. Aliquots of material were removed, heated to 77°C and tested for functionality. After 3 hr of incubation, additional aliquots were removed and retested.

One additional test, gelation, was assessed for specific samples under selected conditions. Gelation studies were made on aqueous dispersions of 10% protein in the same ratios as used for other functional tests. The variants included: 1) no treatment, 2) pH 8.5, 3) 0.1% $Na_2HPO_4$, 4) 0.1% $Na_3$Citrate, 5) 0.1M $CaCl_2$, 6) pH 4.5 and 7) pH activated (Fleming, 1974). The experimental techniques which were used for each functional test are described in the following section.

Whipping

The whipping ability of the samples were obtained by taking a quantity of the protein sol which upon whipping did not result in clogging of the blades. For blends composed of 3.2, 5.0 and 8.0% protein this amounted to 75, 50 and 40 ml, respectively. The mixtures were whipped at speed 8 in a Kitchen Aid Model 3-C mixer, equipped with a wire whip. A whipping time of 6 min proved optimal. Specific volumes (ml/g) and ½ stability times (min) were determined. After whipping was completed, the material was transferred to a tared beaker of known volume and reweighed to determine specific volume. To determine ½ stability the beaker was covered with ½ in stainless steel mesh screen and inverted over a funnel which collected liquid draining from the foam. The time required for collection of liquid equal to ½ of the weight of the original foam was recorded. Stability times of less than 5 min were reported as zero.

Emulsion Capacity

Emulsion capacity was determined by a procedure similar to that of Webb et al. (1970). An aliquot of the protein sol, equivalent to 10 mg of protein was taken from each sample. This was added to 100 ml of 1.0M NaCl solution in a 600 ml beaker at room temperature. This mixture was then weighed. The beaker was covered with a rubber stopper

fitted with holes for a propeller, two electrodes and a delivery tube. The propeller (resting in the sample) was attached to a Talboy T line variable speed, laboratory stirrer. Refined corn oil was added from a 500 ml separatory funnel and delivered via tubing into the beaker at a constant rate of 0.1 ml/sec. Stirring was maintained at 3000 rpm during delivery of the oil until the emulsion inverted. The inversion point was monitored with a voltmeter which was set to record electrical resistance. After the break point was reached, the beaker was detached from the apparatus and reweighed to determine the amount of corn oil added. The emulsifying capacity was calculated as the g of oil required to reach an infinite electrical resistance minus a blank (100 ml of a 1.0M NaCl solution) divided by the amount of protein.

Soluble Protein

The percent soluble protein in each sample was determined by filtering several ml through Whatman no. 42 filter paper. An appropriate portion of filtrate was removed for analysis by a Lowry protein assay (Lowry et al., 1951). Bovine serum albumin dissolved in distilled water was used to prepare the standard curve. Absorbance was read at 540 nanometers on a Beckman DKU spectrophotometer.

Viscosity

Viscosity was measured at room temperature with a Brookfield RVF rotary viscometer. The rotor speed was kept constant at 50 rpm using spindle no. 4 (except in those circumstances where the viscosity exceeded the capacity of the spindle). Measurement was made in a 400 ml beaker filled with the appropriate amount of sample. Values were recorded in centipoise.

Solubility Index

The solubility index was determined according to a procedure from the American Dry Milk Institute, Inc. (1954). Conical graduated centrifuge tubes were filled to the 50 ml mark and centrifuged for 5 min at 1000 rpm. Immediately after centrifugation, the supernatant liquid was poured off leaving approximately 5 ml above the sediment. Twenty five ml of distilled water were added to the tubes and the sediment dispersed. The tubes were then filled to the 50 ml mark and centrifuged again. After 5 min the tubes were removed from the centrifuge and the ml of sediment in the tube reported to the nearest graduation.

Gelation

In evaluating the gelation capacity of the blends, the procedure followed was that of Fleming *et al*. (1975). Aqueous dispersions of 10% protein were prepared by mixing at 2500 rpm for 10 min. Gelation experiments were conducted

by heating the slurries in sealed glass containers to 90°C for 45 min in a water bath. After heating, the containers were quickly cooled in an ice water bath to 25°C. The firmness of the gel was measured with a plummet dropped from a height of ten inches. With this device a score of 10 indicated firmness while 1 indicated softness.

Sensory Subjective Analysis

In this study subjective analysis was carried out on the foam produced during whipping of the samples. A total of thirty points was assigned, 10 each for color, flavor and texture. High scores indicated greater acceptability while samples with less than 60% of the total were unsatisfactory.

Examination for Heat Induced Interactions

A set of experiments was designed to examine for heat induced interactions between the water soluble protein of soy isolate and milk proteins. The milk proteins were prepared from sweet whey, acid whey, ammonium sulfate precipitated casein and sodium caseinate. Samples were prepared and heated separately and in a 1 to 1 combination with soy for each of the milk protein materials. Five heat treatments were used: no treatment, 68°C/30 min, 77°C/20 sec, 94°C/10 sec and 121°C/5 sec.

After treatment, the sols were centrifuged at 25,000 x g for 30 minutes and filtered through Whatman no. 42 filter

paper. Approximately 8 ml of filtrate were pumped upward through a Pharmacia column packed with sephacryl S-200 superfine. After resolution of the proteins, the fractions were collected and polyacrylamide disc gel electrophoresis (PAGE) and nitrogen analysis performed on each. Proteins that eluted in the void volume of the sephacryl column were concentrated 10 to 1 and reapplied over a column packed with sepharose 4B. The analyses previously described were repeated.

## Preparation of Products

### Soy Protein

Soy soluble protein was prepared from soy isolate according to a modified technique from Soybeans: Chemistry and Technology. The soy isolate was suspended in distilled water at a protein concentration of 3.2% (w/w). The dispersion was stirred at 2500 rpm for 15 min followed by centrifugation at 1500 rpm for 10 min. After centrifugation the supernatant was filtered through Whatman no. 42 filter paper. The filtrate was dialyzed for 48 hours at 4$^{o}$C followed by freeze drying at 7 $\mu$ absolute pressure. The freeze-dried material was stored at -20$^{o}$C.

### Whole Casein

Colloidal casein was prepared according to the method described by McKenzie, 1972. Ammonium sulfate (26.4 g) was added with stirring to 100 ml of skimmilk over a period

of 40 min at 2°C. After the ammonium sulfate was added the milk was stirred for an additional 90 min. The precipitate was collected after centrifugation at 14,600 x g for 35 min. The precipitate was redissolved in distilled water making certain the total volume did not exceed 90 ml. With mechanical agitation and stirring this was accomplished in approximately 50 min. The volume was increased to 100 ml, holding the temperature at 3°C. The casein was reprecipitated with 24.0 g ammonium sulfate and stirred for 90 min after addition of the salt. The casein was separated by centrifugation as before and redissolved in water to a final volume of 60 ml. This was dialyzed against distilled water for 48 hr at 4°C and freeze-dried. The material was stored at -20°C.

Sodium Caseinate

Whole, raw milk was obtained from the Michigan State dairy farm. The cream was removed by centrifugal separation at 30°C. The casein was precipitated by addition of 1N HCl to a pH of 4.6. The casein was collected with cheese cloth, washed with water, and redissolved in water by adjusting the pH to 7.0 with 1N NaOH. This procedure was repeated 3 times. The final solution was dialyzed against distilled water for 48 hr at 4°C and freeze-dried. The dried material was stored at -20°C.

Whey Proteins from Acid Whey

After precipitation of the casein from skimmilk by addition of 1N HCl the whey was gathered and centrifuged at 2000 rpm for 20 min. This was followed by filtration through Whatman no. 42 filter paper. The filtrate was dialyzed for 96 hr at 4°C and freeze-dried. The whey proteins were stored at -20°C.

Whey Proteins from Sweet Whey

Whole raw milk was obtained from the MSU dairy farm. The milk was warmed to 30°C and incubated with 1% cheese starter. After the acidity had risen 0.03% rennet was added and the milk allowed to coagulate. In approximately 45 min the curd was cut. After cooking to 39°C (following a 15 min resting period), the whey was drained and the casein removed by filtration through cheese cloth. The whey was centrifuged at 2000 rpm for 20 min and filtered throuth Whatman no. 42 filter paper. The filtrate was dialyzed for 96 hr at 4°C and freeze-dried. The whey proteins were stored at -20°C.

Gel Filtration

A 2.5 cm x 100 cm Pharmacia column was packed with sephacryl S-200 superfine. Sephacryl S-200 superfine (Pharmacia, Inc.) is a gel filtration media which can withstand high flow rates. The exclusion limit is approximately 200,000 daltons. This material purchased as a slurry was

washed with phosphate buffer, defined 3 times and degassed for five hours. After degassing was completed, the material was slurried into the column and packed in a downward flow at 200 ml/hr using a Pharmacia peristalic 3-P pump. After several volumes of buffer had passed through the material, the flow was reversed and the procedure repeated. For sample separation, flow was in the upward direction at a rate of 125 ml/hr. An Isco Model UA-2 ultraviolet analyzer and recorder were employed to monitor the proteins at 280 nm. An Isco Model 326 fraction collector, equipped with automatic actuator was used to simplify collection of the fractions. Approximately 70 mg of protein was applied to the column and eluted with phosphate buffer, pH 7.0, $\mu$ = 0.1. Mercaptoethanol and human serum were used to determine total volume and void volume, respectively. Proteins that eluted in the void volume were concentrated approximately 10 to 1 with a millipore immersible separator. Approximately 4 ml of this material were pumped upward through a 2.5 cm x 45 cm Pharmacia column packed with sepharose 4B (Pharmacid, Inc.). Sepharose 4B, a gel filtration material with an effective range of 100,000-10,000,000 daltons was prepared and packed at 60 ml/hr. The proteins were eluted from the column using upward flow at a rate of 50 ml/hr. Phosphate buffer was used as the mobile phase. The UV analyzer and fraction collector used were those previously described. Mercaptoethanol and Blue dextran G-2000 were

used to determine total volume and void volume, respectively.

Polyacrylamide Gel Electrophoresis

All electrophoretic analyses were made using a Bio-Rad model 400 power supply and model 150 gel electrophoresis cell. Disc gel electrophoresis was carried out by modification of Melachouris (1969) procedure. For systems containing whey, soy and whey-soy blends the procedure consisted of preparing gel buffer pH 8.9 (see appendix) and adding to it the proper amount of cyanogum to give either 5.0 or 7.0 percent gels. Five percent gels were run on all fractions eluted from the sepharose 4B column while 7.0 percent gels were run on those eluted from the sephacryl S-200 column. To initiate polymerization 0.05 ml of a 5 percent ammonium persulfate solution was added to the mixture. Temed (N,N,N,N-tetramethylethylenediamine) was added (10 µl/ml of gel solution) to accelerate polymerization. The gel solution was deposited in glass tubes to a depth of 6.5 to 7.0 cm. Water was layered on top to prevent a meniscus and to prevent inhibition of polymerization. The electrode buffer, pH 8.3 was composed of trisglycine (see appendix). Reagent grade sucrose was added to the samples to increase the density. One drop of Bromophenol Blue (1% in phosphate buffer) was added as the marker dye. After polymerization was completed and the

tubes assembled in the cell, sample was layered onto the top of the gel. It was found that satisfactory results could be obtained without switching to tris-HCl buffer, pH 6.7, from the phosphate buffer, pH 7.0 (Aldrich, 1977). Constant current was applied to the gels at the rate of 2.5 mA/tube for approximately 60 min. The gels were stained according to the Malik-Berrie (1974) staining procedure (see appendix) using Coomassie Brilliant Blue R-250.

Samples containing either casein, soy or casein-soy blends were examined in a system containing 7M urea. The gel buffer, tris-HCl pH 8.9 was made to contain 7M urea. Five percent and seven percent gels were prepared for the purpose previously discussed. All other conditions and buffers remained as previously mentioned.

Gel Scanning

Casein, soy and casein-soy gels from no treatment and 94$^{o}$C/5 sec samples were analyzed densitometrically. The gels were scanned at 2 cm/min by a Gilford gel scanner attached to a Beckman DU spectrophotometer at 550 nm. The bands were monitored by a Hewlett-Packard automatic integrator, model 3380S. Chart speed was set at 2 cm/min, with slope sensitivity at 0.3 mV/min and an attentuation of 32.

Nonprotein Nitrogen

Casein, whey, soy and their blends were separated by gel filtration into many fractions. Nonprotein nitrogen (NPN) was determined on the fraction eluting closest to the experimental total volume (determined with 2 mercaptoethanol). NPN was determined by taking 10 ml of the fraction and mixing with it an equal amount of 24% trichloroacetic acid (TCA). Blank determinations were made by adding 10 ml of TCA (24% w/w) to phosphate buffer. After standing 30 min the sol was filtered through Whatman no. 42 filter paper. Five ml of the filtrate was then examined for nitrogen content by a semi-micro Kjeldahl procedure.

In Vitro Enzymatic Hydrolysis

Samples of NFDM, soy isolate and their blend were prepared, treated, dialyzed and freeze-dried. The purpose of which was to examine release of total amino nitrogen from intact proteins as affected by various processing treatments and possible interactions between protein systems. The soy isolate used in this study was identical to that utilized in the functional tests. Nonfat dry milk was chosen as the milk protein product over sodium caseinate and the whey products because of its extensive use in the food industry and because it contains both protein systems found in the other products. The procedure used to assess

release of α amino nitrogen was the enzymatic approach as modified by Dimmler (1974). With this method both pepsin, pH optimum 1.8 and pancreatin, pH optimum 7-8 were added to the digestion mixture.

Preparation of Samples

All samples were made up to 500 g in distilled water with a protein concentration of 3.2% (w/w). Samples were divided into three groups, which included: 1) samples made entirely from soy isolate, 2) samples made entirely from NFDM and 3) samples made from a 1 to 1 combination of each. The mixtures were subjected to 24 different treatments which totaled 72 separate samples. The treatments were designed to simulate various processes, which included: 1) no treatment (control), 2) heated at 68°C/30 min, 4) heated at 94°C/4 sec, 5) heated at 121°C/4 sec, 6) heated at 113°C/15 min, 7) heated (microwave) 77°C/no hold, 8) sunlight oxidized 2 hr, 9) pH adjusted to 8.5 and heated at 68°C/30 min, 10) pH adjusted to 11.5, 11) addition of 0.1% $Na_3$Citrate and heated at 68°C/30 min, 14) addition of 5.0% NaCl and heated at 68°C/30 min, 15) addition of 0.1% CMC and heated at 68°C/30 min, 16) addition of 0.1M $CaCl_2$ and heated at 68°C/30 min, 17) addition of 0.06% $H_2O_2$, followed by catalase treatment and heating at 68°C/30 min, 18) homogenization at 2000 psi and heated 68°C/30 min, 19) addition of 3.5% glycan (yeast cell wall polysaccharide),

homogenized at 2000 psi and heated at 68°C/30 min, 20) addition of 3.5% partially hydrogenated soybean oil, homogenized at 2000 psi and heated at 68°C/30 min, 21) addition of 0.01% L cysteine, followed by 0.06% $H_2O_2$ and catalase, 22) addition of maleic anhydride at one gram per gram protein, 23) addition of succinic anhydride at one gram per gram protein, 24) protein mixed with safflower oil and held at 45°C for four days to allow oxidation to occur. After preparation the samples were dialyzed for 72 hours, freeze-dried and stored at -20°C.

Hydrolysis Procedure

Samples for enzymatic hydrolysis were prepared by dissolving 300 mg of the freeze-dried material in 25 ml of deionized distilled water. The pH of the mixtures was lowered to 1.8 with 1N HCl. Additional water was added, which increased the volume to 30 ml, representing a sample concentration of 1.0%. Twenty-five mg of pepsin was added to the solution. After swirling, the flask was placed in a 37°C oven for 20 hours of incubation. Peptic digestion was terminated by the addition of 0.5M $NaHCO_3$ buffer, and 1.0N NaOH to a final pH of 8.0. A final incremental addition of water brought the total volume to 35.0 ml. Thirty mg of pancreatin was dissolved into the reaction mixture. The samples were reincubated at 37°C for 3 hours followed by a second addition of 30 mg of pancreatin and an

additional 3.5 hours of incubation at 37°C. After incubation was completed, a 30% TCA solution was added to the reaction mixtures to a final concentration of 15%. Enzyme blanks were treated identically except for the absence of the specimen protein. Protein blanks were treated identically except for the absence of the enzymes. After addition of the TCA, the samples were centrifuged and filtered to yield a crystal clear supernatant. When the proper dilution was made the samples were assayed for total alpha amino nitrogen by the ninhydrin test (Clark, 1964). The assay consisted of adding ninhydrin solution (see appendix) to the amino acid mixture and boiling in a water bath for 20 minutes. After cooling, 8 ml of 50 percent aqueous n-propanol were added. After ten minutes, absorbances were read at 570 nm against a blank. Glycine was used to prepare the standard curve. Acid hydrolysis (see appendix) was used to correlate percent enzymatic hydrolysis with total hydrolysis on the control samples.

## Protein Determinations

### Lowry Procedure for Soluble Protein

Percent soluble protein was determined by the method of Lowry *et al.* (1951) with one modification. Reagent C was prepared daily from a mixture of 50 parts reagent A and 1 part reagent B (see appendix). A standard curve, ranging in protein concentration from 50-500 µg/ml was prepared

from crystallized bovine serum albumin (BSA). The protein content of BSA was determined by Kjeldahl analysis. Absorbance was measured at 540 nm.

Lowry Procedure for Insoluble Protein

Percent total protein in the freeze-dried samples used in the *in vitro* enzymatic hydrolyses was determined according to the method of Lowry *et al.* (1951). Reagent E was prepared daily from a mixture of 50 parts reagent D and 1 part reagent B (see appendix). A standard curve was prepared with BSA and absorbance read at 540 nm.

Kjeldahl Analysis

Total nitrogen was determined using a semimicro Kjeldahl method (Swaisgood, 1963). Samples containing approximately 15 mg of nitrogen were digested by adding 5 ml of digestion mixture (see appendix) and heating for 90 min. After the initial digestion, the samples were cooled and 2 ml of 30% $H_2O_2$ added. Digestion was continued for an additional 60 min. Following heating the flasks were cooled and rinsed down with 10 ml of water. The flasks were then connected to a steam distillation apparatus. The mixture was made alkaline by addition of approximately 25 ml of 40% NaOH. The released ammonia was steam distilled into 15 ml of 4% boric acid containing 4 drops of indicator (see appendix). The ammonium borate complex was titrated with 0.02 N HCl to an olive green endpoint. Factors of

6.25 and 6.38 were used to convert nitrogen to protein for samples containing soybean and milk protein respectively. Recovery of nitrogen was checked with DL tryptophan.

# RESULTS AND DISCUSSION

## Investigation of Functional Properties

The results and discussion pertaining to examination of functional properties will be presented in the same manner as outlined in the experimental section pages 68. Each property will be discussed relevant to the treatments described in Functionality Part I. Immediately following, the results of Functionality Part II (pertinent to the second set of treatments) will be presented.

In Part I, dispersions were prepared at protein levels of 3.2, 5.0 and 8.0% (w/w) in ratios of 0:100, 25:75, 50:50, 75:25 and 100:0 milk product to soy isolate. The products included: nonfat dry milk (NFDM), sodium caseinate (NAC), electrodialyzed whey (EW), sweet whey powder (SW), whey protein concentrate (WPC) and soy isolate (SI). In Part II eleven protein systems were chosen as representative of the samples studied in Part I. A protein concentration of 3.2% was employed. The samples included: NFDM, NFDM 50:50 SI (NFDM/50), NAC; NAC 50:50 SI (NAC/50), EW, EW 75:25 SI (EW/75), WPC, WPC 75:25 SI (WPC/75), SW, SW 50:50 SI (SW/50) and SI.

The soy isolate used in this study was Edi-ProN from Ralston Purina. This isolate was chosen because of ease of

dispersion, protein solubility (Table 1) and flavor.

Solubility

The solubility of a protein is affected by pH, temperature, ionic strength, compounds which change their dielectric constant and by interaction with other substances. Solubility is an important physical parameter to measure because of its substantial effect on the many other functional properties of proteins. Measurement of solubility provides the researcher with important information concerning the effect of various processing treatments on proteins.

Data pertaining to solubility are shown in Tables 2-9. SI dispersions had the lowest solubilities of any of the products tested. The solubility of untreated samples ranged from 57.8 to 62.5% for dispersions containing 3.2 and 8.0% protein respectively. Shen (1976) reported that the solubility of this isolate was 54.0% and did not vary appreciably as the protein concentration changed. Heating at moderate temperatures slightly increased solubilities except for those samples subjected to Ultra High Temperature (UHT) heating. Solubilities decreased significantly at 121°C/ 4 sec. Shen (1976) and Hermannson and Akesson (1975a) both reported that low temperature heating slightly improved solubilities probably due to increased hydration or dissociation of the protein into subunits. Aldrich (1977), Mann and Briggs (1955), Catsimpoolas et al. (1969), Wolf (1970) and Catsimpoolas et al. (1971) found that the amount of

protein insolubilized increased with increased heat treatment. Loss of solubility was due to aggregation of the proteins.

Adjustments of the pH of SI sols to 8.5 increased their solubility. Wolf (1961), Wolf (1970), Fukushima and Van Buren (1970), Franzen and Kinsella (1976) and Amilara et al. (1977) all reported that alkaline pH treatment improved the solubility of soy protein. As the pH of the sols was raised into the alkaline region the net negative charge of the proteins increased thus increasing their relative attraction toward water molecules. This increased the hydration of the water molecules and hence their solubility. Wolf (1970) suggested that alkaline pH treatment of soy protein involved dissociation through rupture of weak secondary forces such as hydrogen bonds and van der Waals forces. This in turn lead to electrostatic repulsion between positively and negatively charged subunits. Lowering the pH of soy sols to 4.5 practically eliminated their solubility. Paulsen and Horan (1965), McWatters and Cherry (1977) and Hutton and Campbell (1977a) found that the solubility of soy protein was minimal near its isoelectric point. At pH 4.5 (the isoelectric point of soy protein) there was maximum attraction of the positive and negative forces of adjacent protein molecules which resulted in precipitation and loss of protein solubility. Addition of $CaCl_2$ to the SI sols also markedly reduced their solubilities. Paulsen and Horan (1965), Aldrich (1977), Mann and Briggs (1950)

and Smith and Circle (1972) reported that divalent cations such as calcium significantly reduce the solubility of soy protein. Heavy metal ions such as calcium, probably function to precipitate proteins by neutralization of charges or by forming crosslinkages with the protein molecules (Whitaker, 1972). The addition of either $Na_2HPO_4$ or $Na_3$Citrate slightly increased the solubilities of SI samples, especially at the higher salt levels. This may have been due to a salting in effect. Homogenization increased the solubility of SI dispersions at all three protein concentrations. Amilari et al. (1977) also reported that homogenization increased the solubility of soy proteins.

NFDM had the lowest solubility of any milk product at 3.2% protein. Heating increased solubilities to a small extent. Raising the pH of the samples to 8.5 elevated solubilities while lowering the pH to 4.5 markedly reduced this property due to isoelectric precipitation of the casein. Addition of $CaCl_2$ had little effect upon the solubility of NFDM dispersions probably because of the influence of whey proteins and the ionic environment of NFDM sols. The addition of $Na_2HPO_4$ or $Na_3$Citrate, at low concentrations, increased the solubility of NFDM dispersions probably by increasing the net charge of the protein and thus their hydration. Homogenization increased the solubility of these samples. Untreated NAC redispersed in distilled water had high solubility at the protein concentrations studied. Solubilities were close to those found for EW and WPC.

Table 1. Dispersible protein (%) of several soy isolates at 4.0% protein in water

| Soy Isolate | pH | | |
|---|---|---|---|
| | 3.0 | 7.0 | 9.0 |
| Edi-ProN[1] | 86.9 | 88.7 | 92.1 |
| Supro 610[1] | 24.1 | 23.6 | 29.5 |
| Promine D[2] | 49.1 | 55.9 | 58.6 |
| Promine F[2] | 39.1 | 41.3 | 51.8 |
| Pro-FAM 90 HS[3] | 27.9 | 73.6 | 74.1 |
| Pro-FAM 90 LS[3] | 6.8 | 14.2 | 19.3 |
| Protein Max 90[4] | 3.1 | 11.1 | 11.7 |

[1]Edi-ProN and Supro 610 are products of Ralston Purina.

[2]Primine D and F are products of Central Soya, Inc.

[3]Pro-FAM 90 HS and LS are products of Grain Processing Corp.

[4]Protein Max 90 is a product of Worthington Foods.

Heat treatment and adjustment of the pH to 8.5 had little effect upon solubility of NAC sols. Lowering the pH to the isoelectric region of casein precipitated nearly 100% of the protein. Addition of $CaCl_2$ also reduced the solubility though not to the same extent as found for SI dispersions. The major casein components bind calcium extensively at the normal pH of milk. The instability of caseinate sols at high calcium concentrations has been attributed either to their zeta potential or to cross-links established by the divalent cation (El-Negoumy, 1971).

Sweet whey powder (SW) had the lowest solubilities of any of the whey products, while EW had the highest. The solubility of WPC was slightly less than that recorded for EW. Marvopoulous and Kosikowski (1973) reported that the solubility of whey powders ranged from 91.4 to 99.3%. The solubilities of EW were 92.2, 91.1 and 93.6% at pH 4, 6 and 8 (Morr et al., 1973). The solubilities of WPC at the same pH values were 89.5, 90.2 and 91.2. Heating generally decreased the solubility of the whey products, especially at higher temperatures. WPC was the most vulnerable to heat denaturation. Howat and Wright (1933), Dill and Roberts (1964), Guy et al. (1967) and Morr (1969) reported that the denaturation of whey protein increased dramatically as heating temperature increased. McDonough et al. (1974) found that pasteurization of WPC solutions decreased their solubilities and Hidalgo and Gamper (1977) reported that the solubility of WPC sols decreased rapidly when they were

heated from 80-134°C. Adjustment of the pH to either 8.5 or 4.5 had little effect upon the solubilities of WPC or EW sols. This concurred with data reported by Morr et al. (1973). The solubility of SW samples increased slightly when the pH was raised and decreased to a small extent when the pH was lowered. The solubility of SW also decreased when dispersed in $CaCl_2$. Addition of $Na_2HPO_4$ or $Na_3$Citrate had little effect upon the solubilities of any of the whey products. At the protein concentrations studied the solubilities of the different whey products remained similar.

NAC:SI blends had solubilities (Table 3) intermediate between SI and NAC. Samples containing more SI had solubilities very similar to SI, while blends containing more NAC had solubilities close to those of NAC. Hoffman (1974) reported that the solubility of casein:soy blends decreased as soy protein displaced casein. At 5.0 and 8.0% protein solubilities differed only slightly compared to samples containing 3.2% protein. Heating had little effect upon the solubilities of these samples. Raising the pH to 8.5 slightly increased, while lowering the pH to 4.5 drastically decreased their solubilities. Adjustment of the samples to 0.1M $CaCl_2$ decreased solubilities markedly. The addition of stabilizing salts or homogenization only slightly affected solubilities. NFDM:SI blends had lower solubilities (Table 4) than NAC:SI blends. Solubility increased as the amount of NFDM in these blends increased. As protein concentration rose, solubilities decreased. Heating had little

effect upon the solubility of these blends, while alkaline treatment at pH 8.5 resulted in higher values. Lowering the pH to 4.5 or addition of $CaCl_2$ to the samples substantially decreased their solubility.

EW and WPC:SI blends had higher solubilities (Tables 5 and 6) than SW:SI (Table 7) blends. Solubilities increased as the proportion of whey product in the blend increased. The amount of soluble protein remained approximately the same at all three protein concentrations. Heating in general, decreased the solubility of the samples, especially for those blends having ratios composed of 75% whey product. Raising the pH of the blends to 8.5, generally increased their solubilities, particularly for samples containing 75% SI. Lowering the pH to 4.5 decreased the solubility of the blends especially for samples containing 75% SI. Addition of $CaCl_2$ also reduced solubilities in much the same manner. These same effects were seen regardless of the whey product. Addition of stabilizer salts did not appreciably change their solubilities while homogenization was responsible for slight improvement.

The solubilities of the samples exposed to the treatments from functionality Part II are shown in Tables 8 and 9.

The addition of ethylenediamine tetracetic acid (EDTA), $Na_2HPO_4$ or $Na_3$Citrate to samples containing $CaCl_2$ prevented the drastic loss of solubility noted for many of the samples. In general, solubilities were slightly lower than the

controls. Addition of EDTA increased the solubility of NFDM and NFDM/50 dispersions. Aoki and Imamura (1976) and Morr and Josephson (1968) found that EDTA increased the solubility of caseinate sols by removal of calcium from the micelle. This lead to disaggregation of the micelle which increased the amount of soluble casein. El-Negoumy (1974), Sommer (1952) and Morr (1975) reported that EDTA and citrate complexed calcium and thus prevented its destabilizing effect.

The solubility of the whey products was reduced by heating samples dispersed in 0.1M $CaCl_2$. The solubility of the blends was also decreased. Richert (1975), Monica et al. (1958) and Townsend and Gyuricsek (1974) reported that heating whey proteins in the presence of $CaCl_2$ caused substantial precipitation of the protein. This was due to the combination effect of heat-salt denaturation. The solubilities of NFDM, NAC, NFDM/50, NAC/50 and SI were all markedly reduced by this treatment.

The addition of either $Na_2HPO_4$ or $Na_3$Citrate prior to heating at 68°C/30 min had minimal effect upon the solubilities of the whey products or their SI blends. The solubility of NFDM, SI and their blend was increased by this treatment. Sommer (1952) reported that heating calcium caseinate sols in the presence of $Na_3$Citrate or $Na_2HPO_4$ increased their solubilities probably by substituting sodium for calcium. Sodium caseinate was more soluble than calcium caseinate.

Raising the pH to 8.5 prior to heating at 68°C/30 min had negligible effect upon the solubilities of the whey

products or their SI blends. This treatment improved the solubility of NFDM and SI.

Addition of $H_2O_2$ (followed by catalase) increased the solubility of NFDM and SI. Fish and Michelsen (1967) found that skimmilk treated with catalase before heating caused a decrease in the denaturation of the whey proteins. Fukushima and Van Buren (1970) found that $H_2O_2$ increased the dispersibility of soy milk due to its effect upon disulfides.

The addition of reducing agents, such as L-cysteine or mercaptoethanol increased the solubilities of NFDM, NAC and SI. The whey products maintained their high solubility. Fukushima and Van Buren (1970), Wolf (1970), Wolf (1961), Nash and Wolf (1967), Kelley and Pressey (1967) and Amilari et al. (1977) reported that the addition of reducing agents to soy protein sols increased their solubilities. This was found to be due to the disruption of disulfide bonds which resulted in the depolymerization of soy protein. The addition of SDS to the samples improved the solubilities of SI and its milk product blends to a small extent. Kelley and Pressey (1966), Catsimpoolas (1969), Wolf and Tamura (1969), and Koshiyama (1970) observed that detergents disrupted the quaternary structure of soy protein which increased their solubility.

Addition of succinic or maleic anhydride to the samples increased the solubilities of NFDM, SI and their blend. The whey products maintained their high solubilities while the solubilities of their SI blends increased. Franzen and

Kinsella (1976) found that succinylation improved the solubility of soy sodium proteinate. Succinylation of a protein converts the cationic amino groups to anionic residues. The increase in net negative charge alters the physicochemical character of the protein resulting in enhanced aqueous solubility. The ammonium cations of lysine are replaced by succinate ions which due to their negative charge repel native carboxyl groups thus reducing protein-protein interaction and increase protein-water interaction.

The addition of NaCl to the samples reduced the solubility of NAC, SI and the whey products. Hermannson and Akeson (1975b) reported that NaCl markedly reduced the solubility of soy sodium proteinate. They ascribed the reduced solubility to changes in the quaternary structure of the protein. WPC became slightly less soluble as the concentration of NaCl increased in the samples, though the solubility of NAC was not affected.

Enzymatic hydrolysis markedly improved the solubility of both SI and its milk product blends. Digestion for three hours did not appreciably alter the results. Jost (1977) and Monti and Jost (1978) increased the solubility of proteins by enzymatic digestion. The remaining treatments had little affect upon the solubilities of the samples.

In general, milk products demonstrated the highest degree of solubility, with milk product:soy isolate blends having appreciably higher solubility than SI. Specific treatments resulted in widely varying solubilities. This

Table 2. Protein solubility (%) of samples of NFDM, NAC, SI, WPC, EW and SW at a protein concentration of 3.2%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | NFDM | NAC | SI | WPC | EW | SW |
| Control | 65.6 | 99.5 | 57.8 | 96.4 | 99.8 | 80.5 |
| 68°C/30' | 68.8 | 99.7 | 58.1 | 94.6 | 95.3 | 90.6 |
| pH 8.5 | 70.1 | 94.3 | 60.5 | 99.1 | 96.9 | 82.5 |
| pH 4.5 | 25.0 | 0.5 | 4.2 | 99.2 | 98.4 | 63.0 |
| 0.1M $CaCl_2$ | 66.2 | 24.1 | 7.8 | 92.4 | 99.5 | 47.5 |
| 0.1% $Na_2HPO_4$ | 72.2 | 99.5 | 58.0 | 91.0 | 99.8 | 76.0 |
| 0.1% $Na_3$Citrate | 73.1 | 97.3 | 59.6 | 93.4 | 99.7 | 77.2 |
| Homogenization | 81.2 | 99.6 | 62.6 | 99.5 | 98.4 | 84.3 |

Table 3. Protein solubility (%) of blends of NAC and SI at a protein concentration of 5.0%

| Variant | NAC:SI | | |
|---|---|---|---|
| | 75:25 | 50:50 | 25:75 |
| Control | 76.0 | 70.0 | 65.0 |
| $68^{o}C/30'$ | 78.1 | 72.5 | 66.5 |
| pH 8.5 | 81.0 | 71.5 | 68.0 |
| pH 4.5 | 0.2 | 0.1 | 0.1 |
| 0.1M $CaCl_2$ | 22.5 | 27.0 | 27.0 |
| 0.1% $Na_2HPO_4$ | 80.6 | 67.5 | 66.0 |
| 0.1% $Na_3$Citrate | 81.6 | 73.2 | 68.0 |
| Homogenization | 92.5 | 90.0 | 72.4 |

Table 4. Protein solubility (%) of blends of NFDM and SI at a protein concentration of 3.2%

| Variant | NFDM:SI | | |
|---|---|---|---|
| | 75:25 | 50:50 | 25:75 |
| Control | 59.4 | 57.9 | 58.1 |
| $68^{o}C/30'$ | 56.6 | 56.2 | 56.5 |
| pH 8.5 | 72.5 | 64.5 | 60.1 |
| pH 4.5 | 12.0 | 12.2 | 12.3 |
| 0.1M $CaCl_2$ | 54.7 | 46.5 | 35.0 |
| 0.1% $Na_2HPO_4$ | 56.5 | 56.3 | 54.5 |
| 0.1% $Na_3$Citrate | 55.7 | 54.1 | 54.7 |
| Homogenization | 78.3 | 79.7 | 75.6 |

Table 5. Protein solubility (%) of blends of NAC and SI at a protein concentration of 5.0%

| Variant | NAC:SI | | |
|---|---|---|---|
| | 75:25 | 50:50 | 25:75 |
| Control | 71.4 | 67.2 | 64.0 |
| 68°C/30' | 74.0 | 70.0 | 67.3 |
| pH 8.5 | 75.0 | 71.6 | 67.1 |
| pH 4.5 | 72.6 | 64.0 | 49.0 |
| 0.1M $CaCl_2$ | 63.0 | 54.6 | 33.4 |
| 0.1% $Na_2HPO_4$ | 72.0 | 68.3 | 61.0 |
| 0.1% $Na_3$Citrate | 72.4 | 67.1 | 64.5 |
| Homogenization | 89.0 | 74.6 | 71.0 |

Table 6. Protein solubility (%) of blends of EW and SI at a protein concentration of 3.2%

| Variant | EW/SI | | |
|---|---|---|---|
| | 75:25 | 50:50 | 25:75 |
| Control | 85.9 | 65.4 | 59.4 |
| $68^{o}C/30'$ | 81.3 | 70.1 | 65.3 |
| pH 8.5 | 92.2 | 80.1 | 68.0 |
| pH 4.5 | 85.0 | 60.0 | 43.0 |
| 0.1M $CaCl_2$ | 84.0 | 65.0 | 42.8 |
| 0.1% $Na_2HPO_4$ | 90.6 | 67.2 | 59.3 |
| 0.1% $Na_3$Citrate | 87.5 | 70.1 | 56.3 |
| Homogenization | 90.6 | 66.1 | 62.3 |

Table 7. Protein solubility (%) of blends of SW and SI at a protein concentration of 5.0%

| Variants | SW:SI | | |
|---|---|---|---|
| | 75:25 | 50:50 | 25:75 |
| Control | 74.5 | 67.5 | 49.0 |
| 68°C/30' | 73.5 | 62.0 | 48.0 |
| pH 8.5 | 75.0 | 71.5 | 57.5 |
| pH 4.5 | 75.0 | 44.6 | 29.0 |
| 0.1M $CaCl_2$ | 67.4 | 66.4 | 51.4 |
| 0.1% $Na_2HPO_4$ | 73.0 | 67.5 | 49.4 |
| 0.1% $Na_3$Citrate | 74.5 | 68.0 | 47.0 |
| Homogenization | 75.5 | 71.2 | 58.3 |

Table 8. Protein solubility (%) of WPC, WPC/75, EW and EW/75 at a protein concentration of 3.2%

| Variant | Samples | | | |
|---|---|---|---|---|
| | WPC | WPC/75 | EW | EW/75 |
| 0.1M $CaCl_2$-0.1% $Na_2HPO_4$ | 85.9 | 68.8 | 96.8 | 84.3 |
| 0.1M $CaCl_2$-0.1% EDTA | 91.3 | 77.2 | 99.4 | 87.5 |
| 0.1% $Na_2HPO_4$-68°C/30' | 90.6 | 81.3 | 98.4 | 92.2 |
| pH 8.5-68°C/30' | 93.8 | 81.3 | 99.2 | 89.1 |
| 0.06% $H_2O_2$-Cat. | 94.4 | 78.1 | 99.8 | 88.1 |
| 0.01% Mercaptoethanol | 96.9 | 90.0 | 99.3 | 90.6 |
| Maleic anhydride | 92.8 | 80.6 | 89.6 | 72.8 |
| Enzyme hydrolysis | 98.4 | 90.6 | 95.4 | 83.8 |
| 5.0% NaCl | 89.0 | 75.0 | 91.3 | 84.1 |
| 0.5% SHMP | 89.4 | 72.5 | 93.8 | 77.2 |

Table 9. Protein solubility (%) of NFDM, NFDM/50, SI, NAC, and NAC/50 at a protein concentration of 3.2%

| Variants | Samples | | | | |
|---|---|---|---|---|---|
| | NFDM | NFDM/50 | SI | NAC/50 | NAC |
| 0.1M $CaCl_2$-0.1% $Na_2HPO_4$ | 59.5 | 40.6 | 51.6 | 26.2 | 4.7 |
| 0.1M $CaCl_2$-0.1% EDTA | 51.5 | 53.6 | 51.6 | 0.5 | 39.1 |
| 0.1% $Na_2HPO_4$-68°C/30' | 84.3 | 74.4 | 65.6 | 89.1 | 90.6 |
| pH 8.5-68°C/30' | 75.0 | 64.3 | 61.9 | 88.5 | 90.0 |
| 0.06% $H_2O_2$-Cat. | 77.2 | 69.2 | 64.7 | 84.4 | 98.6 |
| 0.01% Mercaptoethanol | 81.9 | 78.2 | 73.4 | 84.3 | 90.9 |
| Maleic anhydride | 82.9 | 71.0 | 67.5 | 75.8 | 80.9 |
| Enzyme hydrolysis | 70.3 | 63.1 | 79.1 | 70.3 | 66.6 |
| 5.0% NaCl | 75.0 | 58.4 | 56.8 | 56.1 | 54.7 |
| 0.5% SHMP | 69.2 | 55.3 | 53.1 | 72.5 | 79.7 |

made it possible to achieve the desired solubility by selecting the appropriate treatment for the protein.

Solubility Index

Solubility index is a measure of the material sedimented during centrifugation at 1000 rpm. It is measured by centrifugating 50 ml samples in graduated centrifuge tubes and recording the ml of sediment. High index numbers correspond to low dispersibility. If a protein or ingredient is to be functionally active in a liquid system that material must be dispersible. The solubility index (SBI) is a good measurement of this characteristic.

NFDM had low index values at all protein levels (Table 10) with the exception of those samples acidified to pH 4.5. At this pH some coagulation occurred which resulted in higher values. NAC had low index values at all three protein concentrations except for samples acidified to pH 4.5 or dispersed in 0.1M $CaCl_2$ where some coagulation took place.

Electrodialyzed and sweet whey also had low indexes at the protein levels studied. Solubility index increased slightly as the percent protein rose to 8.0. Samples receiving heat treatment, acidification to pH 4.5 or dispersed in 0.1M $CaCl_2$ were also higher. Untreated WPC had low index values. Heat treatment, addition of $CaCl_2$ or adjustment of the pH to 4.5 increased the SBI. Values also increased slightly as the percent protein rose to 8.0.

Table 10. Solubility index of NFDM, NFDM/50, NAC, NAC/50, EW, EW/75, WPC, WPC/75, SW, SW/50 and SI at a protein concentration of 3.2%

| Sample | Variant | | | | | |
|---|---|---|---|---|---|---|
| | Control | 68°C-30' | 0.1M-$CaCl_2$ | 0.1M $CaCl_2$ 68°C/30' | Homog. | 5.0% Pro.-Control |
| NFDM | 0.0 | 0.0 | 0.2 | 4.0 | 0.0 | 0.1 |
| NFDM/50 | 2.5 | 2.3 | 0.8 | 4.8 | 0.4 | 2.5 |
| NAC | 0.1 | 0.1 | 2.0 | 2.5 | 0.1 | 0.1 |
| NAC/50 | 2.0 | 2.0 | 2.0 | 4.4 | 0.5 | 4.4 |
| EW | 0.1 | 0.1 | 0.1 | 1.0 | 0.0 | 0.1 |
| EW/75 | 1.3 | 1.3 | 1.1 | 2.1 | 1.0 | 1.8 |
| WPC | 0.1 | 0.1 | 0.2 | 1.3 | 0.1 | 0.1 |
| WPC/75 | 1.0 | 1.2 | 1.1 | 2.0 | 1.0 | 1.6 |
| SW | 0.1 | 1.0 | 1.0 | 1.5 | 0.1 | 0.5 |
| SW/50 | 3.8 | 4.0 | 3.5 | 4.0 | 2.5 | 3.5 |
| SI | 3.5 | 2.5 | 3.5 | 5.0 | 0.3 | 6.0 |

The SBI of soy isolate were substantially higher than those of the milk products. Heat treatment lowered the SBI due to the greater dispersibility of the protein. Homogenization markedly reduced SBI. This was also found by Guy et al. (1969) who used homogenization to reduce the SBI of soy-whey systems. As the amount of soy protein increased to 8.0% SBI increased almost linearly. Addition of stabilizing salts had little effect upon these values.

Blends of NFDM and SI had values intermediate (Table 10) between the unblended materials. As the amount of NFDM in the blends increased SBI decreased. Solubility index increased with increasing protein concentration. Processing treatments had approximately the same effect upon the blends as was indicated for the unblended materials. Blends between NAC and SI had results very similar to those recorded for NFDM:SI blends. Scores increased as protein concentration increased. Blends having the greater percentage NAC had lower SBI.

Blends between WPC and SI had higher index values than the unblended WPC. This was true for all whey protein:soy isolate blends. SBI decreased as the amount of whey product in the samples increased. Proportionally the values were slightly lower than the same blend ratios of NFDM and NAC. This may have been due to the high solubility maintained by the whey product:soy isolate blends. SBI values increased as percent protein rose.

Many of the processing variants employed in functionality Part II, did not substantially affect the SBI. However, the treatments involving added $CaCl_2$ did appreciably affect SBI. These treatments generally resulted in higher measurements for almost all samples. Many of the samples subjected to these treatments suffered substantial denaturation which resulted in measurable precipitation. In these instances solubilities decreased markedly. Addition of 3.5% glycan to the dispersions resulted in very high measurements. This was because the glycan settled out. After homogenization of these samples scores were lowered to approximately their former values. Very few of the remaining treatments had any significant affect upon the SBI values of the samples.

Solubility index is a useful parameter for relating the amount of sedimentable material to dispersibility. SBI is affected by protein composition and processing treatments. In this study, certain treatments did affect SBI though values obtained were more related to the amount of SI in the sample.

## Viscosity

Viscosity is related to the degree of protein hydration which is affected by pH, ionic strength, temperature and by agents which affect the water of hydration. Viscosity is an important functional property of many products in addition to its obvious effect upon processing equipment and techniques.

Viscosity measurements are presented in Tables 11-14. As protein concentration increased the viscosity (Table 11) of SI sols rose. Values more than doubled as the protein concentration went from 3.2 to 8.0%. Sosulski et al. (1976), Circle et al. (1964), Fleming et al. (1975) and Hermannson (1975) reported that increasing the soy protein concentration caused substantially higher viscosities. Hermannson (1975c) felt this increase was due to protein-protein interaction which led to the formation of a protein network at elevated protein levels. Heating the soy sols further increased the viscosity measurements. Hermannson (1975), Circle et al. (1964), Hutton (1977a) and Bau et al. (1978) found that heating dramatically increased the viscosities of soy protein, especially at temperatures of 90°C or more. The increases noted were probably due to increased swelling of the proteins. Circle et al. (1964), Kelley and Pressey (1966), Fleming et al. (1975), Ehninger and Pratt (1974) and Hermannson (1975c) observed that alkali treatment of soy protein (pH 8-9) resulted in higher viscosities. This effect was confirmed in research reported in this thesis. The greater viscosities were probably due to the greater net charge and increased swelling of the proteins. Homogenization of the samples reduced viscosities slightly while addition of either $Na_2HPO_4$ or $Na_3$Citrate had little effect. Guy et al. (1969), who examined soy flour-whey blends, also found that homogenization reduced viscosity.

Viscosities of NFDM dispersions increased as protein concentration increased to 8.0%, though this increase was much less than that observed with SI. Viscosities were also slightly higher as the pH of sols were adjusted to 8.5. The remaining treatments had little effect upon the viscosity measurements of NFDM. At 3.2% protein NAC had viscosities approximately the same as other milk products. Substantial increases were noted as the protein concentration rose to 8.0%. The samples were slightly syrupy at this protein level, with values similar to those recorded for SI. Hermannson (1975c) reported that the viscosity of NAC was very concentration dependent with nearly logarithmic increase over a broad protein range. This researcher felt that the increased viscosities were due to an increase in the number of solvated caseinate particles. Adjustment of the pH to 8.5 resulted in higher measurements as was also reported by Hermannson (1975c). The addition of stabilizer salts had little effect.

WPC sols had the lowest viscosities (Table 12) of any of the products tested. This was also observed by Hermannson (1972) who reported that WPC had lower viscosities than either SI or NAC when examined over a broad protein concentration. Increasing the amount of protein in the samples had very little effect upon viscosity measurements. At 8.0% protein, heating increased viscosities to a small extent. None of the other treatments employed had appreciable effect upon the viscosities of WPC sols. Hermannson (1975c)

demonstrated that WPC dispersions maintained low viscosity until a protein concentration of 18-20% was reached. Factors such as pH and ionic strength had little effect upon viscosities. Viscosity measurements of EW and SW increased tremendously as protein content was raised to 8.0%. This was because of the paste-like products which resulted due to the high total solids levels. At 3.2% protein SW had the highest viscosities of any of the products tested, while EW had values similar to the other milk products. Addition of $CaCl_2$ substantially increased viscosities at 8.0% protein for both products, possibly due to interaction between protein and calcium ions. Heating also increased the viscosity measurements.

NFDM:SI blends had viscosities only slightly different from the unblended systems. At 3.2% protein, viscosities were very close regardless of the blend ratio. At 5.0% protein, viscosities rose slightly, with no major differences found due to blend ratio. At 8.0% protein viscosities were higher for those samples containing the greater percent SI. The viscosities of samples composed primarily of NFDM or SI had values close to those recorded for the unblended protein systems. The viscosity of the blends was affected by the treatments employed in much the same manner as the individual products. The viscosities of NAC:SI blends rose as total protein increased. At 3.2 and 5.0% protein slight differences were noted due to blend ratio. At 8.0% protein the samples containing the greater percent NAC had slightly

higher viscosities, but were not substantially different than those measurements recorded for the individual proteins. Heating and pH adjustment increased the viscosity of the blends as previously reported for the unblended products.

There were only small differences in the viscosities of the various blend ratios of WPC:SI. Viscosities rose slightly as the percent protein increased. At 8.0% protein, blends containing 75% of the total as SI had slightly higher viscosities. WPC:SI blends had lower viscosities than NAC:SI blends which was in agreement with Hoffman (1974). Hoffman (1974) also found that as soy protein replaced whey protein in blends, viscosities rose slightly. There were slight differences in the viscosities of EW:SI blends at 3.2 and 5.0% protein. At 8.0%, blends containing 75% EW had higher viscosities due to the high total solids content. Addition of $CaCl_2$ further enhanced these higher values. The viscosity of these blends (at all ratios) increased slightly with increased protein. At the protein concentrations studied, SW:SI blends which contained 75% SW had higher viscosities. Viscosity rose with increased protein content due to the high total solids. Blending SI and SW lowered the viscosities of the samples as compared to unblended SW systems. Addition of $CaCl_2$ to the samples resulted in higher viscosities.

The viscosities of the samples exposed to the treatments from Functionality Part II are shown in Tables 13 and 14. Treatments which involved the utilization of $CaCl_2$ with either $Na_2HPO_4$ or $Na_3$Citrate resulted in higher viscosities

for SW and EW samples. The remaining samples had viscosities approximately the same.

Addition of $CaCl_2$ and heating to 68°C/30 min increased the viscosity of SW. The viscosity of SW also increased when either $Na_2HPO_4$ or $Na_3$Citrate were added to the samples prior to heating at 68°C/30 min.

Addition of maleic anhydride to the samples increased the viscosities of NFDM, NFDM/50, NAC, NAC/50 and SI, though Franzen and Kinsell (1976) reported that succinylation did not significantly alter the viscosity of soy proteins. The viscosity of whey products was much less affected by this treatment.

Addition of 0.1% CMC increased the viscosities of all samples approximately by the same magnitude. Heating at 68°C/30 min had no appreciable effect upon the measurements. Addition of 0.5% CMC to the samples markedly increased the viscosities. The increase in viscosity caused by addition of CMC was due to the stabilizing and thickening characteristics of this hydrocolloid. Lang et al. (1976) reported that addition of stabilizer to skimmilk increased its viscosity.

Addition of glycan was ineffectual because it settled out. Homogenization following addition of glycan dispersed the material and resulted in higher viscosities. This was probably due to the polysaccharide nature of this material.

The addition of 5.0% NaCl was responsible for causing a slight decrease in the viscosity of SI. This also was found

by Hermannson (1975c) and Ehninger and Pratt (1974). They postulated that salt must have a general effect on the structure of soy proteins. Swelling and solubility were reduced. The presence of less swollen, more rigid aggregates and less solvated protein molecules could lead to lower viscosities. NAC sols were slightly more viscous in 5.0% NaCl dispersions. Hermannson (1975c) felt that the effect of salt might be due to dehydration, resulting in changes in the repulsive balance or the micellar structure of the proteins. Addition of mercaptoethanol or cysteine as reducing agents were responsible for slight viscosity reductions. Hermannson (1975c) and Kelley and Pressey (1966) also reported that reducing agents lowered the viscosity of soy protein dispersions. This reduction was probably due to the breaking of disulfide bonds resulting in some dissociation. Enzymatic hydrolysis of SI lowered its viscosity to a small extent probably due to release of peptides. The remaining treatments had little or no affect upon the viscosities of the samples.

The viscosity of protein dispersions is of prime importance because 1) information is obtained relating to quality control; 2) insight concerning changes in the molecular structure is provided; 3) data may be obtained regarding suitable fields of application for a new product; 4) information necessary for the optimal design of unit processes may result; and 5) information relevant to mouthfeel and hence acceptability characteristics may be obtained. In general, viscosities of milk products, soy isolates and milk

Table 11. Viscosity (cp) for selected samples of NFDM, NFDM/50, NAC, NAC/50 and SI at 3.2, 5.0 and 8.0 percent protein

| Variant | Sample | | | | |
|---|---|---|---|---|---|
| | NFDM | NFDM/50 | SI | NAC/50 | NAC |
| | | | 3.2% | | |
| Control | 10 | 10 | 9 | 10 | 11 |
| 68°C/30' | 10 | 10 | 9 | 11 | 12 |
| pH 8.5 | 12 | 12 | 12 | 11 | 12 |
| | | | 5.0% | | |
| Control | 12 | 12 | 11 | 12 | 15 |
| 68°C/30' | 12 | 12 | 12 | 11 | 14 |
| pH 8.5 | 13 | 12 | 14 | 15 | 19 |
| | | | 8.0% | | |
| Control | 14 | 17 | 19 | 19 | 28 |
| 68°C/30' | 14 | 19 | 25 | 22 | 26 |
| pH 8.5 | 19 | 23 | 28 | 25 | 35 |

Table 12. Viscosity (cp) for selected samples of EW, EW/75, WPC, WPC/75, SW and SW/50 at 3.2, 5.0 and 8.0 percent protein

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | EW | EW/75 | WPC | WPC/75 | SW | SW/50 |
| | | | 3.2% | | | |
| Control | 10 | 11 | 9 | 9 | 19 | 10 |
| 0.1M $CaCl_2$ | 11 | 10 | 9 | 9 | 23 | 10 |
| 0.1% $Na_2HPO_4$ | 10 | 10 | 8 | 9 | 17 | 10 |
| | | | 5.0% | | | |
| Control | 10 | 13 | 9 | 9 | 89 | 11 |
| 0.1M $CaCl_2$ | 16 | 13 | 8 | 9 | 660 | 15 |
| 0.1% $Na_2HPO_4$ | 15 | 12 | 8 | 9 | 68 | 12 |
| | | | 8.0% | | | |
| Control | 790 | 160 | 10 | 10 | 780 | 41 |
| o.1M $CaCl_2$ | 2,100 | 300 | 11 | 11 | 7,900 | 1,700 |
| 0.1% $Na_2HPO_4$ | 540 | 62 | 10 | 10 | 1,500 | 420 |

Table 13. Viscosity (cp) for selected samples of NFDM, NFDM/50, NAC, NAC/50 and SI at a protein concentration of 3.2%

| Variant | Sample | | | | |
|---|---|---|---|---|---|
| | NFDM | NFDM/50 | SI | NAC/50 | NAC |
| Homogenization | 9 | 10 | 8 | 10 | 11 |
| pH 8.5-68$^{o}$C/30' | 11 | 10 | 10 | 11 | 11 |
| Maleic anhydride | 13 | 14 | 15 | 15 | 12 |
| 0.1% CMC | 17 | 19 | 24 | 22 | 22 |
| 0.5% CMC | 114 | 126 | 170 | 125 | 105 |
| 3.5% glycan-Homog. | 189 | 241 | 258 | 237 | 230 |
| 0.1M $CaCl_2$-1.0% EDTA | 15 | 14 | 9 | 8 | 9 |
| Enzyme hydrolysis | 10 | 8 | 7 | 8 | 8 |

Table 14. Viscosity (cp) for selected samples of EW, EW/75, WPC, WPC/75, SW and SW/50 at a protein concentration of 3.2%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | EW | EW/75 | WPC | WPC/75 | SW | SW/50 |
| 68°C/30' | 12 | 11 | 8 | 8 | 23 | 10 |
| 0.1M $CaCl_2$-0.1% $Na_3$Cit. | 12 | 10 | 9 | 10 | 41 | 11 |
| 0.1M $CaCl_2$-68°C/30' | 13 | 10 | 10 | 9 | 28 | 10 |
| Maleic anhydride | 13 | 12 | 9 | 9 | 18 | 15 |
| 0.1% CMC | 23 | 24 | 15 | 15 | 29 | 27 |
| 0.5% CMC | 134 | 143 | 79 | 99 | 215 | 160 |
| 3.5% glycan-homog. | 327 | 310 | 275 | 241 | 471 | 433 |
| Enzyme hydrolysis | 10 | 11 | 8 | 8 | 19 | 10 |

product:soy isolate blends were quite close regardless of the blend ratio, with the exception of the whey products at 8.0% protein. Viscosities increased moderately as the percent protein increased. Specific treatments resulted in higher (or lower) viscosity, but the majority had little effect.

Gelation

Gelation is an important functional property pertaining to many food products. The objective of this study was to evaluate the gelation capability of soy isolate:milk product blends. Edi-ProN, the soy isolate utilized through out this research did not demonstrate any gelling ability under the conditions used. Yasumatsu et al. (1972) reported that denatured soy isolates were more amenable to gelation because partial denaturation was believed to be conducive to preparation of firm gels. Soy isolates with a nitrogen solubility index (NSI) of 50 had better gel formation qualities than one of 80. Edi-ProN with high nitrogen solubility did not form firm gels, therefore, a substitution was made. Promine D, a SI manufactured by Central Soya, Inc., was used in all experiments dealing with gelation.

The data pertaining to gelation are presented in Table 15. SI formed firm gels except when the dispersion was adjusted to pH 8.5 prior to heating. Under the influence of this treatment the sample turned black and remained fluid. The decrease in gelation at this pH may have been caused by hydrolytic action (Circle and Meyer, 1964). Circle and

Meyer (1964) formed soy gels by heating 10% dispersions at 100°C for 45 min. These researchers described the gelatin phenomenon as being primarily dependent on heat denaturation. Gel rigidity was principally due to protein concentration. Gels of denatured proteins involve first an unfolding or extension of the globular protein into a more unsymmetric shape, exposing reactive groups and nonpolar amino acids. Association of the chains by crosslinks and by localized and nonlocalized attractive forces lead to the formation of a three dimensional network. The irreversibility of the soy gels indicated that primary covalent bonds were involved in crosslinking. The addition of $Na_2HPO_4$, $Na_3$Citrate or $CaCl_2$ increased gel strength. Saio (1975b) reported that addition of calcium to soy increased strength of gels perhaps due to greater crosslinking. Hermannson and Akesson (1975a), Sosulski et al. (1976), Catsimpoolas and Meyer (1970) and Fleming and Sosulski (1975) all demonstrated the gelation capability of soy protein.

Untreated dispersions of NFDM demonstrated weak gel properties. Addition of $CaCl_2$ substantially increased gel strength. Kalab and Emmons (1972) reported that calcium promoted gelation. Firm gels were produced by heating NFDM at 100°C (Kalab et al., 1971) though 50% dispersions were used. High total solids content were necessary in the development of these gels. Sodium caseinate sols did not demonstrate any gelation ability in this study. Hermannson

Table 15. Gelation strength, measured with a Plummett*, was determined for SI, NFDM, WPC, WPC/50 and EW/25

| Sample | Variant | | | | |
|---|---|---|---|---|---|
| | Control | 0.1% $Na_2HPO_4$ | 0.1% $CaCl_2$ | pH 8.5 | pH activ. |
| SI | 4.5 | 7.0 | 6.8 | NG | 4.8 |
| NFDM | 2.0 | NG[a] | 7.5 | NG | NG |
| WPC | 10.0 | 10.0 | 8.8 | 10.0 | 9.0 |
| WPC/50 | 6.5 | 10.0 | 7.5 | 9.0 | 9.0 |
| EW/25 | 7.0 | 7.5 | 6.2 | 8.0 | 7.1 |

*A score of 10 = very firm and a score of 1 = very soft.

a; NG = no gel formation.

and Akesson (1975a) also reported that NAC failed to gel when heated at 100°C. Sweet and electrodialyzed whey dispersions failed to show any gel formation in this research. The remaining milk product WPC, did form very strong gels when subjected to the conditions used. The gels were firm, (almost rubbery) translucent and possessed good sensory qualities. McDonough et al. (1974) prepared firm, resilent gels from 10% WPC solutions which did not whey off. Modler and Emmons (1977) reported gelation of a WPC following heating at 95°C for 20 min. Hermannson and Akesson (1975a) also showed that WPC formed firm gels when heated. Schmidt et al. (1978), in examining the gelation characteristics of peanut/whey protein blends, observed that whey protein formed firm gels when heated.

Very limited success was achieved when blends between SI and the milk products were examined for gelation ability. Blends containing 25% EW formed gels of moderate strength though wheying off occurred. WPC:SI blends demonstrated substantial gelling capability. A small amount of wheying off did occur, with partial visual separation of the two gel systems. Of the milk products examined only WPC, in association with or without SI, appeared to have substantial gelation characteristics.

Emulsion Capacity

The ability of a protein to emulsify fat is an important functional characteristic of that protein. This property is

extremely important in meat products, salad dressings, spreads, etc., where ever emulsification is required. Kinsella (1976) described emulsification as a surfacant property related to the capacity of proteins to lower the interfacial tensions between the hydrophobic and hydrophilic components in foods. Generally, surfactant properties are related to the aqueous solubility of proteins.

Emulsion capacities (EC) were determined for the samples at 3.2% protein. The results are shown in Tables 16 and 17. NFDM and WPC had the highest EC of the products tested. SW and SI had the lowest. The apparent discrepancy between the EC of the whey products was probably due to compositional and processing differences. Sodium caseinate had slightly less EC than NFDM. Kuehler and Stine (1974) reported that WPC had greater EC than casein with NFDM having EC intermediate between the two. Smith et al. (1973) and Crenweldge et al. (1974) found that NFDM had more emulsifying capacity than SI at neutral pH.

NFDM:SI blends which were composed of not more than 50% NFDM had lower EC than NFDM. Blends containing 75% NFDM had EC resembling those found for NFDM. Sodium caseinate: soy isolate blends had less EC than NAC except for those blends containing 75% NAC. Hoffman (1974) reported that the EC of casein-soy blends decreased as the amount of soy protein increased. Blends of EW:SI had almost identical EC regardless of the blend ratio. This also was found to be true with SW:SI blends. Blends of WPC:SI had lower EC than

WPC regardless of the blend ratio, though EC increased as the percent WPC in the blend increased. Hoffmann (1974) found that the ability of WPC to emulsify oil decreased as SI was substituted into the sample.

Heating had only a slight effect on the EC of SI, NFDM, NAC and there blends. Emulsion capacity increased to a small extent perhaps due to the slightly higher solubilities. The effect of heat on the EC of the whey products was opposite to that observed for the other samples. As the heating temperature rose the EC decreased probably due to decreased solubility. The EC of whey product:soy isolate blends decreased, but less so, when heated at the higher temperatures.

Lowering the pH to 4.5 substantially decreased the EC of SI, NFDM, NAC and their blends. Crenweldge et al. (1974) and Hutton and Campbell (1977b) found that the EC of NFDM and SI were minimal near their isoelectric points due to loss of solubility. WPC and its SI blends also had lower EC. Whey product:SI blends had lower EC proportional to the amount of SI in the blend.

Raising the pH of the samples to 8.5 increased the EC of NFDM, NAC and SI. Inklaar (1969) and Pearson et al. (1965) reported that soy protein, NFDM and sodium caseinate had greater EC at higher pH probably due to increased solubility. The whey products were much less affected.

Addition of $CaCl_2$ to samples of SI, NAC and NAC:SI blends markedly reduced their EC due to loss of solubility. The EC of NFDM also decreased (though not nearly to the

same extent as for the previous samples). Blends of NFDM:SI had decreased EC proportional to the amount of SI in the sample. This treatment had little effect upon the EC of the whey products but did decrease the EC of their SI blends.

Homogenization of the samples resulted in only minor changes in EC. Addition of stabilizing salts did not appreciably alter the emulsion capacities.

The emulsion capacities of the samples exposed to the treatments from Functionality Part II are summarized in Tables 16 and 17. Addition of 0.2% monoglycerides increased the EC of all samples. Increased EC may have been due to the emulsification activity of monoglycerides. Addition of 125 ppm SDS may have also improved the EC of the samples due to the surfactant nature of this compound.

Addition of succinic or maleic anhydride increased the EC of the samples. Franzen and Kinsella (1976) observed that succinylation enhanced the EC of soy isolate dispersions. They concluded that it was due to the increased solubility of the protein.

The EC of many of the samples was improved by the addition of 5.0% NaCl. Addition of anions may increase EC by enhancing the unfolding of protein molecules, thereby enlarging their effect surface area available for interfacial membranes (Kinsella, 1976). Soy protein is known to be dissociated by high concentrations of NaCl.

Enzymatic hydrolysis did not significantly affect the EC of the majority of samples. Digestion for 1 hr did

Table 16. Emulsion capacity measured in g oil/mg protein for NFDM, NFDM/50, SI, NAC/50 and NAC at a protein concentration of 3.2%

| | Sample | | | | |
|---|---|---|---|---|---|
| | NFDM | NFDM/50 | SI | NAC/50 | NAC |
| Control | 3.5 | 3.0 | 2.4 | 3.0 | 3.2 |
| 77°C/30' | 3.9 | 3.1 | 2.6 | 2.9 | 3.4 |
| pH 4.5 | 1.7 | 1.4 | 1.0 | 0.4 | 0.1 |
| 0.1M $CaCl_2$ | 2.7 | 2.5 | 0.8 | 1.0 | 1.2 |
| 0.1M $CaCl_2$-0.1% $Na_2HPO_4$ | 5.1 | 3.1 | 2.1 | 2.1 | 1.7 |
| Homogenization | 3.1 | 2.9 | 2.1 | 2.3 | 2.8 |
| 0.1% $Na_2HPO_4$-68°C/30' | 5.3 | 4.0 | 2.2 | 3.9 | 5.1 |
| pH 8.5-68°C/30' | 4.9 | 3.4 | 2.1 | 3.6 | 5.1 |
| Maleic anhydride | 5.1 | 4.5 | 3.0 | 4.6 | 5.2 |
| 0.5% SHMP | 2.8 | 2.4 | 2.3 | 2.6 | 3.0 |
| 125 ppm SDS | 3.6 | 3.1 | 3.0 | 3.2 | 3.4 |
| 0.2% monoglyceride | 4.6 | 3.5 | 2.7 | 3.6 | 4.1 |
| 5.0% NaCl | 4.3 | 3.8 | 3.0 | 2.9 | 2.4 |
| Enzymatic hydrolysis | 3.5 | 3.0 | 3.1 | 3.1 | 3.5 |

Table 17. Emulsion capacity measured in g oil/mg protein for EW, EW/75, WPC, WPC/75, SW and SW/50 at a protein concentration of 3.2%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | EW | EW/75 | WPC | WPC/75 | SW | SW/50 |
| Control | 2.9 | 2.8 | 3.7 | 3.3 | 2.4 | 2.6 |
| 77°C/30' | 2.6 | 2.7 | 1.5 | 2.3 | 2.0 | 2.4 |
| pH 4.5 | 3.3 | 2.8 | 1.7 | 2.9 | 2.6 | 2.6 |
| 0.1M $CaCl_2$ | 2.9 | 2.8 | 4.0 | 1.8 | 2.5 | 2.1 |
| 0.1M $CaCl_2$-0.1% $Na_3$Cit. | 4.1 | 3.3 | 5.0 | 2.7 | 2.5 | 2.5 |
| 0.1M $CaCl_2$-1.0% EDTA | 4.5 | 3.9 | 5.3 | 3.4 | 3.6 | 2.7 |
| 0.1% $Na_2HPO_4$-68°C/30' | 4.4 | 4.0 | 4.7 | 3.2 | 2.6 | 2.2 |
| 0.1% L cysteine | 2.8 | 2.3 | 3.1 | 2.0 | 2.4 | 2.1 |
| 125 ppm SDS | 4.2 | 3.4 | 3.9 | 3.3 | 3.8 | 3.0 |
| 0.2% monoglyceride | 4.2 | 4.0 | 4.2 | 4.3 | 3.2 | 2.8 |
| Maleic anhydride | 4.3 | 4.1 | 4.4 | 4.2 | 4.3 | 3.6 |
| 5.0% NaCl | 4.5 | 3.5 | 5.1 | 4.1 | 3.1 | 2.5 |
| 0.5% SHMP | 3.2 | 3.1 | 4.7 | 3.6 | 3.5 | 2.5 |
| Enzyme hydrolysis | 2.7 | 2.4 | 2.8 | 2.6 | 3.1 | 2.7 |

increase the EC of SI probably due to the increase in solubility. After 3 hr of digestion EC decreased slightly. Kuehler and Stine (1974) reported that enzymatic digestion (Prolase) did not significantly affect the EC of whey protein, NFDM or NAC.

Addition of either $Na_2HPO_4$ or $Na_3$Citrate prior to heating at 68°C/30 min increased the EC of the samples. Adjustment of the pH to 8.5 and heating also increased the EC of most of the samples. Increased solubility may have been responsible for the improvement noted.

The EC of SI, NAC and their blend remained the same or decreased when any of the treatments employing $CaCl_2$ were tested. The EC of NFDM and NFDM/50 increased when these materials were dispersed in 0.1M $CaCl_2$ containing either $Na_2HPO_4$ or $Na_3$Citrate. The EC of the whey products also increased when subjected to these treatments.

Emulsification is a primary functional requirement in several food proteins. In general, the soy isolate had less EC than the milk products. Blends between the two often had nearly as much EC as the milk product and usually always more than the SI. By selecting the treatment corresponding to a particular protein it was possible to obtain the higher EC associated with the milk products.

Whipping Ability

Foaming or whipping, i.e. the capacity to form stable foams with air, is an important functional property of

proteins. This property is important in angel food cakes, confections, candy, meringues, souffles and toppings. Foaming properties include whippability (or foamability) and foam stability. Food foams usually consist of air droplets dispersed in and enveloped by a liquid containing a soluble surfactant. The surfactant lowers the surface tension of the liquid, thereby facilitating deformation of the liquid and the marked expansion in its total surface area against its own surface tension (Kinsella, 1976). In this study, specific volume (SV) in ml/g was used to assess the increase in volume following whipping. Foam stability was measured by recording the one-half stability time (½t) in min. Stability times of less than 5 min were reported as 0.

SI foams had low SV with zero stability times at all three protein concentrations. Watts (1937) reported that several commercial soy flours had little or no whipping ability and Sosulski et al. (1976) found that several soy isolates did not exhibit good foaming properties. Samples which were dispersed in 0.1M $CaCl_2$ were the only SI sols to have stable foams. Substantial loss of solubility was suffered by these samples. Improved foam stability may have been due to a charge effect, crosslinking or partial denaturation. Kinsella (1976) observed that proteins forming foams must exhibit a critical balance between their ability to engage in limited intermolecular cohesion required to form a stable elastic membrane and the tendency

to self-associate excessively, which would result in aggregation and breakdown of the foam. Soy protein in the presence of calcium may have had sufficient protein-protein interaction to stabilize a foam structure.

Reconstituted SW powder exhibited minimal foam production and stability. Sols of sweet whey did not whip into stable foams, regardless of the treatment, until the protein concentration was 8.0%. At this concentration the total solids level was very high which resulted in very viscous samples. When these samples were whipped paste like foams were produced with low SV but long stability times.

Blends of SW:SI did not whip into stable foams except at 8.0% protein where paste like foams were produced. These highly viscous foams were not observed unless 75% of the blend ratio was derived from SW.

Heating was necessary to produce stable foams (Table 18) from WPC sols. Some heat denaturation appeared to be a prerequisite to whipping. Partial heat denaturation may be necessary to increase the degree of cohesion, i.e. protein-protein interaction necessary in the formation of a protein film (Kinsella, 1976). The optimum time/temperature relationship appeared to be 68-77$^{o}$C/30 min. Increased heat treatment reduced stability times. Stability times increased as the percent protein increased. Peter and Bell (1930), Jelen (1973), Devilbiss _et al_. (1974), Kuehler and Stine (1974) and McDonough _et al_. (1974) reported that heat treatment significantly improved the whipping properties of whey

and WPC. McDonough et al. (1974) observed that partial denaturation of the protein was apparently necessary to produce stable foams from WPC.

Blends of WPC:SI did not whip into stable foams at 3.2% protein unless 75% of the protein was derived from WPC. From these samples stable foams were prepared when the samples were heated at temperatures not higher than 77°C. The stability of the foams diminished with increased heat treatment. At 5.0% protein, stable foams were produced from those blends containing at least 50% WPC which were heated. Specific volume and ½t increased as the WPC in the blends increased. Foam stability (FS) declined when the samples were subjected to UHT heating. At 8.0% protein SV and ½t stability increased. Foam stability and specific volume increased with increased WPC in the blends. Hoffman (1974) reported that WPC-soy isolate blends maintained the SV associated with WPC but were so unstable that drip could not be measured. McDonough et al. (1974) found that the stability of WPC sols increased as protein concentration increased.

Samples of EW whipped into stable foams (Table 19) at all three protein concentrations. Heating had much less effect upon the whipping properties of this whey product compared to WPC. Adjustment of the pH to 8.5 or 4.5 increased both SV and ½t. Hansen and Black (1972) and Kuehler and Stine (1974) observed that the greater the net charge, the greater was the tendency to whip. Peter and Bell (1930)

and Jelen (1973) found that acid whey sols had improved whipping properties. Acidification enhanced the vulnerability of whey proteins to denaturation during the whipping process. This may have accentuated protein-protein interaction, thus increasing the cohesiveness of the proteins during membrane formation. Addition of $CaCl_2$ to EW sols increased the stability of the foams. This may have been due to denaturation, crosslinking or charge distribution. At 5.0% protein FS substantially improved. At 8.0% protein, untreated EW foams had viscous, paste like properties. These foams had very long stability times though SV were markedly reduced.

Blends of EW:SI whipped into stable foams (Table 20), but had much shorter stability times than EW foams. At a blend ratio of EW 25:75 SI stable foams were produced from those samples which were modified by addition of stabilizer salts, $CaCl_2$, or which were acidified to pH 4.5. As the percent EW in the blend increased so did the specific volume and stability of the foam. Little improvement was noted in the whipping properties as the concentration of protein increased. In general, displacement of EW for SI substantially depressed both SV and ½t of the whips.

Sodium caseinate whipped into stable foams (Table 21) at all protein levels. Untreated caseinate sols had shorter stability times than NFDM. Neither SV or ½t changed appreciably as the percent protein increased from 3.2-8.0% protein. Heating had little effect upon the whips though

UHT heating did depress both SV and FS. Lowering the pH to 4.5 destroyed all foaming tendencies while addition of $CaCl_2$ to the sols resulted in markedly improved whipping properties. Since both treatments significantly lowered the solubilities of the samples the improvement noted for $CaCl_2$ treated sols may have been due to crosslinking of proteins. Min and Thomas (1977) reported that addition of calcium to NAC sols increased foam overrun and firmness. Homogenization slightly decreased FS.

Sodium caseinate:soy isolate blends whipped into foams (Table 22) having poor stability. Specific volumes were lower than those recorded for NAC. Specific volumes and stability times were similar regardless of the blend ratio. Stability times remained approximately the same as the amount of protein increased while SV decreased slightly. Heating had little effect upon the whipping properties of these blends. Lowering the pH to 4.5 eliminated the whipping properties of the blends while addition of $CaCl_2$ to the dispersions markedly improved their whippability. Hoffman (1974) reported that replacement of casein with SI had little effect upon specific volume but drastically reduced foam stability.

Untreated NFDM sols whipped into foams (Table 23) having the longest stability times of any of the products tested. The specific volumes of these foams were second only to those of NAC. Foam stability increased as the percent protein increased, though SV decreased. Webb (1941)

reported that SV decreased but stability times increased as the concentration of NFDM solids increased. Whipping properties were reduced when severe heat treatments were employed. Tamsma et al. (1969) found that foam stability of NFDM sols was optimal at 30% total solids. Lowering the pH of the sols to 4.5 drastically reduced whipping properties. The addition of $CaCl_2$ to the samples failed to have the same affect on the whipping properties of NFDM that it did on NAC foams. Addition of stabilizing salts increased both SV and ½t possibly due to their effect on solubilities. Homogenization reduced foam stability slightly.

Blends of NFDM:SI had decreased foam stability compared to NFDM foams (Table 24). Specific volumes were approximately the same. Foam stability improved as the percent NFDM in the blends increased. The stability of NFDM:SI foams was greater than the foams from the other product blends. Addition of stabilizer salts increased FS.

The whipping properties of the samples exposed to the treatments from Functionality Part II are shown in Tables 25 and 26. Addition of $Na_3$Citrate (or $Na_2HPO_4$) to WPC sols dispersed in 0.1M $CaCl_2$ improved both the SV and ½t of its whips. This may have been due to the partial denaturation which resulted or crosslinking of the proteins. The whipping properties of the remaining samples were either unaffected or slightly reduced.

Addition of EDTA to WPC and WPC/75 sols dispersed in 0.1M $CaCl_2$ markedly improved both the SV and ½t of these

whips. The whipping properties of the other samples were reduced.

Addition of either $Na_2HPO_4$ or $Na_3$Citrate to the samples prior to heating resulted in the production of stable foams from SW and SW/50. The SV and ½t of WPC and WPC/75 whips also increased.

Raising the pH of the sols to 8.5 prior to heating resulted in the formation of stable whips from SW and SW/50. The stability and specific volumes of NFDM foams also increased. McDonough et al. (1974) reported that samples subjected to both heat and alkaline pH treatment had excellent whips. Increased whippability may have been due to greater solubility.

Heating following addition of $H_2O_2$ to the samples increased the whipping properties of SW and SW/50. The foam stability of EW foams also increased. This may have been due to oxidation of the heated (partially denatured) proteins. Hansen and Black (1972) reported that addition of $H_2O_2$ to whey protein sols improved their whipping properties. These researchers reported that $H_2O_2$ caused definite changes in the electrophoretic patterns of the proteins and that these changes were probably important in the improved whipping properties.

Addition of reducing agents such as L-cysteine or mercaptoethanol to the samples resulted in greater foam stability and expanded specific volumes for many of the sols. The reducing agents may have affected the whipping

properties of these products by their direct effect upon disulfides or indirectly through increased solubility. Peter and Bell (1930) found that reducing agents improved the whipping characteristics of whey proteins.

The addition of succinic or maleic anhydride markedly improved the foamability of SI sols. The specific volumes of NFDM and NAC sols increased though the FS of NAC foams decreased. The whipping properties of EW were practically eliminated. Franzen and Kinsella (1976) found that succinylation markedly enhanced the FS and SV of soy protein dispersions, probably due to the increase in solubility of these sols.

The foams from NAC and NAC/50 were more stable when 5.0% NaCl was added to these samples. The solubilities of these dispersions were reduced, thereby possibly enlarging their effective surface area.

Addition of 0.5% sodium hexametaphosphate (SHMP) markedly improved the SV and ½t of WPC and WPC/75 sols. Melachouris (1972) observed that whey protein acts as a cation while SHMP acts as an anion. Under the right conditions they bind and form sizable aggregates. These aggregates may be of sufficient size to increase the strength of the protein membrane.

Addition of glycan to the samples was ineffectual because the material settled out. Homogenization was necessary to disperse the glycan. This treatment improved the whipping properties of many of the sols. Foams produced

from these samples had long stability times but reduced SV.

The addition of CMC to the samples increased the FS of practically all the samples (except SI at.1% CMC). Specific volumes were essentially unchanged. Heating following addition of CMC further increased the stability of the whey products and their SI blends. Foams with long stability times were noted after addition of 0.5% CMC. The whips had paste-like properties with low overrun. SI produced foams of the least stability. Morr et al. (1973), Kuehler and Stine (1974) and Hansen and Black (1972) reported that utilization of CMC in protein sols resulted in prolonged stability times.

Enzymatic hydrolysis (prolase) for 1 hr increased the FS of the materials except for NAC and its SI blend. Specific volumes also increased. After digestion for 3 hr FS decreased slightly. Cooney (1976) and Kuehler and Stine (1974) used enzymatic hydrolysis to improve the whipping properties of proteins. These researchers postulated that the increased foamability was probably due to the greater polypeptide content which increased the available surface of the protein. This allowed more air to be incorporated into the foam structure. Extensive hydrolysis resulted in a greater number of small peptides which lacked the strength to maintain the protein membrane.

In general, stable foams were produced from NFDM, NAC, EW and their SI blends. Whey protein concentrate subjected to specific treatments also produced stable foams. The

Table 18. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for WPC at protein levels of 3.2, 5.0 and 8.0%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | 3.2 | | 5.0 | | 8.0 | |
| | SV | ½t | SV | ½t | SV | ½t |
| Control | 2.6 | 0 | 2.3 | 0 | 5.4 | 0 |
| 68°C/30' | 10.3 | 19 | 7.0 | 23 | 7.9 | 45 |
| 77°C/NH | 7.7 | 20 | 6.9 | 25 | 7.1 | 43 |
| 77°C/30' | 6.9 | 18 | 6.9 | 22 | 6.7 | 68 |
| 94°C/4" | 4.6 | 0 | 6.0 | 11 | 6.6 | 11 |
| 121°C/4" | 5.1 | 0 | 7.2 | 7 | 5.6 | 10 |

Table 19. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for EW at protein levels of 3.2, 5.0 and 8.0% protein

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | 3.2 | | 5.0 | | 8.0 | |
| | SV | ½t | SV | ½t | SV | ½t |
| Control | 6.4 | 30 | 7.3 | 28 | 3.1 | 150* |
| 68°C/30' | 6.6 | 28 | 6.3 | 24 | 3.3 | 150* |
| 77°C/30' | 5.1 | 32 | 5.7 | 30 | 3.5 | 150* |
| pH 8.5 | 7.0 | 40 | 7.3 | 70 | 4.5 | 150* |
| pH 4.5 | 9.9 | 35 | 9.5 | 120 | 5.6 | 150* |
| 0.1M $CaCl_2$ | 6.2 | 50 | 8.3 | 94 | 3.1 | 150* |
| 0.1% $Na_2HPO_4$ | 7.0 | 29 | 6.6 | 52 | 2.5 | 150* |
| 0.1% $Na_3$Citrate | 6.8 | 30 | 6.6 | 65 | 3.6 | 150* |

*did not breakdown

Table 20. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for EW/25, EW/50 and EW/75 at a protein level of 5.0%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | EW/25 | | EW/50 | | EW/75 | |
| | SV | ½t | SV | ½t | SV | ½t |
| Control | 3.5 | 0 | 5.1 | 22 | 6.4 | 27 |
| 68°C/30' | 3.6 | 0 | 4.7 | 20 | 5.4 | 23 |
| 77°C/NH | 3.8 | 0 | 5.2 | 16 | 6.0 | 24 |
| pH 8.5 | 3.6 | 0 | 4.7 | 13 | 4.1 | 45 |
| pH 4.5 | 5.6 | 5 | 7.3 | 19 | 5.1 | 6 |
| 0.1M $CaCl_2$ | 5.5 | 18 | 6.5 | 38 | 6.8 | 41 |
| 0.1% $Na_2HPO_4$ | 4.1 | 5 | 5.6 | 23 | 6.6 | 44 |
| 0.2% $Na_2HPO_4$ | 4.3 | 6 | 5.6 | 24 | 6.6 | 41 |
| 0.1% $Na_3$Citrate | 3.9 | 7 | 5.1 | 19 | 6.4 | 40 |
| 0.2% $Na_3$Citrate | 3.8 | 5 | 5.3 | 25 | 6.4 | 37 |

Table 21. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for NAC at protein levels of 3.2, 5.0 and 8.0%

| Variant | Samples | | | | | |
|---|---|---|---|---|---|---|
| | 3.2 | | 5.0 | | 8.0 | |
| | SV | ½t | SV | ½t | SV | ½t |
| Control | 8.7 | 17 | 9.3 | 16 | 8.5 | 15 |
| 94°C/4" | 8.4 | 15 | 8.2 | 10 | 8.1 | 8 |
| 121°C/4" | 8.1 | 9 | 8.1 | 10 | 8.1 | 8 |
| pH 4.5 | 2.4 | 0 | 2.3 | 0 | 2.1 | 0 |
| 0.1M $CaCl_2$ | 16.8 | 50 | 21.2 | 49 | 19.5 | 45 |
| 0.1% $Na_2$Citrate | 9.0 | 20 | 8.4 | 18 | 9.1 | 16 |
| Homogenization | 8.7 | 13 | 8.5 | 13 | 8.1 | 12 |

Table 22. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for NAC/25, NAC/50 and NAC/75 at a protein level of 3.2%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | NAC/25 | | NAC/50 | | NAC/75 | |
| | SV | ½t | SV | ½t | SV | ½t |
| Control | 7.2 | 7 | 8.2 | 8 | 9.3 | 10 |
| 77°C/NH | 6.3 | 6 | 8.2 | 10 | 8.0 | 15 |
| pH 8.5 | 7.3 | 6 | 8.4 | 7 | 7.7 | 12 |
| pH 4.5 | 2.8 | 0 | 1.9 | 0 | 3.3 | 0 |
| 0.1M $CaCl_2$ | 10.2 | 25 | 9.5 | 28 | 15.6 | 17 |
| 0.1% $Na_2HPO_4$ | 8.1 | 16 | 8.5 | 12 | 8.6 | 10 |
| 0.1% $Na_3$Citrate | 7.8 | 12 | 8.3 | 10 | 8.9 | 11 |

Table 23. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for NFDM at protein levels of 3.2, 5.0 and 8.0%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | 3.2 | | 5.0 | | 8.0 | |
| | SV | ½t | SV | ½t | SV | ½t |
| Control | 7.5 | 35 | 7.1 | 48 | 6.8 | 65 |
| 68°C/30' | 7.2 | 32 | 7.2 | 46 | 7.2 | 58 |
| 77°C/30' | 7.2 | 22 | 6.3 | 37 | 5.8 | 48 |
| pH 4.5 | 3.0 | 5 | 3.1 | 5 | 3.1 | 5 |
| 0.1M $CaCl_2$ | 6.4 | 32 | 6.6 | 53 | 6.7 | 60 |
| 0.2% $Na_2HPO_4$ | 7.7 | 40 | 7.8 | 63 | 6.9 | 97 |
| 0.3% $Na_2HPO_4$ | 7.7 | 39 | 7.9 | 60 | 6.8 | 90 |
| 0.2% $Na_3$Citrate | 8.8 | 32 | 8.0 | 61 | 7.4 | 85 |
| 0.3% $Na_3$Citrate | 9.3 | 35 | 8.8 | 63 | 8.7 | 83 |
| Homogenization | 8.1 | 33 | 7.3 | 36 | 6.7 | 61 |

Table 24. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for NFDM/25, NFDM/50, and NFDM/75 at a protein level of 3.2%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | NFDM/25 | | NFDM/50 | | NFDM/75 | |
| | SV | ½t | SV | ½t | SV | ½t |
| Control | 8.0 | 22 | 8.7 | 27 | 7.6 | 30 |
| 77°C/NH | 8.0 | 20 | 8.6 | 25 | 7.9 | 30 |
| pH 8.5 | 7.8 | 20 | 7.2 | 25 | 10.6 | 57 |
| pH 4.5 | 2.8 | 0 | 2.0 | 0 | 3.2 | 5 |
| 0.1M $CaCl_2$ | 4.6 | 5 | 5.3 | 12 | 6.5 | 15 |
| 0.1% $Na_3$Citrate | 8.3 | 22 | 8.9 | 32 | 9.6 | 46 |

Table 25. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for NFDM, NFDM/50, NAC, NAC/50 and SI at a protein level of 3.2%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | SV NFDM | ½t | | | SV NFDM/50 | ½t |
| pH 8.5-68°C/30' | 9.2 | 60 | | | 8.0 | 29 |
| 0.01% L cysteine | 7.7 | 40 | | | 7.9 | 38 |
| 0.01% mercaptoethanol | 6.9 | 32 | | | 7.2 | 23 |
| Succinic anhydride | 11.4 | 27 | | | 8.8 | 21 |
| Maleic anhydride | 12.8 | 17 | | | 8.6 | 20 |
| 0.5% SHMP | 7.8 | 45 | | | 8.9 | 33 |
| 0.1% CMC | 7.6 | 81 | | | 8.8 | 45 |
| 0.5% CMC | 8.4 | 360 | | | 7.7 | 260 |
| 3.5% glycan-homog. | 4.3 | 150 | | | 5.1 | 145 |
| Enzyme hydrol. | 14.4 | 50 | | | 10.1 | 18 |
| | NAC | | NAC/50 | | SI | |
| Succinic anhydride | 10.9 | 17 | 8.6 | 13 | 4.5 | 20 |
| Maleic anhydride | 9.7 | 16 | 8.3 | 13 | 6.0 | 41 |
| 5.0% NaCl | 10.3 | 35 | 7.7 | 37 | 3.7 | 0 |
| 0.5% CMC | 9.7 | 60 | 8.2 | 68 | 4.1 | 20 |
| Enzyme Hydrol. | 8.2 | 15 | 9.6 | 10 | 9.7 | 19 |

Table 26. Whipping ability as specific volume (SV) in ml/g and ½ stability time (½t) in min for WPC, WPC/75, SW, SW/50, EW and EW/75 at a protein level of 3.2%

| Variant | Sample | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SV | ½t | SV | ½t | SV | ½t | SV | ½t |
| | WPC | | WPC/75 | | SW | | SW/75 | |
| 0.1M $CaCl_2$-0.1% $Na_3Cit.$ | 7.7 | 17 | 7.1 | 14 | 2.2 | 0 | 3.4 | 0 |
| 0.1M $CaCl_2$-0.1% $Na_2HPO_4$ | 7.6 | 17 | 6.5 | 14 | 3.0 | 0 | 3.6 | 0 |
| 0.1M $CaCl_2$-0.1M EDTA | 10.7 | 35 | 8.8 | 22 | 4.6 | 0 | 2.3 | 0 |
| 0.1M $CaCl_2$-1.0% EDTA | 8.4 | 22 | 8.2 | 9 | 2.8 | 0 | 2.1 | 0 |
| 0.1% $Na_2HPO_4$-68°C/30' | 7.2 | 47 | 8.4 | 43 | 5.7 | 37 | 4.0 | 5 |
| 0.1% $Na_3Cit.$-68°C/30' | 9.1 | 40 | 8.2 | 32 | 6.3 | 51 | 4.6 | 0 |
| pH 8.5-68°C/30' | 9.0 | 16 | 9.0 | 36 | 6.3 | 35 | 5.2 | 12 |
| $H_2O_2$-68°C/30'-Cat. | 8.2 | 18 | 7.2 | 18 | 5.6 | 40 | 4.3 | 5 |
| 0.01% L cysteine | 4.8 | 0 | 5.7 | 0 | 5.7 | 39 | 5.2 | 6 |
| 0.5% SHMP | 8.8 | 30 | 6.5 | 22 | 4.9 | 0 | 4.1 | 0 |
| Maleic anhydride | 6.5 | 0 | 2.2 | 0 | 5.7 | 6 | 4.2 | 0 |
| 0.1% CMC | 5.3 | 5 | 3.5 | 0 | 5.2 | 24 | 4.3 | 12 |
| 0.1% CMC-68°C/30 | 6.3 | 17 | 5.5 | 22 | 4.4 | 140 | 4.5 | 20 |
| 0.5% CMC | 4.0 | 38 | 4.9 | 78 | 5.8 | 300 | 3.4 | 28 |
| 3.5% glycan-hom. | 4.6 | 5 | 4.0 | 0 | 3.4 | 0 | 2.7 | 0 |
| Enzyme Hydrol. | 10.6 | 47 | 10.1 | 19 | 4.8 | 5 | 5.2 | 5 |

| | EW | | EW/75 | |
|---|---|---|---|---|
| $H_2O_2$ | 7.1 | 35 | 5.8 | 14 |
| 0.1% CMC | 5.7 | 47 | 5.3 | 43 |
| 0.5% CMC | 3.0 | 338 | 4.5 | 140 |
| 3.5% glycan homog. | 4.1 | 240 | 4.1 | 65 |

blends of these products with SI generally had similar SV values with slightly shorter ½t. By utilization of the appropriate treatment it was possible to substantially improve the whipping properties of the sample.

Sensory Evaluation

Subjective sensory analysis is an important property to measure in any food system. The product should be appealing, attractive and palatable. One of the major problems preventing greater utilization of soy isolates has been their offensive flavor and odor (Johnson, 1970). Therefore, in any system employing soy isolates, sensory evaluation is imperative.

For the purposes of this study the foam produced during whipping was used as the test material. A possible 10 points was awarded to each characteristic, flavor, color and texture. The stiffest, whitest and most bland foam received maximum scores of 10. The scores presented in this study were not taste panel averages but the opinion of a limited group of panelists. A score of 6 was considered acceptable.

The data in Tables 29-30 present the sensory evaluation scores for the products tested in this study. In general, flavor, color and texture scores were highest for the milk products, particularly NFDM and NAC. Foams made from NFDM and NAC received high flavor, color and texture scores at all three protein concentrations. The only exceptions were for samples adjusted to pH 4.5 or to which $CaCl_2$ was added.

Foams produced from blends of NAC and SI received acceptable flavor, color and texture scores at all protein concentrations. Flavor acceptability increased slightly as total protein increased. As the amount of NAC in the blends increased, sensory properties improved. Hoffmann (1974) also reported that sensory evaluation of casein-soy sols showed flavor acceptability to decrease as casein was replaced with soy protein. The odor was described as beany. Foams produced from NFDM:SI blends received similar sensory scores. Color, flavor and texture scores were still acceptable when as much as 75% of the total blend protein was from SI. Scores rose slightly as the amount of NFDM increased in the blends and as total protein increased. There were only slight differences in the sensory properties of samples subjected to the different treatments. Lowering the pH to 4.5 imparted a bitter-acid flavor to the samples while addition of $CaCl_2$ resulted in very bitterly flavored samples due to the nature of this salt. NFDM foams had greater acceptability, though the sensory characteristics of NFDM:SI blends were satisfactory.

The SI foams received low flavor, color and texture scores regardless of the protein concentration. Kalbrener et al. (1971) reported that in test panel results commercial SI had flavor scores ranging from 5.9-6.4. It was agreed that beany and bitter flavors persisted in isolates. Cowan et al. (1973) reported that beany and bitter flavors were still detectable in soybean products after an initial

extraction. Yasumatsu et al. (1972) observed that almost all types of soybean products have some undesirable flavor characteristics. Even though SI was the most purified form of soy protein, the characteristic soy flavor was present in the isolate. The SI foams had poor texture characteristics and were definitely off-white. Some improvement in texture was noted for those samples having stable whips. In general, the processing variants had little effect though alkali treatment slightly improved flavor. Bourne et al. (1970) reported that alkali treatment increased flavor scores of soymilk. In practically all cases SI had unacceptable flavor properties which were always lower than NFDM and NAC. Maga and Lorenz (1973a) reported that in major sensory studies of NFDM, NAC and SI, NFDM and NAC had the most bland flavor and odor characteristics. The milk group was substantially more bland than the vegetable proteins.

Electrodialyzed whey foams had very acceptable sensory properties. Color, flavor and texture scores were high, though slightly less than those recorded for NFDM. The whips were less stiff and faintly yellow. Texture scores rose as total protein increased. The sensory properties of these foams were not substantially affected by any of the processing treatments. SW foams received much lower flavor scores because of the saltiness associated with this product. Color scores were also lower due to yellowness of the whips. Most samples had low texture scores because they did not whip into stable foams. Treatment variation had negligible

effect upon the sensory properties of SW. WPC foams received moderately high flavor and color scores for practically all variants (except pH 4.5 and $CaCl_2$ samples). Texture scores were low except for samples subjected to heating. Heat treatment was necessary for the production of stable foams. Increasing the protein concentration did not affect sensory properties. The whey products had good sensory characteristics; this was also noted by Marvopoulous and Kosikowski (1973). Maga and Lorenz (1972) examined the flavor and odor intensities of milk and soy protein supplements. Milk products included NFDM, NAC, whey powder and demineralized whey powder. There were no statistical differences between odor intensities of the milk products. NFDM was judged to be the most bland. The flavor of SI was statistically inferior. Demineralized whey powder was more bland than standard whey powder.

Blends prepared between SW and SI produced foams of low quality. Color and texture scores were unacceptable regardless of protein content or blend ratio. Blends of WPC:SI whipped into foams receiving medium to high scores for color and flavor at the protein levels studied. Foams were given low texture scores except for those samples heated. Texture, flavor and color improved as the amount of WPC increased in the samples and as total protein increased. Variants other than heating had little effect upon the sensory properties of WPC:SI blends. Foams prepared from EW:SI blends scored higher than any other whey product blends. Flavor scores

were higher probably because the sweetness from the EW masked the beany flavor of the SI. Sensory improvement was noted as the blend ratio changed to contain more EW.

The subjective analyses of samples exposed to the treatments from Functional characterization Part II are shown in Tables 27-30. Many of the treatments did not affect the sensory properties of the samples. However, any of the treatments which employed $CaCl_2$ resulted in low flavor scores due to the bitter nature of this salt. Several treatments improved the flavor scores of SI, these included: raising the pH to 8.5 and heating, addition of $H_2O_2$, addition of CMC and heating, addition of $Na_2HPO_4$ and heating, addition of NaCl and addition of glycan. However, the flavor characteristics of these SI foams were still considered unacceptable. These same treatments generally increased the flavor scores of foams produced from milk product:SI blends. Sucrose raised the flavor scores of SI foams to an acceptable level.

Addition of maleic anhydride to the samples reduced the flavor scores of many foams due to the salty-bitter flavor properties of this compound. This was in contrast to that reported by Franzen and Kinsella (1976) who submitted that there were no flavor problems associated with the succinylation of soy protein. Enzymatic hydrolysis markedly decreased the flavor scores of NFDM, NAC and their SI blends. Foams produced from these samples were very bitter. Arai et al. (1970), Yamashita et al. (1969), Fujimaki et al.

(1968), Johnson (1975) and Pelissier and Manchon (1976) all reported bitterness in protein hydrolysates of soy and casein. The whey products and their SI blends did not have bitter flavored foams. Color and texture scores of SW and SW/50 foams were improved by treatments such as addition of CMC and heating, addition of stabilizer salts and heating, and pH adjustment to 8.5. Color and texture scores of SI foams were improved by many of these treatments including enzymatic hydrolysis.

Flavor scores of NFDM and NAC were the highest of any milk products. Electrodialyzed whey and NFDM blended with soy isolate had the most favorable sensory properties of any of the blends. The sensory characteristics of SI foams were unacceptable. However, when SI was blended with the milk products the flavor scores were only slightly less than those recorded for the respective milk product. Milk products were used to effectively improve the color, flavor and texture of SI sols. Many treatments did not substantially improve the sensory properties of the samples. Several treatments while markedly improving functionality resulted in low flavor scores for many of the samples. This may in fact limit or modify the use of such treatments in actual food products.

Table 27. Flavor scores* of the whips from samples of EW, EW/75, WPC, WPC/75, SW and SW/50 at a protein level of 3.2%

| Variant | Sample | | | | | |
|---|---|---|---|---|---|---|
| | EW | EW/75 | WPC | WPC/75 | SW | SW/50 |
| Control | 8 | 8 | 8 | 7 | 6 | 6 |
| 0.1M $CaCl_2$ | 2 | 2 | 3 | 2 | 3 | 4 |
| pH 4.5 | 7 | 8 | 6 | 7 | 5 | 3 |
| pH 8.5-68°C/30' | 9 | 8 | 9 | 7 | 8 | 8 |
| Maleic anhydride | 4 | 4 | 3 | 3 | 4 | 4 |
| 0.1% CMC-68°C/30' | 10 | 8 | 8 | 9 | 7 | 8 |
| Enzyme hydrol. | 9 | 8 | 9 | 8 | 7 | 7 |
| 0.5% SHMP | 8 | 6 | 8 | 8 | 6 | 4 |
| 5.0% NaCl | 7 | 8 | 5 | 6 | 6 | 6 |
| 3.5% glycan-Hom. | 8 | 8 | 9 | 7 | 8 | 7 |

*A scale of 1-10 was used; 10=most desirable

Table 28. Flavor scores* of the whips from samples of NFDM, NFDM/50, NAC, NAC/50 and SI at a protein level of 3.2%

| Variant | Sample | | | | |
|---|---|---|---|---|---|
| | NFDM | NFDM/50 | SI | NAC/50 | NAC |
| Control | 10 | 8 | 4 | 6 | 10 |
| 0.1M $CaCl_2$ | 1 | 1 | 1 | 3 | 3 |
| pH 4.5 | 4 | 4 | 4 | 2 | 3 |
| Maleic anhydride | 4 | 3 | 4 | 3 | 3 |
| 0.1% CMC-68$^{o}$C/30' | 10 | 8 | 5 | 7 | 10 |
| pH 8.5-68$^{o}$C/30' | 10 | 8 | 5 | 7 | 10 |
| Enzyme hydrol. | 3 | 2 | 3 | 2 | 2 |
| 0.1% L cysteine | 9 | 8 | 5 | 6 | 10 |
| 5.0% Sucrose | 10 | 10 | 6 | 8 | 10 |
| 3.5% glycan-Hom. | 7 | 7 | 5 | 6 | 6 |

*A scale of 1-10 was used; 10=most desirable

Table 29. Texture scores* of the whips from samples of NFDM, NFDM/50, NAC, NAC/50, EW, EW/75, WPC, WPC/75, SW, SW/50 and SI at a protein level of 3.2%

| Sample | Variant | | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.1M $CaCl_2$ | Maleic anhyd. | Enzyme Hydrol. | 0.1% CMC 68°C/30' | 0.1% $Na_2HPO_4$ 68°C/30' |
| NFDM | 8 | 7 | 7 | 9 | 10 | 9 |
| NFDM/50 | 8 | 7 | 8 | 7 | 8 | 9 |
| NAC | 8 | 10 | 8 | 4 | 8 | 8 |
| NAC/50 | 6 | 7 | 7 | 5 | 8 | 6 |
| EW | 6 | 8 | 7 | 8 | 9 | 8 |
| EW/75 | 5 | 9 | 7 | 7 | 7 | 6 |
| WPC | 2 | 3 | 2 | 8 | 7 | 8 |
| WPC/75 | 6 | 2 | 2 | 8 | 6 | 8 |
| SW | 2 | 5 | 6 | 5 | 6 | 5 |
| SW/50 | 2 | 6 | 3 | 4 | 6 | 4 |
| SI | 2 | 6 | 6 | 8 | 5 | 3 |

*A scale of 1-10 was used; 1-=most desirable

Table 30. Color scores* of the whips from samples of NFDM, NFDM/50, NAC, NAC/50, EW, EW/75, WPC, WPC/75, SW, SW/50 and SI at a protein level of 3.2%

| Sample | Variant | | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.1M $CaCl_2$ | Maleic anhyd. | Enzyme hydrol. | 0.1% CMC 68°C/30' | 0.1% $Na_2HPO_4$ 68°C/30' |
| NFDM | 10 | 10 | 8 | 10 | 10 | 10 |
| NFDM/50 | 8 | 7 | 8 | 9 | 8 | 8 |
| NAC | 10 | 10 | 9 | 9 | 9 | 10 |
| NAC/50 | 8 | 8 | 8 | 8 | 8 | 8 |
| EW | 7 | 7 | 7 | 7 | 7 | 7 |
| EW/75 | 6 | 6 | 7 | 7 | 7 | 6 |
| WPC | 7 | 7 | 6 | 9 | 8 | 7 |
| WPC/75 | 6 | 2 | 6 | 9 | 7 | 7 |
| SW | 4 | 7 | 6 | 6 | 7 | 6 |
| SW/50 | 4 | 7 | 6 | 6 | 7 | 6 |
| SI | 4 | 8 | 7 | 8 | 7 | 5 |

*A scale of 1-10 was used; 10=most desirable

## Examination of Milk and Soy Protein Blends for Heat Induced Interaction

The purpose of this study was to examine milk and soy protein blends for heat induced interactions. Gel filtration, disc polyacrylamide gel electrophoresis (PAGE) and densitometry were used as the primary analytical tools.

After preparation and freeze-drying of the materials used in this study, portions were taken and analyzed for nitrogen content, by a semimicro Kjeldahl procedure. Factors of 6.38 and 6.25 were used for milk and soy protein respectively. Several buffers were prepared to determine in which medium the proteins should be resuspended. These included: 1) phosphate buffer, pH 7.0, $\mu$=0.1, 2) milk ultrafiltrate prepared from fresh skimmilk, 3) Koop's buffer and 4) deionized distilled water. Soy protein was used as the test protein because of its vulnerability to calcium ions. One percent suspensions were made by stirring the protein into each buffer for 10 min at 1200 rpm. Aliquots of each were then centrifuged at 14,000 x g for 30 min. Following centrifugation portions were taken from the supernatant of each buffer-protein system and analyzed for nitrogen. The results are shown in Table 31. Soy protein was the most soluble in the phosphate buffer system. Solubility was substantially reduced in both Koop's buffer and milk ultrafiltrate probably due to the presence of calcium ions. The other test proteins were then resuspended in phosphate buffer and examined for total soluble protein.

Table 31. Solubility (%) of water soluble soy protein in selected buffers at 1.0% protein

| Sample | Buffer | | | |
|---|---|---|---|---|
| | D.D. water | Koop's | Ultrafiltrate | phosphate |
| Soy protein | 85.3 | 63.4 | 64.3 | 97.9 |

Table 32. Solubility (%) of NAC, WC, AC, and SW in phosphate buffer pH 7.0, μ=0.1 at 1.0% protein

| Buffer | Protein | | | |
|---|---|---|---|---|
| | NAC[a] | WC[b] | AC[c] | SW[d] |
| phosphate | 99.8 | 99.6 | 100.0 | 99.1 |

a = sodium caseinate

b = whole casein

c = whey protein from acid whey

d = whey protein from sweet whey

The results are shown in Table 32. Due to the excellent solubility of all the test proteins in phosphate buffer (pH 7.0, $\mu$=0.1) it was chosen as the buffer system. The proteins included: 1) sodium caseinate (NAC), 2) whole casein (WC), 3) whey protein from acid whey (AC), 4) whey protein from sweet whey (SW) and 5) soy protein from a water extract of soy isolate. The proteins were resuspended in phosphate buffer at a concentration of 1.0%. Milk and soy protein blends were prepared by combining the respective proteins at a 1 to 1 ratio (2% total protein). The samples were heated at temperatures of: 1) control-unheated, 2) 68$^{o}$C/30 min, 3) 77$^{o}$C/20 sec, 4) 94$^{o}$C/10 sec and 5) 121$^{o}$C/5 sec. These temperatures were chosen because they approximated similar treatments used in the pasteurization and UHT heating of fluid milk products. Heating was accomplished by immersing a test tube (containing the sample) into a silicone oil bath adjusted to the appropriate temperature. A delay time of approximately one and a half min could not be avoided in bringing the sample to the desired temperature.

After heating, cooling, centrifugation and filtration, the nitrogen solubility was determined for each sample. The results for AC (acid whey protein), AS (the acid whey-soy protein combination), SW (sweet whey protein), SS (the sweet whey-soy protein combination) and soy protein (SP) are shown in Table 33. The initial solubilities were nearly

Table 33. Solubility of AC, AS, SP, SS and SW in phosphate buffer pH 7.0, μ=0.1 at a protein level of 1.0%

| Treatment | Soluble protein % | | | | |
|---|---|---|---|---|---|
| | AC[a] | AS[b] | SP[c] | SS[d] | SW[e] |
| Control | 100.0 | 98.5 | 97.9 | 98.1 | 99.1 |
| 68°C/30' | 98.9 | 99.3 | 99.8 | 98.3 | 97.4 |
| 77°C/20" | 98.0 | 96.7 | 99.9 | 94.9 | 94.9 |
| 94°C/10" | 94.0 | 92.8 | 94.1 | 86.2 | 83.6 |
| 121°C/5" | 89.1 | 86.7 | 85.8 | 81.6 | 80.3 |

[a]AC - whey protein from acid whey

[b]AS - acid whey:soy protein combination

[c]SP - soy protein

[d]SS - sweet whey:soy protein combination

[e]SW = whey protein from sweet whey

100 percent. The high solubilities of the blended systems indicated apparent compatibility of the different proteins. Acid whey protein retained high solubility as the heat treatment increased to 77°C/20 sec. At 94°C/10 sec and 121°C/5 sec there was a decrease in the solubility of this protein system. Sweet whey protein was not as stable to heat treatment as AC. Loss of solubility was more drastic at the higher temperatures. Previously in this research it was shown (solubility data pertaining to functionality) that heating generally decreased the solubility of whey protein, especially at elevated temperatures. Howat and Wright (1933), Dill and Roberts (1964), Guy et al. (1967) and Morr (1969) reported that the denaturation of whey protein increased dramatically as heating temperature increased. Disc gels of whey protein are presented in Figure 1. The gels are ordered from right to left in terms of least to most severe heat treatment. The whey proteins were identified according to their migration rates. Heating the protein at 68°C/30 min did not substantially affect band prominence. This was also found when the protein sol was heated at 77°C/20 sec. The immunoglobulins and serum albumin bands were slightly less intense. It appeared that less material entered the gel. Major changes occurred when the sol was heated to 94°C/10 sec. The band representing serum albumin had completely disappeared. β-lactoglobulin was much less distinct though recognizable. The least affected whey protein was α-lactalbumin. It appeared

Figure 1. Disc gels of whey protein, soy protein and casein heated at (from left to right): unheated-control, 68°C/30 min, 77°C/20 sec, 94°C/10 sec and 121°C/5 sec.

WHEY
PROTEIN
SOY
PROTEIN


CASEIN
SOY
PROTEIN
(urea)

that more protein had failed to enter the gel. Protein resolution and band intensities were further altered when whey protein was heated at 121°C/5 sec. Extensive smearing was noted as well as an increase in the amount of protein failing to enter the gel. Josephson et al. (1967), Morr (1969) and Kenkare et al. (1964) reported that progressively higher heat treatments resulted in greater denaturation of whey protein which resulted in the formation of aggregates of sufficient size as to prevent their migration into PAGE gels. Hetrick (1950), Melachouris and Tuckey (1966) and Senter et al. (1973) found that α-lactalbumin was the most heat resistant of the major whey proteins. Beta-lactoglobulin, the immune globulins and serum albumin were much more sensitive to heating.

The solubility of soy protein increased when heated at 68°C/30 min. Heating at 94°C/10 sec and 121°C/5 sec decreased the solubility of the soy protein appreciably. Previously in this research it was shown that (solubility data pertaining to functionality) low temperature heating increased the solubility while UHT heating decreased the solubility of soy protein. Shen (1976) and Hermannson and Akesson (1975a) reported that moderate heating slightly improved the solubility of soy protein. Mann and Briggs (1955), Catsimpoolas et al. (1969), Wolf (1970) and Catsimpoolas et al. (1971a) found that the amount of protein insolubilized increased with increased heat treatment. Disc gels of soy protein are presented in Figure 1. The

gels are ordered from right to left in terms of least to most severe heat treatment. The major soy proteins, the 7S and 11S, were identified according to their migration rates. Several faster migrating species were also present including a prominant component near the marker dye. Heating at 68°C/30 min had very little effect upon the resolution or prominence of the proteins. Some change was noted in the electropherogram of soy protein following heating at 77°C/20 sec. The 7S fraction was partially dissociated while the intensity of the 11S band was diminished. These changes were accompanied by an increase in the number and intensity of minor bands. These new components were presumably breakdown products from the 7S and 11S proteins. In addition, the very distinct band located near the marker dye had disintegrated into a smear. The 7S fraction completely disappeared when soy protein was heated at 94°C/10 sec. The 11S fraction had also lost much of its identity and was less concise. The breakdown products were more distinct and intense. The smear located near the marker dye (from the gel representing protein heated at 77°C/20 sec) was less prominant. Heating the protein at 121°C/5 sec resulted in very little change from those observed when the protein was heated at 94°C/10 sec. The species resulting from dissociation of 7S and 11S proteins were stable to this heat treatment. Catsimpoolas et al. (1969) reported that soy protein was stable to temperatures up to 70°C. At temperatures higher than this Catsimpoolas

et al. (1969), Watanbe and Nakayama (1962), Wolf (1970), Saio et al. (1971), Wolf and Tamura (1969), Catsimpoolas et al. (1971a), Cumming et al. (1973), Saio et al. (1975a) and Aldrich (1977) found that soy 7S and 11S proteins suffered significant dissociation. Many of these same researchers reported that the disappearance of these fractions was accompanied by the appearance of faster migrating components.

The solubility of soy isolate:whey protein blends decreased as the heating temperature rose relative to the reduction in the solubility of the individual protein systems. This was also found when these samples were studied during examination of their functional properties. The acid whey:soy protein blend was more stable to heat than the sweet whey:soy sample.

The solubilities of NAC, NS (the sodium caseinate-soy protein blend), WC and WS (the whole casein-soy protein blend) are presented in Table 34. The initial solubilities were nearly 100%. The solubility of both NAC and WC sols remained stable as the heat treatment increased. Heating was also found to have affected the solubility of sodium caseinate dispersions very little during examination of its functional properties. White and Davies (1958), Kresheck et al. (1964) and Alais et al. (1967) studied the behavior of casein and concluded that it was very resistant to heat denaturation.

Table 34. Solubility of NAC, NS, WC and WS in phosphate buffer pH 7.0, μ=0.1 at a protein level of 1.0%

| Treatment | % Soluble protein | | | |
|---|---|---|---|---|
| | NAC[a] | NS[b] | WC[c] | WS[d] |
| Control | 99.8 | 99.5 | 99.6 | 100.0 |
| 68°C/30' | 99.9 | 99.9 | 99.4 | 99.3 |
| 77°C/20" | 99.0 | 100.0 | 98.8 | 99.1 |
| 94°C/10" | 98.1 | 99.1 | 98.3 | 98.7 |
| 121°C/5" | 98.1 | 99.0 | 97.8 | 98.1 |

a = sodium caseinate

b = sodium caseinate:soy protein blend

c = whole casein

d = whole casein:soy protein blend

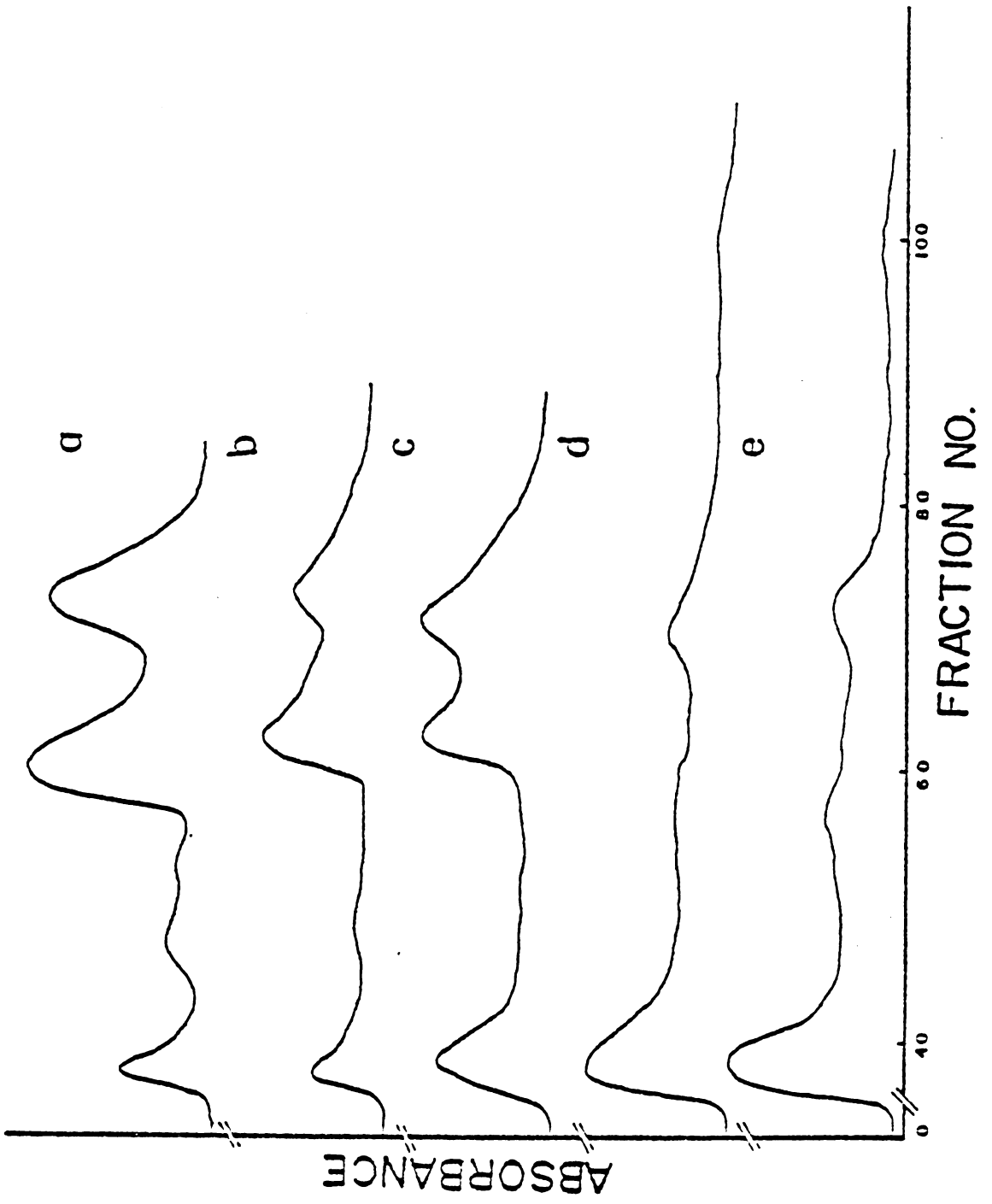
Disc gels of whole casein and soy protein are presented in Figure 1. These are 7% gels in 7M urea. The gels are arranged from left to right in terms of least to most severe heat treatment. Soy protein underwent substantial dissociation when the heat treatment rose to 94°C and higher. There was an appreciable decrease in the intensity of the primary components which was accompanied by a substantial increase in the number of minor constituents. There was similarity between urea-soy gels and nonurea-soy gels. The electropherograms of whole casein depict what other researchers have shown (Kresheck et al., 1964; Hostettler et al., 1965; Alais et al., 1967; Morr, 1969; and Hensen and Melo, 1977). Casein was very stable to heat treatment. Very little change was noted as the heat treatment rose. The gel representing casein heated at 121°C/ 5 sec was inadvertently inverted during photography.

Whey:Soy Protein Blends

After heating, centrifugation and filtration, approximately 7 ml of the clear filtrate was pumped upward through a column packed with sephacryl S-200 superfine. Gel filtration patterns were obtained for each sample.

The gel filtration patterns of acid and sweet whey proteins were almost identical. Elution volumes and peak patterns were extremely similar. For these reasons only the gel filtration chromatograms of AC protein are shown (Figure 2). Each peak pattern is indicative of one of the

Figure 2. Gel filtration chromatograms of whey protein heated at; unheated-control (a), 68°C/30 min (b), 77°C/20 sec (c), 94°C/10 sec (d) and 121°C/5 sec (e).


a
b
c
d
e
ABSORBANCE
0
40
60
80
100
FRACTION NO.

heat treatments employed: unheated, 68°C/30 min, 77°C/20 sec, 94°C/10 sec and 121°C/5 sec. The letters a, b, c, d and e represent these treatments respectively. Five distinct areas were resolved when untreated AC protein was chromatogrammed. Using gel electrophoresis peak no. 1 was identified as primarily containing immunoglobulins. Peak no. 2 may have also been immunoglobulins as this fraction only migrated a short distance into the gel. Peak no. 3 was primarily serum albumin while peaks 4 and 5 were principally β-lactoglobulin and α-lactalbumin, respectively. There was some overlap between peak areas. Very little change was evident when the protein was heated at 68°C/30 min. Peak separation and elution volumes were very similar. Heating at 77°C/20 sec also caused very little change in the gel chromatogram. Slightly less resolution was noted between peaks 4 and 5. Several major changes occurred when the whey protein was heated at 94°C/10 sec. Peak 1 became much broader, and encompassed a much greater area. The remaining peaks were much less distinct and less resolved, especially peak 4. All fractions eluted closer to the void volume. The pattern obtained after heating at 121°C/5 sec was very similar to that observed for the previous treatment. The peaks had lost much of their individual distinction and were compressed together. An additional peak was eluted from samples of whey protein heated at either 94°C/10 sec or 121°C/5 sec. This peak eluted close to the experimental total volume (established

with mercaptoethanol). The elution volume of this peak was roughly the same for both treatments.

The nitrogen distribution of the whey protein fractions discussed are shown in Table 35. Nitrogen was determined by a semimicro Kjeldahl procedure. The largest percent of nitrogen was found in fractions 4 and 5, and accounted for 50.5 and 32.6% of the total nitrogen respectively. These fractions were primarily composed of β-lactoglobulin and α-lactalbumin, though the peaks were not homogeneous. The remaining nitrogen was distributed almost evenly between the other three peaks. Heating whey protein at 68°C/30 changed the nitrogen profile very little which corresponded to the lack of change observed in the gel filtration chromatogram. After heating at 77°C/20 sec there were only slight differences in the nitrogen distribution of the whey protein. Slightly less nitrogen was found in fraction 4 while slightly more was located in peak 1. The major nitrogen shift occurred when whey protein was heated at 94°C/10 sec. The amount of nitrogen in fraction 1 increased substantially with concurrent decreases in peaks 4 (β-lactoglobulin) and 5 (α-lactalalbumin). The nitrogen in fractions 2 and 3 increased relative to the decrease in peaks 4 and 5. Fraction 6, eluting close to the total volume contained 4.5% of the total nitrogen and was mainly composed of nonprotein nitrogen (NPN). Rowland (1937) reported that heating at 95°C produced a small amount of NPN. The gel filtration chromatogram reflected the changes

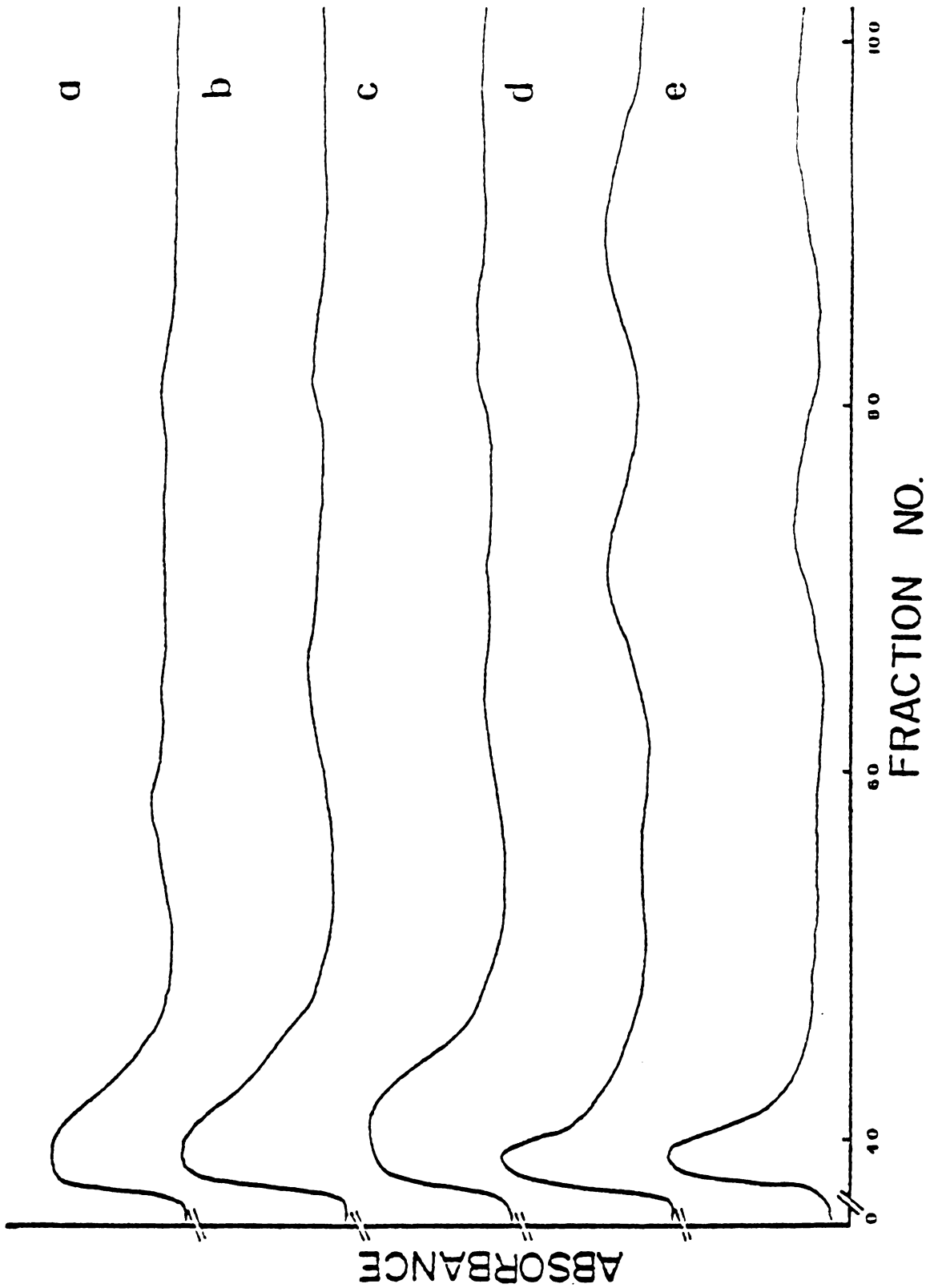
Table 35. Percent nitrogen in each fraction of whey protein separated by gel filtration

| Fraction No. | Treatment | | | | |
|---|---|---|---|---|---|
| | Control | 68°C/30' | 77°C/20" | 94°C/10" | 121°C/5" |
| 1 | 4.8 | 5.1 | 7.2 | 21.9 | 24.0 |
| 2 | 6.1 | 6.0 | 7.2 | 9.7 | 9.7 |
| 3 | 6.0 | 6.1 | 6.4 | 12.9 | 16.5 |
| 4 | 50.5 | 50.3 | 47.3 | 24.9 | 22.3 |
| 5 | 32.6 | 32.5 | 31.9 | 26.1 | 21.4 |
| 6 | - | - | - | 4.5 | 6.1 |

which occurred in nitrogen distribution. Heating whey protein at 121°C/5 sec only magnified the changes which took place when the protein was heated at 94°C/10 sec. The amount of material eluting at the void volume increased while fractions 4 and 5 suffered further losses. Morr (1969), Josephson et al. (1967), Rowland (1937), Hetrick (1950), Harlan et al. (1955), Melachouris and Tuckey (1966) and Dill and Roberts (1964) reported that heating denatured whey protein. Progressively higher temperatures (including UHT) resulted in the gradual reduction of all whey proteins. Kenkare et al. (1964) and Josephson et al. (1967) used gel filtration to demonstrate the aggregation and denaturation of heated whey protein. Melachouris and Tuckey (1966) reported that β-lactoglobulin was the most sensitive whey protein fraction. Hetrick (1950) found that the immunoglobulins and serum albumin were very sensitive to heat while α-lactalbumin was the most resistant of the whey proteins. Larson and Rolleri (1955), heated skimmilk at 70°C/30 min. Their data were in agreement with that of Hetrick (1950).

The gel filtration chromatogram of the water extracted soy isolate protein is presented in Figure 3. The lettering system representing the various heat treatments was previously discussed. Resolution by gel filtration revealed the presence of 4 fractions. Using gel electrophoresis, the components of peak 1 (eluted at the void volume) were identified as primarily 7S and 11S proteins.

Figure 3. Gel filtration chromatograms of soy protein heated at: unheated-control (a), 68°C/30 min (b), 77°C/20 sec (c), 94°C/10 sec (d) and 121°C/5 sec (e).


a
b
c
d
e
0
40
60
80
100
FRACTION NO.
ABSORBANCE

Peaks 2, 3 and 4 contained faster moving species. The fractions were not homogenous. Heat treatment at 68°C/30 min resulted in very little change in the gel filtration chromatogram. Resolution, peak patterns and elution times were approximately the same. Heating at 77°C/20 sec caused a slight change in the gel filtration pattern. Fraction 1 was somewhat larger and broader. The major changes occurred when soy protein was heated at 94°C/10 sec. There was a marked decrease in the size of peak 1 with concurrent increases in the area of the other fractions. Peak 4 eluted closer to the total volume of the column. The gel filtration chromatogram of soy protein heated to 121°C/5 sec was very similar to that obtained from heating at 94°C/10 sec.

The soy protein nitrogen distribution of the fractions previously discussed is presented in Table 36. Fraction 1 (containing the 7S and 11S proteins) was responsible for the major percentage of nitrogen. After heating at 68°C/30 min there were no significant changes in the nitrogen distribution. Heating at 77°C/20 sec increased the amount of nitrogen in fraction 1 which was in agreement with the change in the gel filtration pattern. After heating at 94°C/10 sec, the gel filtration chromatogram of soy protein revealed a major breakdown in fraction 1. The nitrogen shift which occurred was in accord with this. There was a substantial decrease in the amount of nitrogen in this fraction. This was accompanied by increased levels of

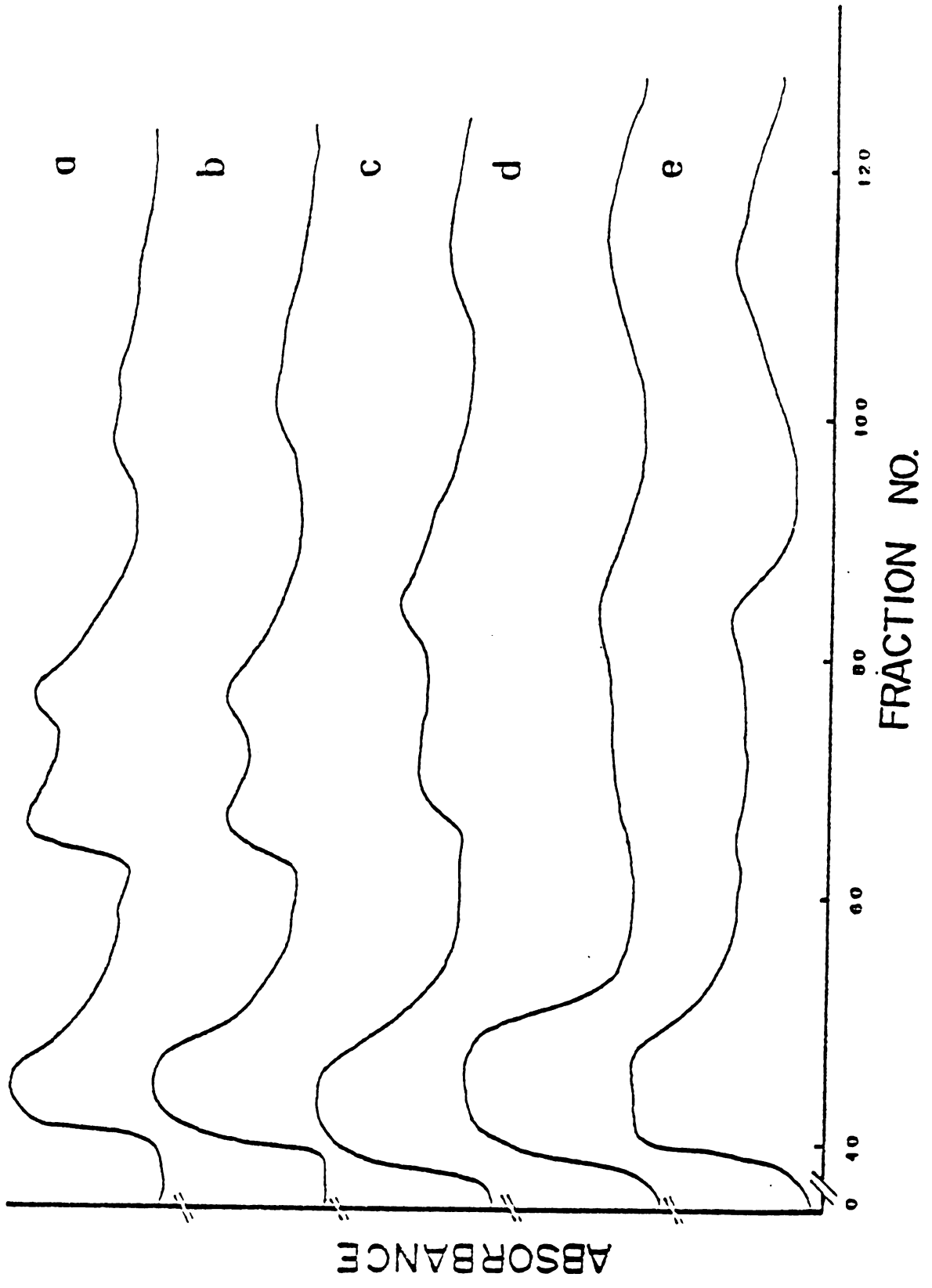
Table 36. Percent nitrogen in each fraction of soy protein separated by gel filtration

| Fraction No. | Treatment | | | | |
|---|---|---|---|---|---|
| | Control | 68°C/30' | 77°C/20" | 94°C/10" | 121°C/5" |
| 1 | 64.1 | 64.6 | 67.9 | 45.0 | 44.4 |
| 2 | 21.4 | 21.6 | 19.8 | 19.5 | 19.0 |
| 3 | 9.3 | 9.0 | 7.2 | 25.5 | 26.1 |
| 4 | 5.2 | 4.8 | 5.1 | 10.0 | 10.5 |

nitrogen in the remaining fractions. The amount of nitgogen in peak 4 increased primarily due to an increase in the amount of NPN. The nitrogen distribution remained very similar after heating at 121°C/5 sec. Catsimpoolas et al. (1969) found that only slight changes occurred to soy protein heated to 70°C. Above 70°C significant dissociation of the protein occurred. Saio et al. (1971) and Saio et al. (1975a) reported that heating soy protein caused it to dissociate into several species.

The gel filtration chromatogram of the whey protein:soy protein blend is shown in Figure 4. The heat treatments and letters of identification remain as previously discussed. Six different peak areas were resolved in the unheated sample. Fraction 1 had the greatest area and was composed of both soy and AC proteins, fraction 1. Fractions 2 and 3 were principally whey protein fractions 2 and 3 while fractions 4 and 5 were primarily β-lactoglobulin and α-lactalbumin respectively. Fraction 6 was derived primarily from soy fraction 4. Heating the blend at 68°C/30 min resulted in little change. There was also little change in the unblended systems when subjected to this treatment. After heating at 77°C/20 sec fraction 1 had broadened while peaks 4 and 5 were less resolved. Heating at 94°C/10 sec resulted in less distinction, blurring and loss of resolution between fractions. Fraction 1 continued to increase in size while fraction 6 eluted closer to the total volume of the column. There was negligible change

Figure 4. Gel filtration chromatograms of the whey:soy protein blend heated at: unheated-control (a), 68°C/30 min (b), 77°C/20 sec (c), 94°C/10 sec (d) and 121°C/5 sec.


a
b
c
d
e
ABSORBANCE
0
40
80
100
120
FRACTION NO.

in the gel filtration chromatogram for the sample heated to 121°C/5 sec. Loss of resolution and disintegration of peaks 4 and 5 were substantial.

The nitrogen distribution of whey:soy protein blends is shown in Table 37. Nitrogen was distributed throughout six fractions. The results shown for the unheated blend are a close approximation relative to the unblended systems. After heating at 68°C/30 min little change was observed regarding the nitrogen distribution or elution profiles. The amount of nitrogen in fraction 1 increased after heating at 77°C/20 sec. Some nitrogen was lost from fractions 4 and 5. Both the blended and unblended systems suffered major change when heated at 94°C/10 sec. The nitrogen in soy fraction 1 decreased while the nitrogen in AC fraction 1 increased. The amount of nitrogen in fraction 1 of the blended system was proportionally higher than it would have been, had there been a strictly linear relationship between the amount of nitrogen lost (soy protein) and the amount gained (whey protein). The nitrogen distribution changed very little when the blend was heated at 121°C/5 sec. Fractions 4 and 5 (corresponding to AC 4 and 5) suffered the greatest nitrogen losses which was in agreement with the change in the gel filtration chromatogram. Fraction 6 had increased nitrogen for samples heated at 94°C/10 sec and 121°C/5 sec. This was primarily due to an increase in NPN. These changes concurred with those observed for the heated, unblended proteins.

Table 37. Percent nitrogen in each fraction of the whey: soy protein blend separated by gel filtration

| Fraction No. | Treatment | | | | |
|---|---|---|---|---|---|
| | Control | 68°C/30' | 77°C/20" | 94°C/10" | 121°C/5" |
| 1 | 37.8 | 38.1 | 41.1 | 47.1 | 50.3 |
| 2 | 7.1 | 6.9 | 6.9 | 7.4 | 10.2 |
| 3 | 5.4 | 5.8 | 6.4 | 10.1 | 12.7 |
| 4 | 25.1 | 23.2 | 22.3 | 14.2 | 8.9 |
| 5 | 20.0 | 21.3 | 17.9 | 13.4 | 9.8 |
| 6 | 4.7 | 5.3 | 5.3 | 7.8 | 8.1 |

The gel diagrams of whey protein (AC), soy protein (SP) and their blend (AS) are shown in Figures 5-7. The prints of the actual gels are shown in Figure 8. The gels were electrophoresed according to a procedure similar to that used by Melachouris (1969). The gels were stained using the Malik-Berrie (1972) staining technique. Disc PAGE was the primary experimental technique used to determine whether milk:soy protein blends had undergone heat induced interaction. Using gel filtration to resolve each protein system into its various components, a portion from each fraction was subjected to disc PAGE. The gel diagrams of the unblended and blended systems were then compared. Gels from the blended systems were examined for the appearance or disappearance of major bands not present in gels of the individual protein systems. Visual examination and calculation of RF values were instrumental in this undertaking. It was assumed that if the bands from the unblended proteins were evident in the gels of the blended system, no interaction had occurred and vice versa.

Gel diagrams are shown for samples heated at: 1) unheated-control, 2) 68$^{o}$C/30 min and 3) 94$^{o}$C/10 sec. Diagrams for samples heated at 77$^{o}$C/20 sec and 121$^{o}$C/5 sec are not shown due to their similarity to the other treatments. The gel diagrams of the unheated protein systems are shown in Figure 5. The fraction number (with reference to gel filtration) is denoted by the number in the upper right hand corner. The gels are identified by their respective symbols

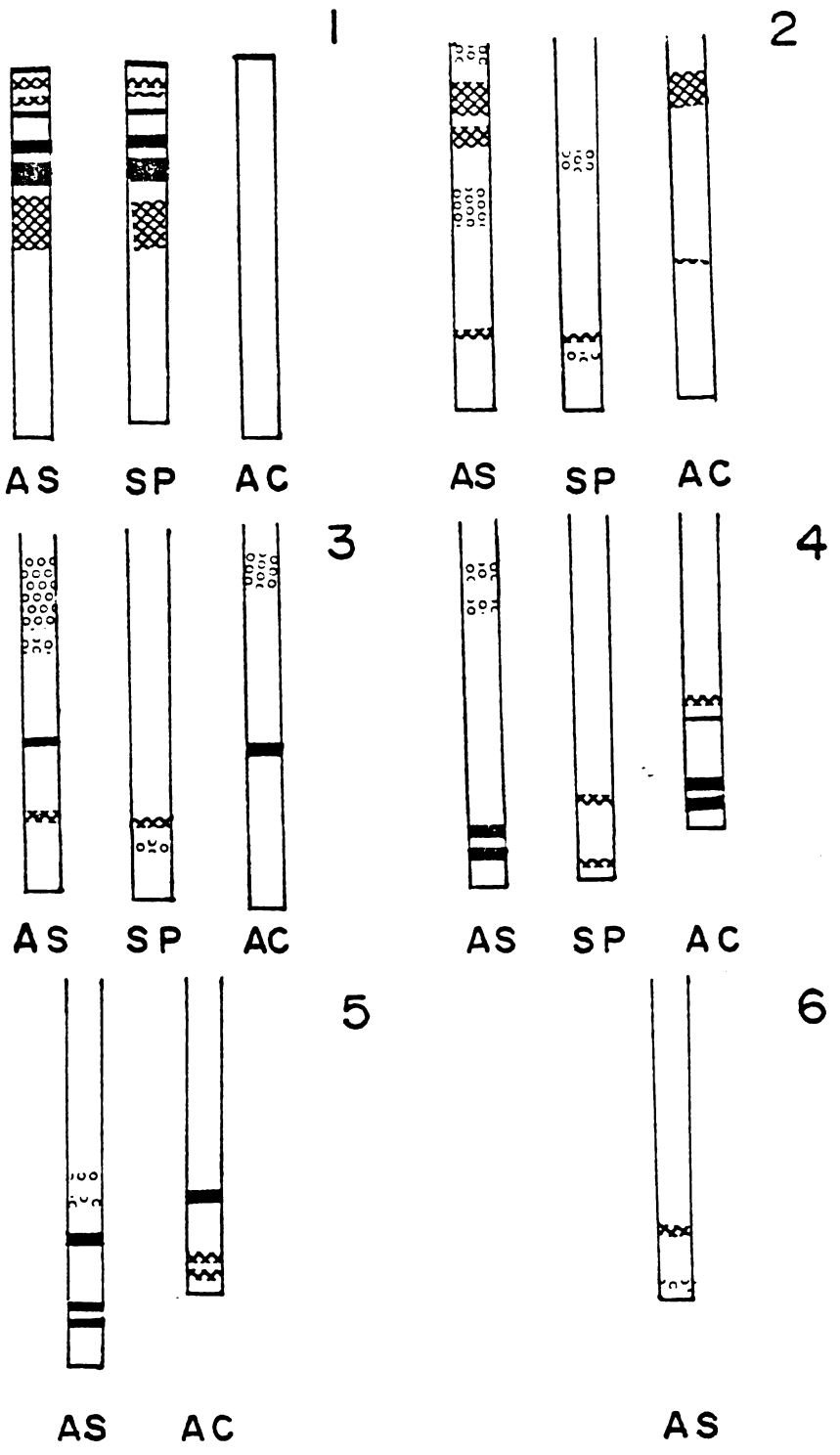



Figure 5. Gel diagrams of unheated whey protein (AC), soy protein (SP) and their blend (AS). The gel filtration fraction is denoted by the number in the upper right hand corner.

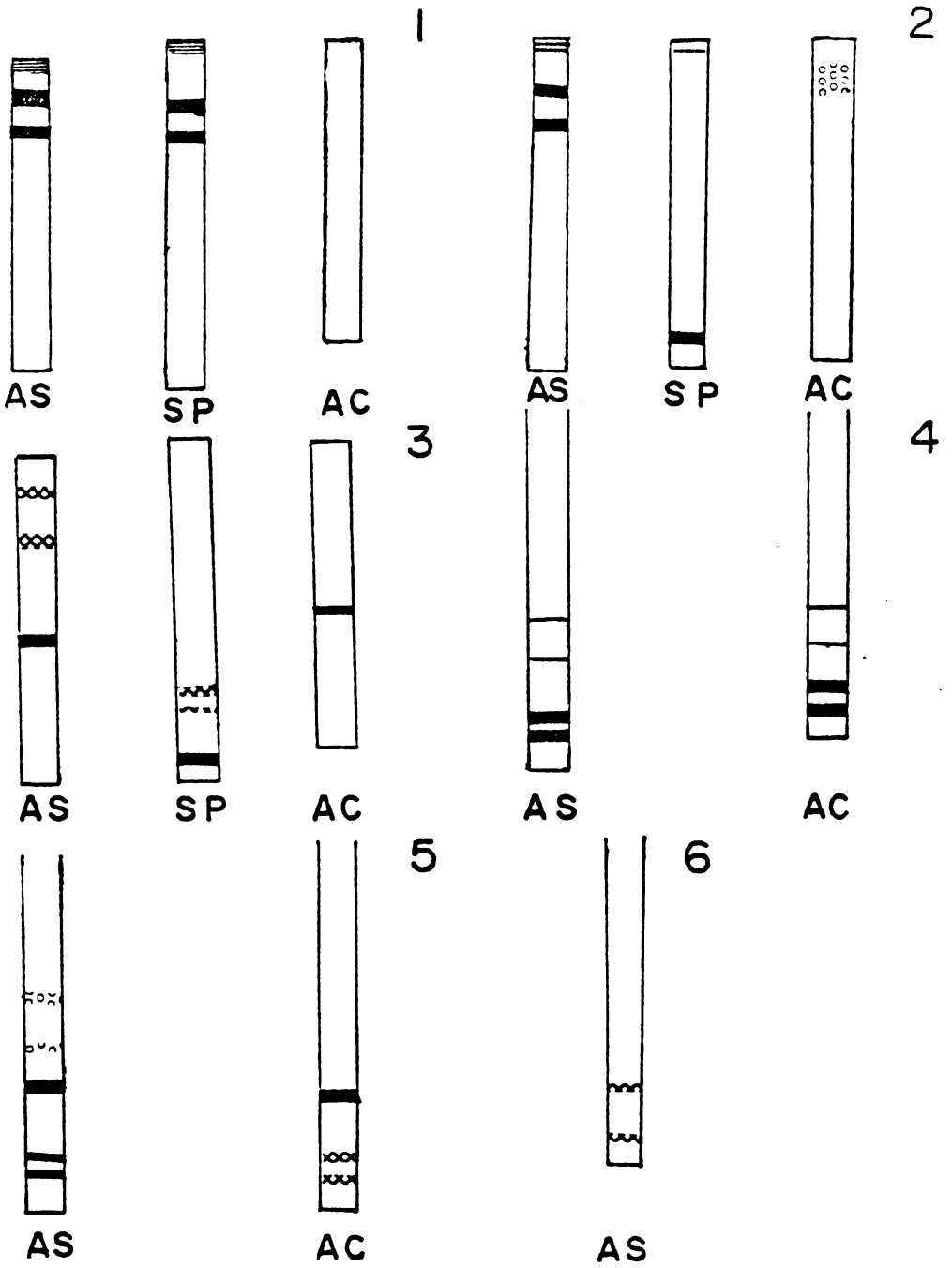



Figure 6. Gel diagrams of whey protein (AC), soy protein (SP) and their blend (AS) heated at 68°C/30 min. The gel filtration fraction is denoted by the number in the upper right hand corner.

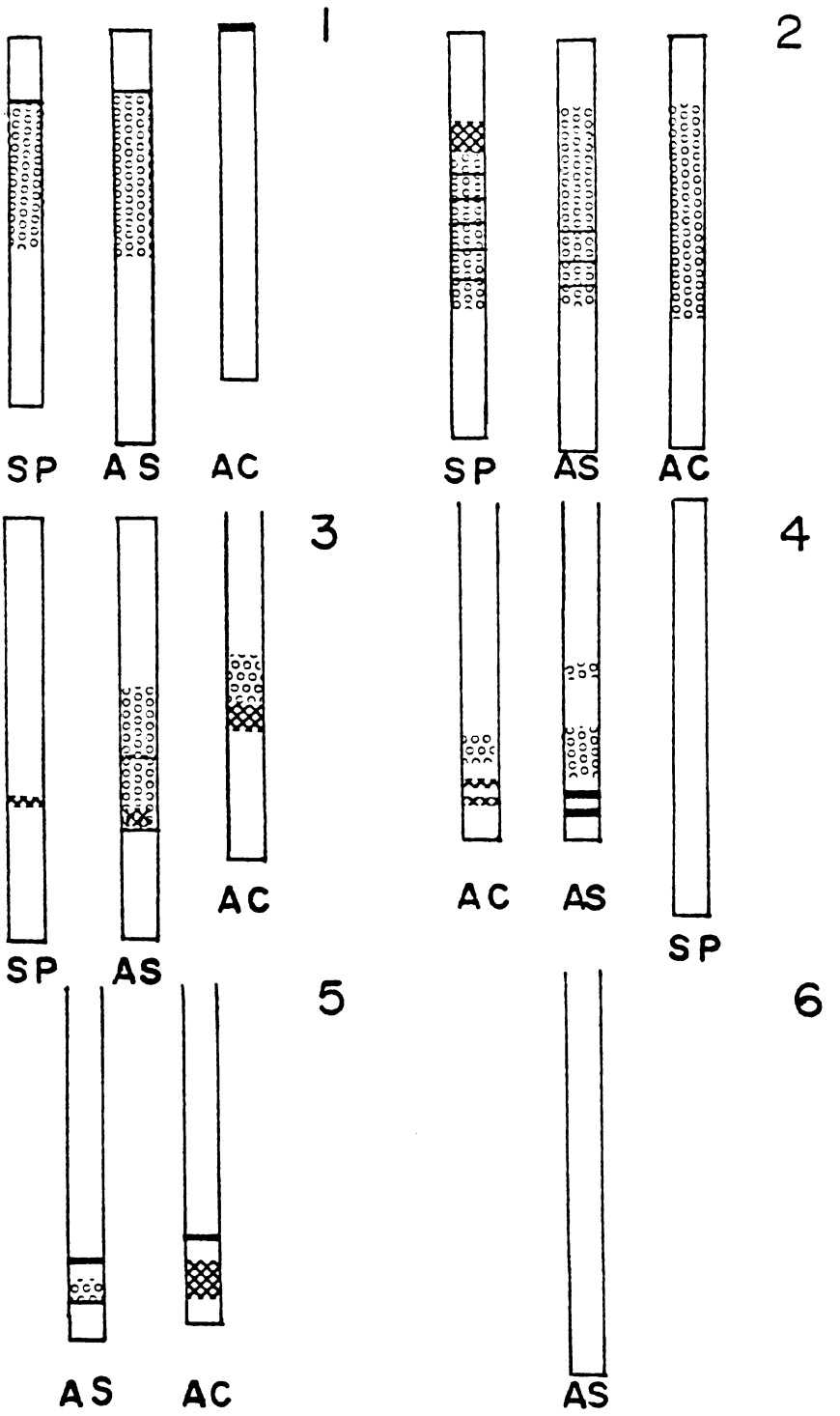
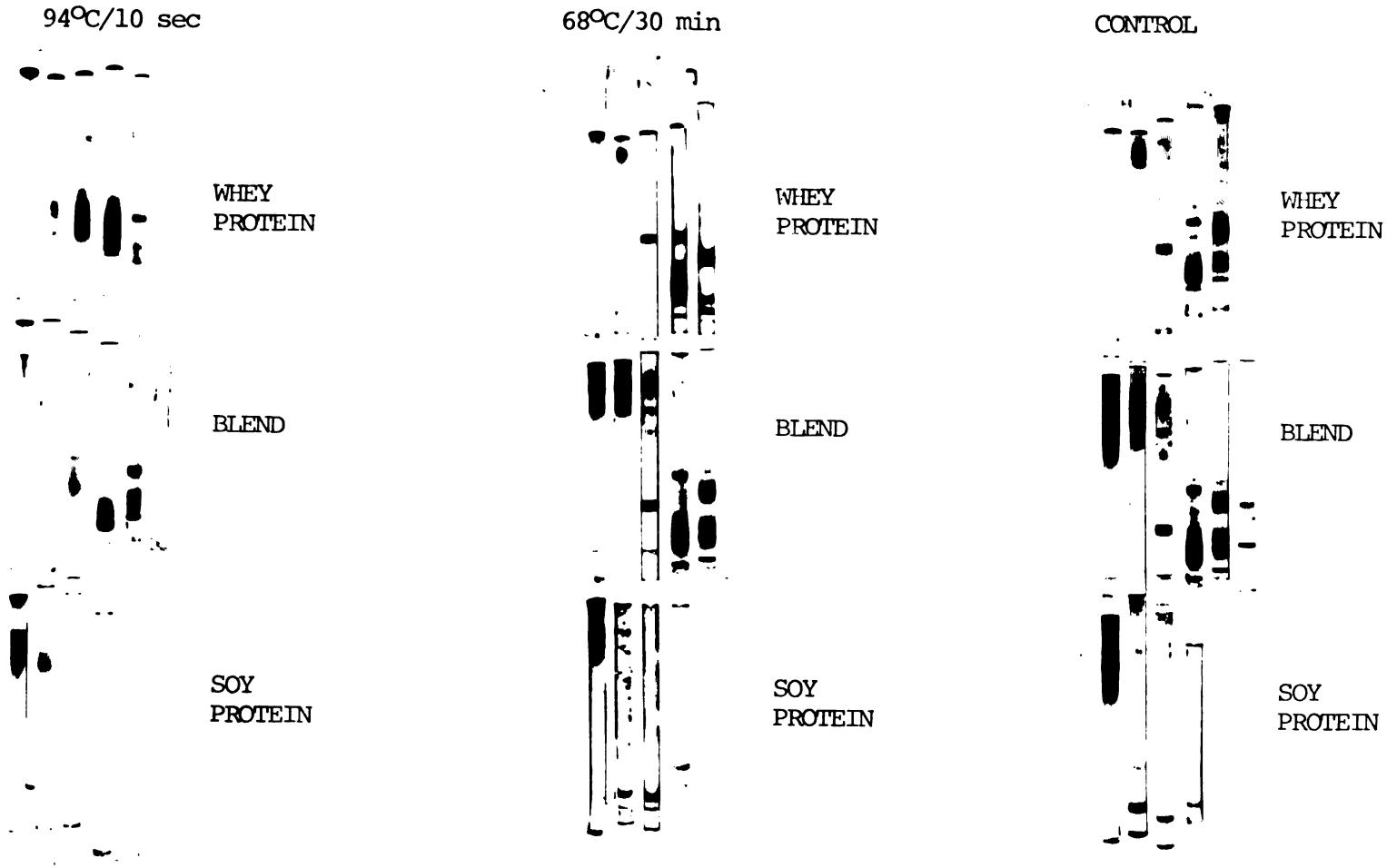



Figure 7. Gel diagrams of whey protein (AC), soy protein (SP) and their blend (AS) heated at 94°C/10 sec. The gel filtration fraction is denoted by the number in the upper right hand corner.

Figure 8. Disc gels of whey protein, soy protein and their blend heated at: unheated control, 68°C/30 min and 94°C/10 sec. The gel bands represent protein present in gel filtration fractions 1-6 (from left to right).


94°C/10 sec
WHEY PROTEIN
BLEND
SOY PROTEIN
68°C/30 min
WHEY PROTEIN
BLEND
SOY PROTEIN
CONTROL
WHEY PROTEIN
BLEND
SOY PROTEIN

located below the diagrams. The print of the actual gels is shown in Figure 8. The protein in AC fraction 1, did not enter the gel material, and thus remained at the origin. The protein in SP fraction 1, resolved into two major fractions, the 7S and 11S. Using visual examination and RF values it was concluded that these same bands were present on AS fraction 1. Whey protein fraction 2 contained one major zone while SP yielded two. These corresponded to areas found on AS fraction 2. Fraction 3 found one major band on the AC gel (serum albumin) which corresponded to the same protein on the AS gel. Two intense bands (β-lactoblobulin) were present on gels from both AC and AS fractions no. 4. The whey protein, α-lactalbumin, was located in fraction 5 of both AC and AS systems. The intensities and RF values of these protein bands were extremely similar. Soy, fractions 3 and 4 contained a fast moving species located near the marker dye which was also apparent in fraction 6 of the AS gels. By calculating the RF values of the bands and examining their visual intensities, all of the major bands from the unblended protein gels were found on the gels of the blended protein systems. Therefore, it appeared that there were no interactions taking place between soy and whey proteins in an unheated blend.

The gel diagrams of AC, SP and AS protein systems heated at 68°C/30 min are shown in Figure 6. Gel identification and fraction numbers are the same as previously mentioned. The print of the actual gels is shown in Figure 8. The

protein in AC fraction 1, remained at the origin while SP fraction 1, yielded two major zones. AC fraction 2 contained one major area which corresponded to an area on AS fraction 2. Protein bands from SP fractions 2, 3 and 4 were also located on gels of the blended proteins. From fraction 3 one major band was found on the AC gel which corresponded to a band on gel 3 of the AS system. AC fractions 4 and 5 contained the prominant bands from β-lactoblobulin and α-lactalbumin, respectively. Visual examination and RF values revealed these to be the same bands located on AS protein gels 4 and 5. There were only slight differences between these gels and those containing the unheated proteins. By calculating the RV values and examining visual intensities, all of the major bands from the unblended proteins were identified on the gels containing the blended systems.

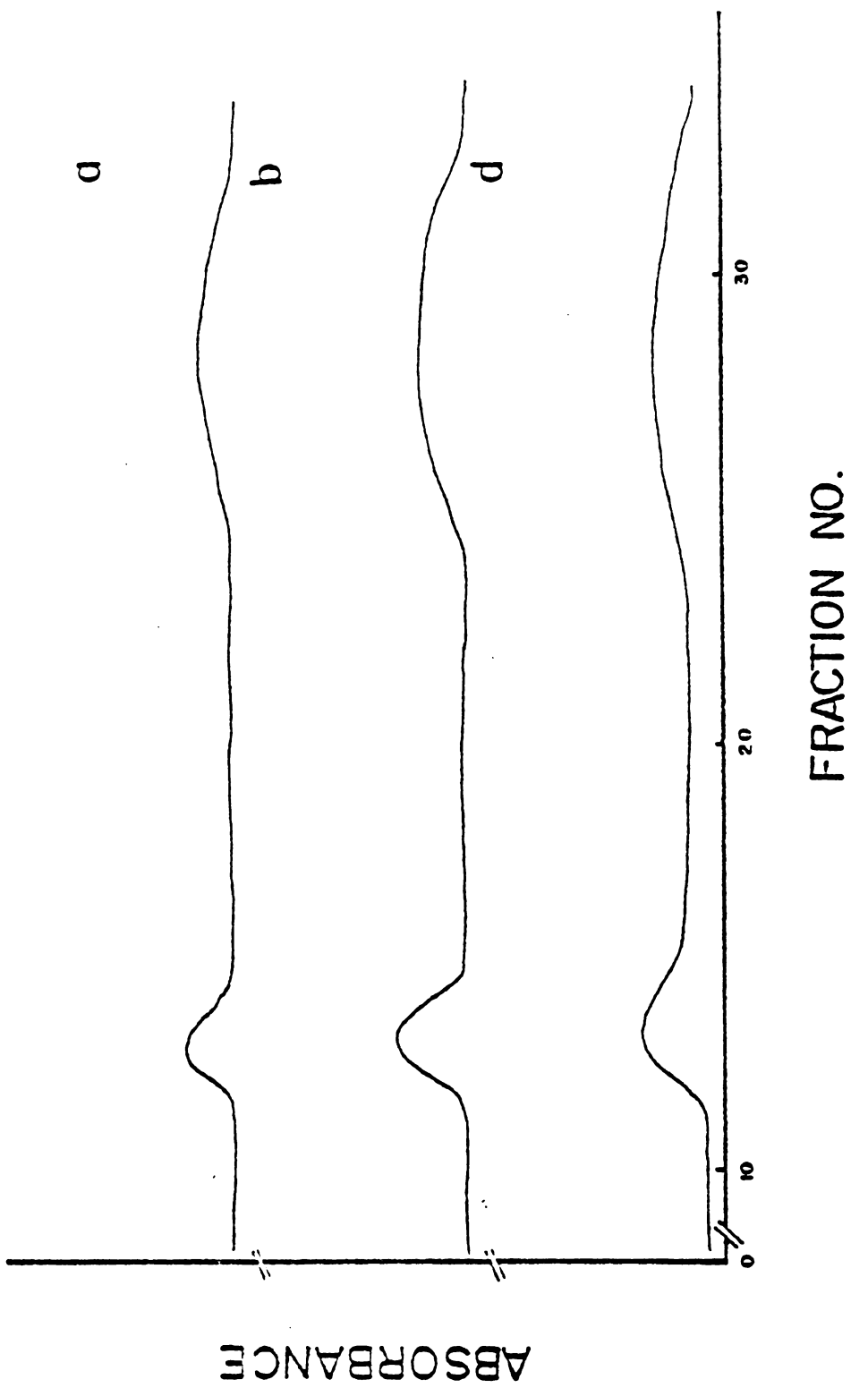
The gel diagrams of AC, SP and AS protein systems heated at $94^{o}$C/10 sec are shown in Figure 7. Gel identification and fraction numbers are as previously described. The print of the actual gels is shown in Figure 8. Gels of both whey and soy proteins heated at this temperature demonstrated the appreciable breakdown of their respective protein systems. Smeared areas were prevalent where clear, distinct zones had been. The gels from (AC) fractions 1, 2 and 3 had several smeared areas which corresponded to zones on the AS gels. AC gels 4 and 5 contained the remnants of β-lactoglobulin and α-lactalbumin. These same areas were

identified on AS gels 4 and 5. Gels containing soy protein also had substantial smearing which corresponded to zones found on the AS gels. SP fraction 4, AC fraction 6 and AS fraction 6 had no visible protein bands. All three fractions eluted at approximately the same volume during gel filtration. These fractions were composed primarily of NPN. Visual examination and determination of RF values demonstrated the non-interaction of whey:soy protein blends heated at 94°C/10 sec. Soy:whey protein blends, heated at 77°C/20 sec, examined by using the same techniques revealed no interaction. Smearing was quite pronounced on the gels of all three protein systems after heating at 121°C/5 sec. There was no indication of any interaction. Aldrich (1977) found that soy 7S and 11S proteins moved independent of β-lactoglobulin when heated in separate systems.

To determine whether or not interaction had occurred in the protein eluted at the void volume, a portion was concentrated and reapplied over a column containing sepharose 4B. Gel filtration, disc PAGE and determination of nitrogen were used in analyzing these samples for possible protein interaction.

The gel filtration chromatogram for whey protein reapplied over sepharose is shown in Figure 9. Only patterns for samples heated at: 1) unheated-control, 2) 68°C/30 min and 3) 94°C/10 sec are shown because samples heated at 77°C/20 sec and 121°C/5 sec did not show appreciable difference from those presented. Gel filtration resolved

Figure 9. Gel filtration chromatograms of whey protein fraction 1, concentrated and reapplied over Sepharose 4B. The heat treatments included: unheated-control (a), 68°C/30 min (b) and 94°C/10 sec (d).


a
b
d
0
10
20
30
FRACTION NO.
ABSORBANCE

two fractions, with the first eluting near the void volume. Gel electrophoresis (5% gels) of the concentrated material was not very helpful in resolving these proteins because they failed to enter the gels. Only those gels from the concentrated material (which was reapplied over sepharose) will be shown. The nitrogen distribution (Table 38) of these fractions did not change appreciably as the heat treatment rose to 68°C/30 min. Upon heating to 121°C/5 sec the nitrogen distribution shifted towards the first peak.

The gel filtration chromatogram for soy protein is shown in Figure 10. Gel filtration resolved the concentrated material into two fractions with the last eluting near the total volume. The majority of the nitrogen (Table 38) was in peak 1. As the heating temperature rose the nitrogen shifted towards the latter peak. Disc PAGE revealed two major zones. The band which migrated farthest down the gel and which eluted first from the column was the 11S protein. The 7S fraction eluted second. After heating the samples at 94°C/10 sec the fractions disintegrated which resulted in substantial smearing.

The gel filtration chromatogram of the whey:soy protein blend is presented in Figure 11. Gel filtration resolved the concentrated material into three fractions, with the first eluting near the void volume and the last near the total volume. The protein in fraction 1 was primarily whey protein while fraction 3 was principally soy protein. The elution pattern did not change appreciably when the

Table 38. Percent nitrogen in each fraction of whey protein, soy protein, and their blend separated by gel filtration with sepharose 4B

| Treatment | Fraction No. 1 | 2 | 3 |
|---|---|---|---|
| | | Whey protein | |
| Control | 49.1 | 50.9 | - |
| 68°C/30' | 49.3 | 50.6 | - |
| 94°C/10" | 58.3 | 41.7 | - |
| | | Soy protein | |
| Control | 74.1 | 25.9 | - |
| 68°C/30' | 68.3 | 31.7 | - |
| 94°C/10" | 20.1 | 79.9 | - |
| | | Whey:Soy blend | |
| Control | 23.7 | 63.2 | 13.1 |
| 68°C/30' | 25.2 | 46.8 | 28.0 |
| 94°C/10" | 30.4 | 29.6 | 41.0 |

Figure 10. Gel filtration chromatograms of soy protein fraction 1, concentrated and reapplied over Sepharose 4B. The heat treatments included: unheated-control (a), 68°C/30 min (b) and 94°C/ 10 sec (d).

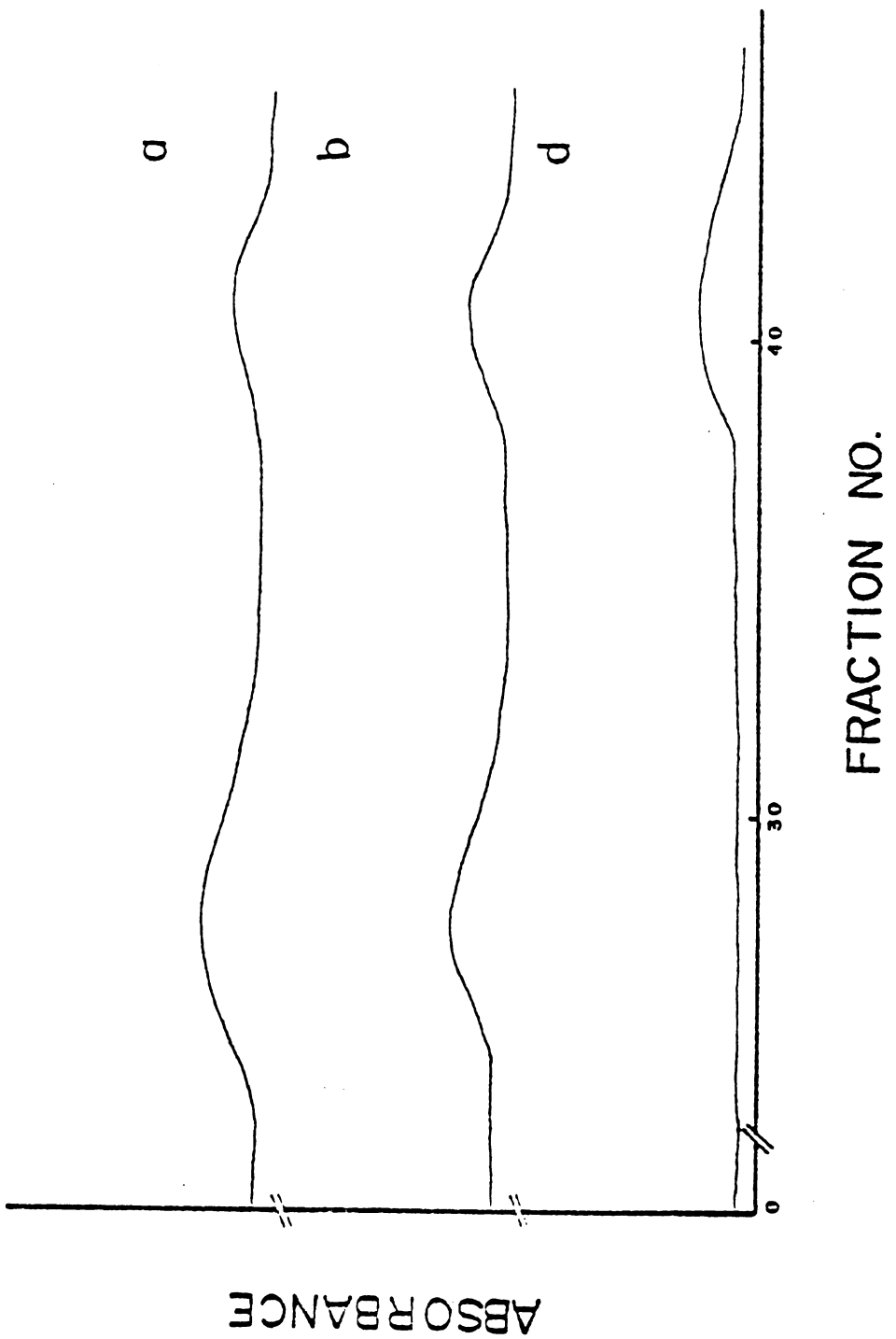
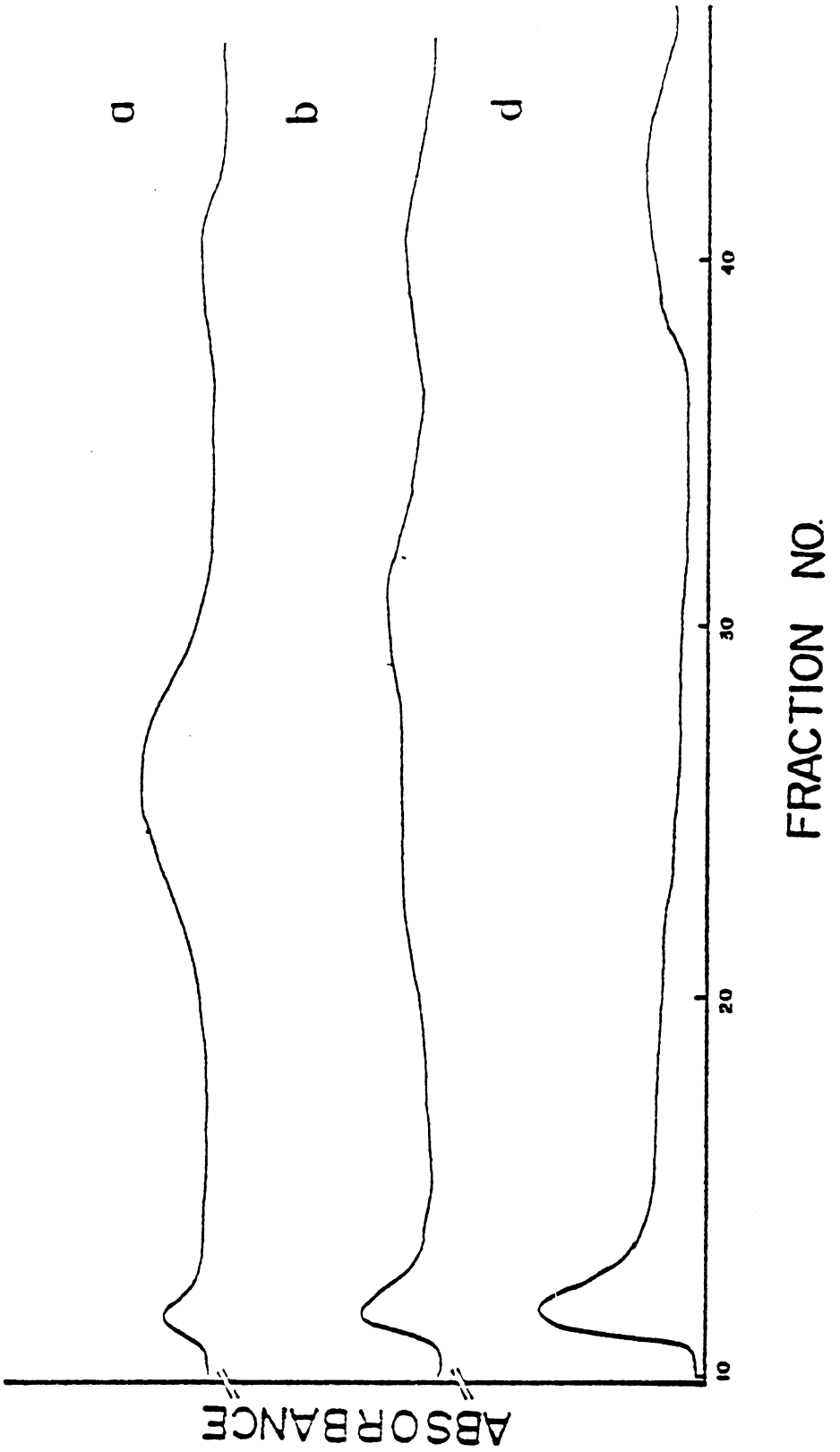

a
b
d
0
30
40
FRACTION NO.
ABSORBANCE

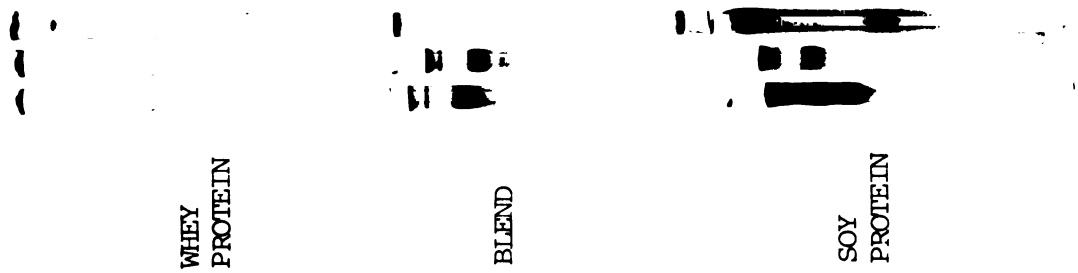
Figure 11. Gel filtration chromatograms of the whey: soy protein blend fraction 1, concentrated and reapplied over Sepharose 4B. The heat treatments included: unheated-control (a), 68°C/30 min (b), 77°C/20 sec (c), 94°C/10 sec (d) and 121°C/5 sec (e).

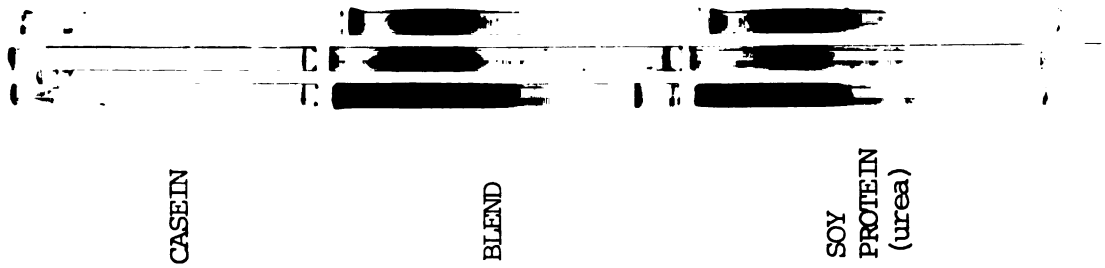

a
b
d
ABSORBANCE
10
20
30
40
FRACTION NO.

blend was heated at 68°C/30 min. The nitrogen profile (Table 38) shifted towards fraction 3 from fraction 2. This was further emphasized when the sample was heated at 94°C/10 sec. The gel filtration chromatogram revealed a substantial decrease in the size of fraction 2 which coincided with the decrease observed for this fraction from the chromatogram of SP. Disc PAGE (Figure 12) of the concentrated material showed several protein fractions. These gels were primarily reflections of the gels from the individual protein systems. It appeared that soy and whey proteins moved independently.

No interaction was detected between any of the major proteins from soy isolate or whey regardless of the heating temperatures employed. However, in concluding that soy and whey proteins do not interact in heated fluid systems, several points should be mentioned. In the system studied only the water soluble soy protein was considered. This fraction accounted for approximately 57% of the total soy protein. Secondly, as the heat treatment rose to 121°C/5 sec less protein was soluble. Whether or not interaction took place in this residue was unknown. Therefore, it can not be stated that under no circumstances would there be interaction between soy and whey protein systems in food products. Simply, in the systems examined, under the temperature conditions employed, no interaction transpired.

Figure 12. Disc gels of casein, whey and soy proteins and the milk:soy protein blends fraction 1, concentrated and reapplied over Sepharose 4B. The heat treatments included: unheated control, 68°C/30 min and 94°C/10 sec.


WHEY PROTEIN
BLEND
SOY PROTEIN


CASEIN
BLEND
SOY PROTEIN (urea)

Casein:Soy Protein Blends

The gel filtration chromatogram of whole casein obtained by pumping a 1% casein sol through sephacryl is presented in Figure 13. The heat treatments and letters of identification remain as previously described. Only the data pertaining to whole casein will be presented due to the extreme similarity between the results gathered from whole casein and sodium caseinate. Gel filtration resolved casein into three major and one minor fraction. The three major fractions eluted close to the void volume while the minor fraction eluted closer to the total volume of the column. The nitrogen distribution (Table 39) had the major proportion of nitrogen located in peak 3. Heating the samples at temperatures of 68°C, 77°C or 94°C resulted in very little change in the gel filtration patterns. Fractions 1 and 2 failed to resolve when the casein was heated at 121°C/5 sec. Nitrogen distribution remained similar regardless of the heat treatment. Even after heating at 121°C/5 sec the nitrogen content of the nonseparated fraction was almost the same as that found when the nitrogen content of fractions 1 and 2 were combined for the other samples. NPN increased to a small extent (Table 41) as the heat treatment rose to 121°C.

The gel filtration chromatogram of the casein:soy protein blend (CS) is presented in Figure 14. The temperature identification scheme remained the same as previously discussed. The elution pattern obtained from the unheated CS

Figure 13. Gel filtration chromatograms of whole casein heated at: unheated-control (a), 68°C/30 min (b), 77°C/20 sec (c), 94°C/10 sec (d) and 121°C/5 sec (e).

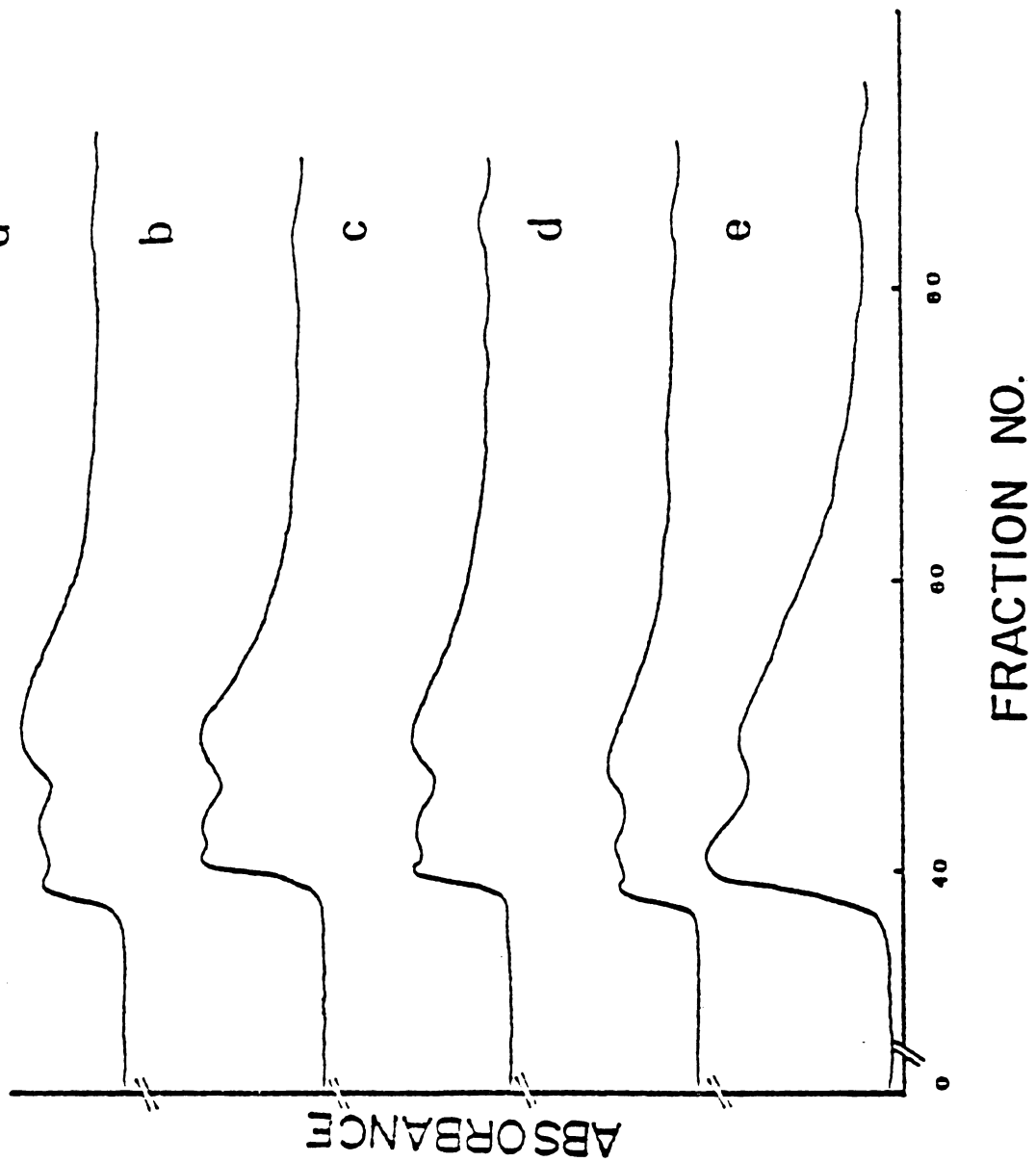
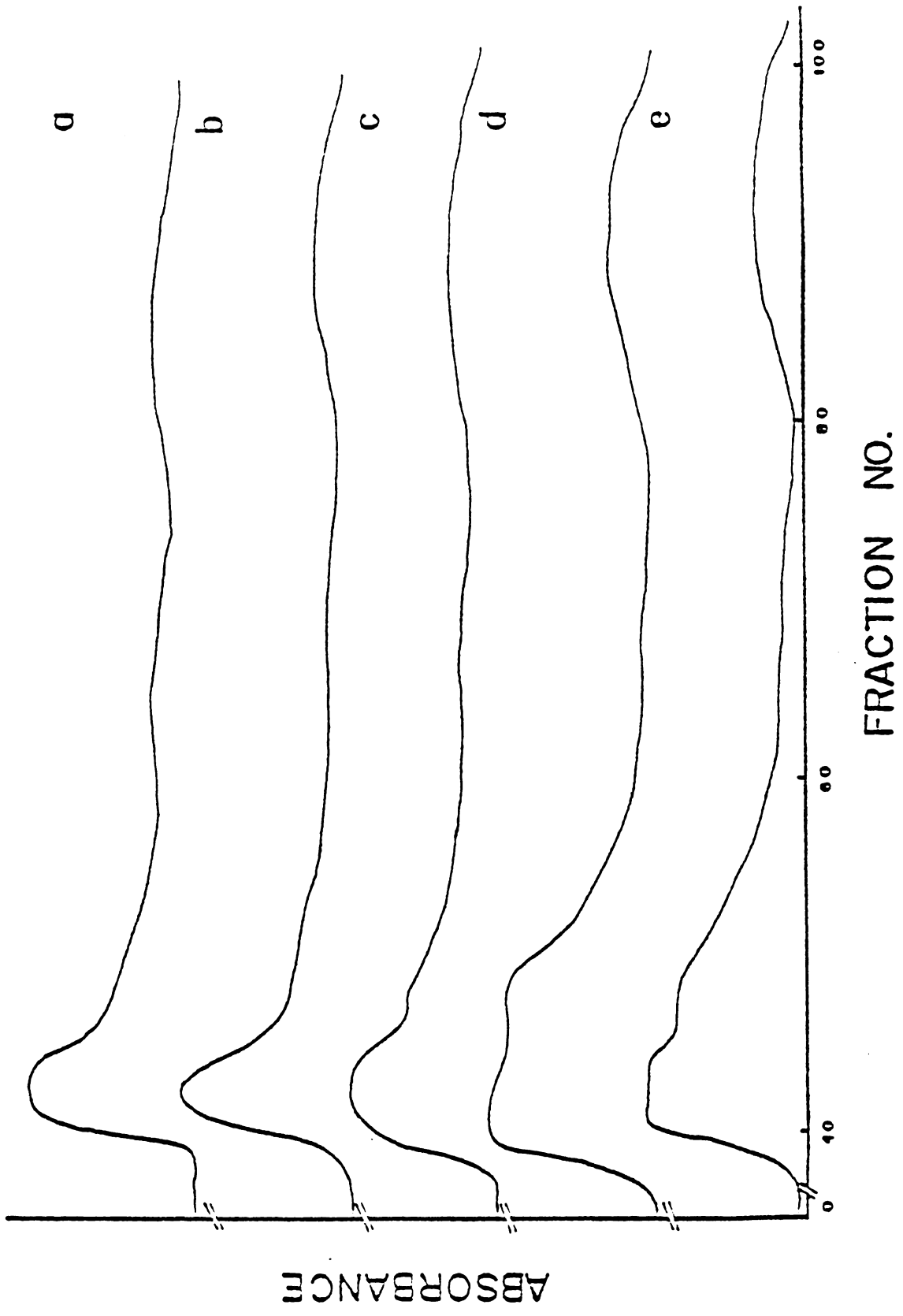

a
b
c
d
e
ABSORBANCE
0
40
80
FRACTION NO.

Table 39. Percent nitrogen in each fraction of whole casein separated by gel filtration

| Fraction No. | Treatment | | | | |
|---|---|---|---|---|---|
| | Control | 68°C/30' | 77°C/20" | 94°C/10" | 121°C/5" |
| 1 | 7.0 | 6.6 | 6.5 | 6.6 | 19.0 |
| 2 | 10.6 | 11.1 | 11.0 | 10.8 | - |
| 3 | 72.3 | 71.4 | 71.6 | 72.0 | 70.5 |
| 4 | 10.0 | 10.9 | 10.9 | 10.6 | 10.5 |

Figure 14. Gel filtration chromatograms of the casein: soy protein blend heated at: unheated-control (a), 68°C/30 min (b), 77°C/20 sec (c), 94°C/10 sec (d) and 121°C/5 sec (e).


a
b
c
d
e
0
40
80
100
FRACTION NO.
ABSORBANCE

blend was similar to the chromatogram observed for (soy protein) SP. Gel filtration revealed the presence of four fractions. The major fraction eluted at the void volume and comprized nearly 50% of the total nitrogen (Table 40). The fourth fractioned contained the least amount of nitrogen, soy fraction 1 and casein, fractions 1 and 2 eluted at approximately the same retention volumes during their respective gel filtration runs. Thus, it could be assumed that these fractions would elute together. The experimental results showed that not only casein, fractions 1 and 2, but that all three of the major casein fractions eluted with soy fraction 1. This could indicate that some interaction had taken place. However, this cannot be concluded from data gathered only from gel filtration. The remainder of the chromatogram was very similar to the gel filtration chromatogram of soy. This was feasible since the majority of the protein left was of soy origin. The gel filtration pattern of the blend changed very little when the sample was heated at 68$^{o}$C/30 min. Nitrogen distribution also remained approximately the same. Following heating at 77$^{o}$C/20 sec the gel filtration pattern showed fraction 1 beginning to separate. The resolution of fraction 1 into two peaks increased after the sample was heated at 94$^{o}$C/10 sec. The chromatogram also revealed that fraction 4 had eluted closer to the total volume of the column. The nitrogen content of this fraction also increased. The changes noted in fraction 4 may have been due to the breakdown of soy protein, which

Table 40. Percent nitrogen in each fraction of the casein: soy protein blend separated by gel filtration

| Fraction No. | Treatment | | | | |
|---|---|---|---|---|---|
| | Control | 68°C/30' | 77°C/20" | 94°C/10" | 121°C/5" |
| 1 | 48.3 | 45.3 | 53.7 | 49.0 | 45.6 |
| 2 | 16.8 | 16.9 | 21.1 | 24.5 | 30.5 |
| 3 | 25.6 | 27.7 | 14.7 | 13.3 | 10.2 |
| 4 | 9.4 | 10.1 | 10.4 | 13.2 | 13.7 |

resulted in greater amounts of NPN. Heating the blend at 121°C/5 sec further emphasized the changes which occurred at the lower temperature. Soy protein subjected to heat treatments of 94°C/10 sec or 121°C/5 sec suffered a major breakdown. This did not occur when the casein:soy blend was heated at these temperatures. Some mechanism, possibly hydrophobic interaction, prevented this from occurring. The solubility of casein:soy blends also remained high when subjected to sufficient heat treatment to reduce the solubility of soy protein.

The gel diagrams of whole casein (WC), soy protein (SP) and their blend are shown in Figures 15-17. The prints of the actual gels are shown in Figure 18. Seven percent gels, containing 7M urea were used. It was necessary to disperse the samples in 7M urea to prevent smearing and obtain good resolution. However, utilization of urea precluded the possibility of detecting other than disulfide interaction. Gel diagrams are shown for samples heated at: 1) unheated-control, 2) 68°C/30 min and 3) 94°C/10 sec. Diagrams for samples heated at 77°C/20 sec and 121°C/5 sec are not shown because of their similarity to the gels of the other treatments. The gels are identified by their respective symbols located above the diagrams. The fraction number (with reference to gel filtration) is denoted by the number in the upper right hand corner.

The three major casein fractions were all multicomponent peaks. Casein, fraction 4, was composed of one major

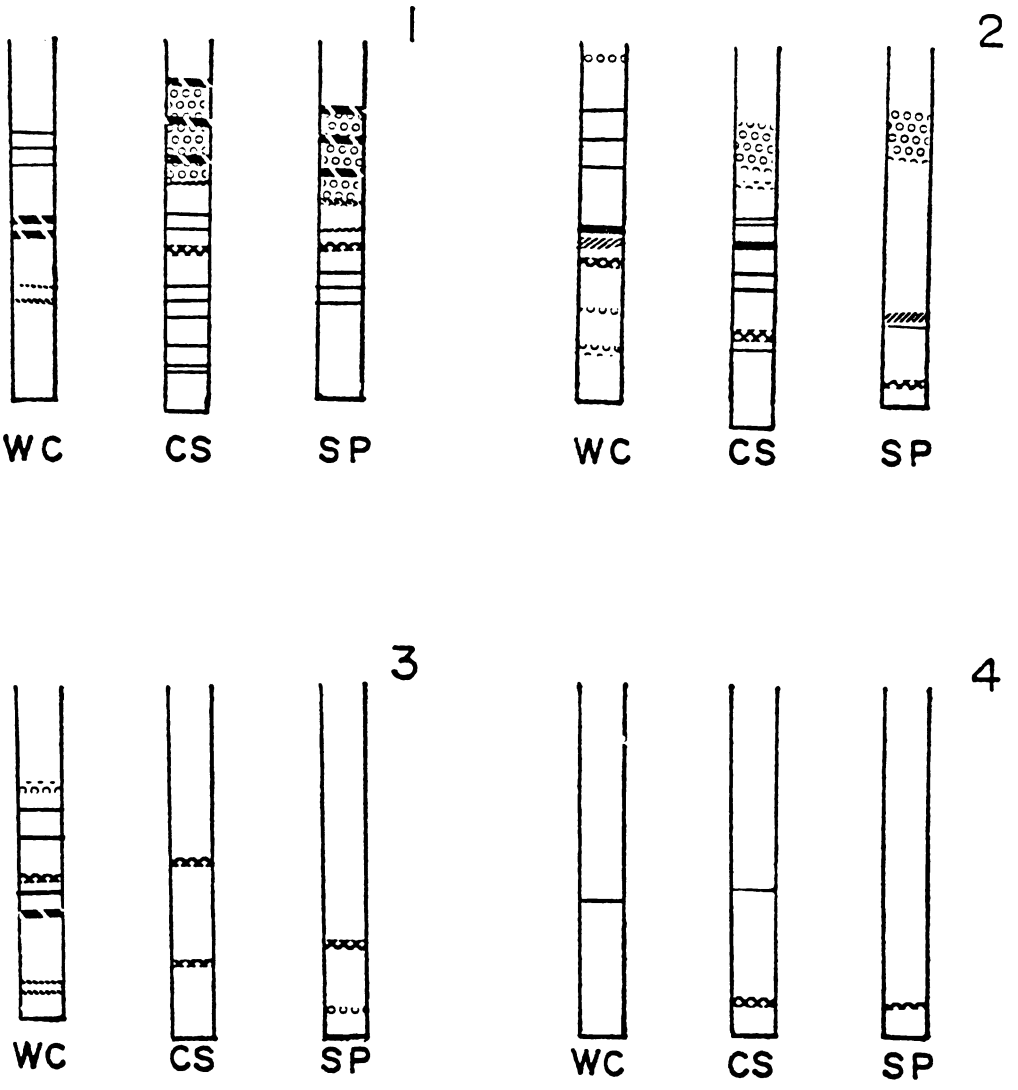



Figure 15. Gel diagrams of unheated whole casein (WC), soy protein (SP), and their blend (CS). The gel filtration fraction is denoted by the number in the upper right hand corner.

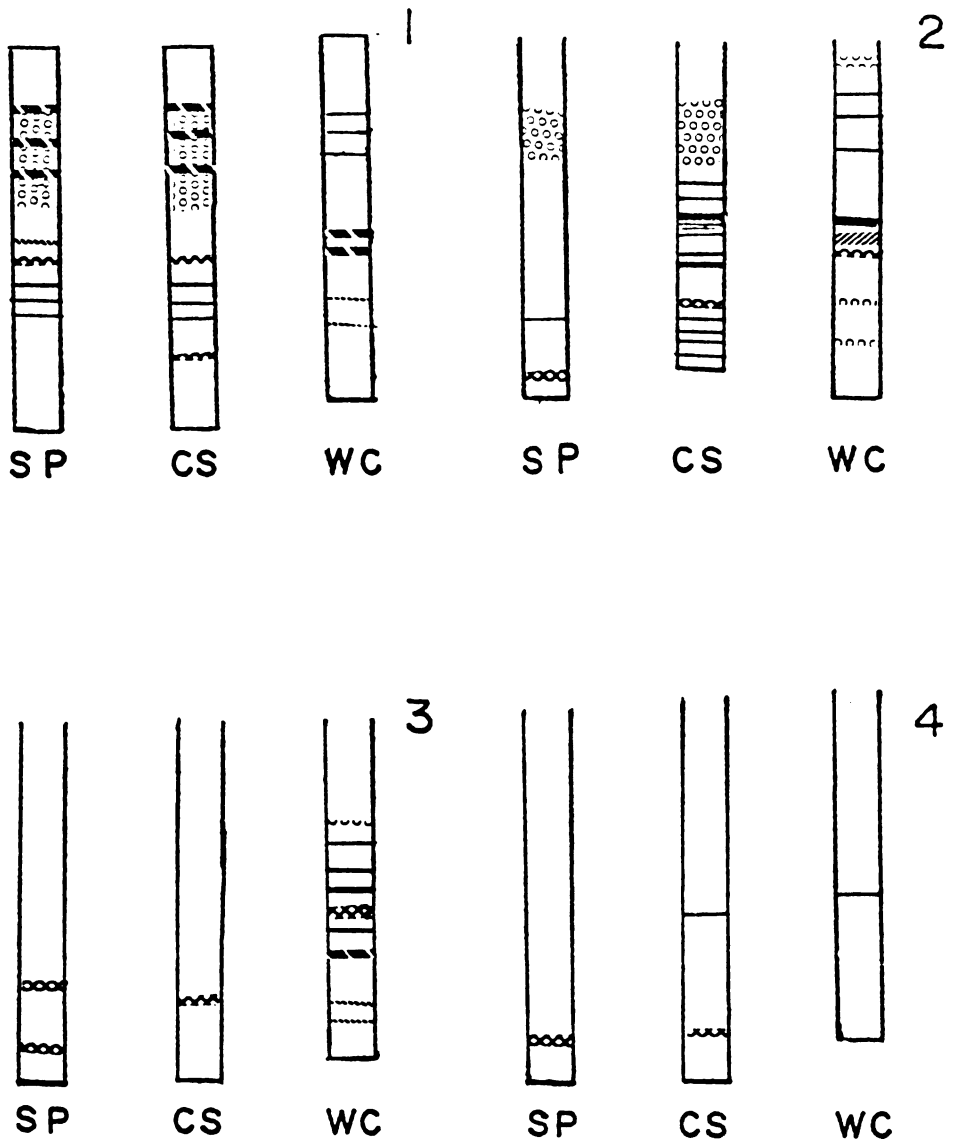



Figure 16. Gel diagrams of whole casein (WC), soy protein (SP) and their blend (CS) heated at 68°C/30 min. The gel filtration fraction is denoted by the number in the upper right hand corner.

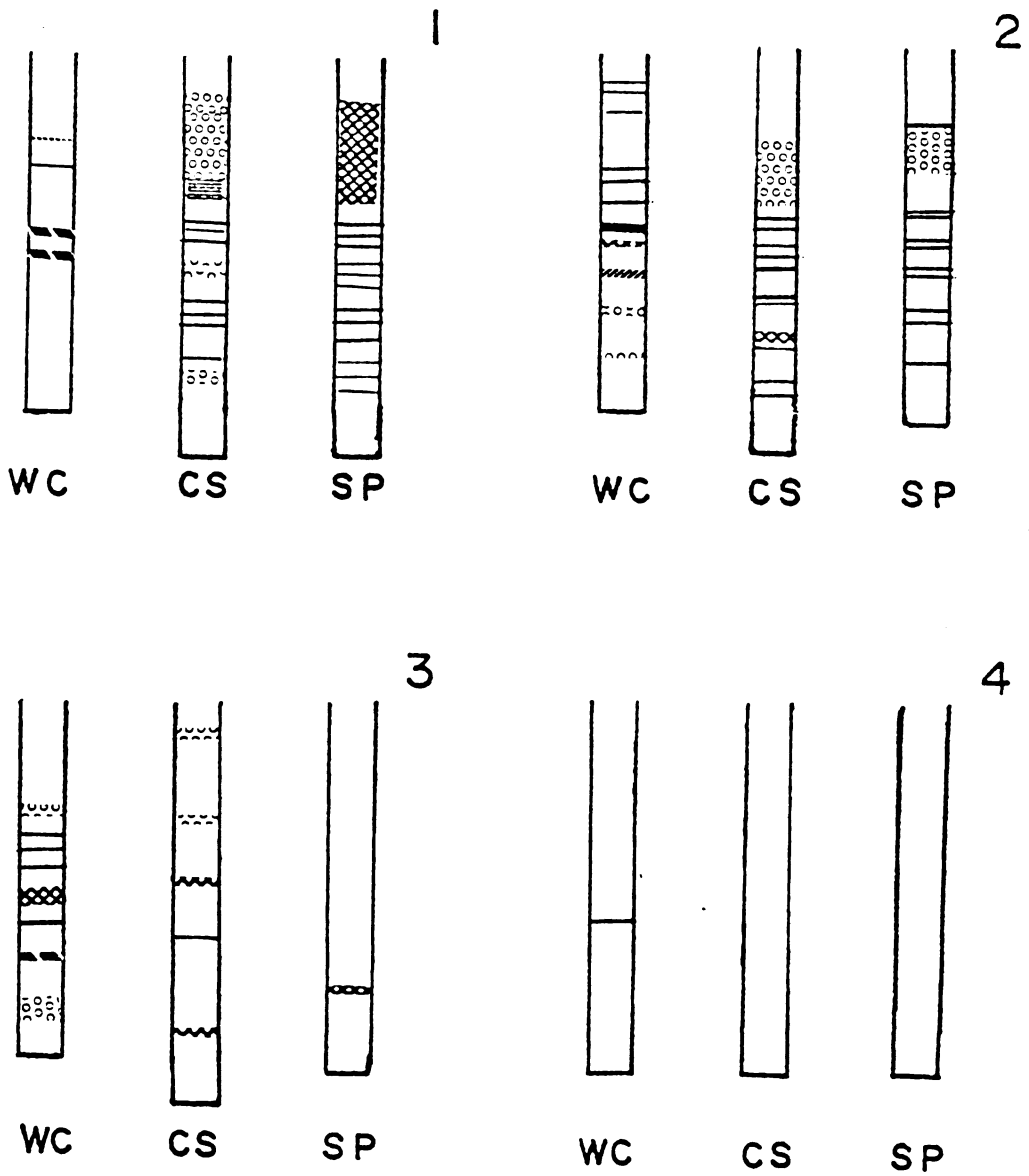
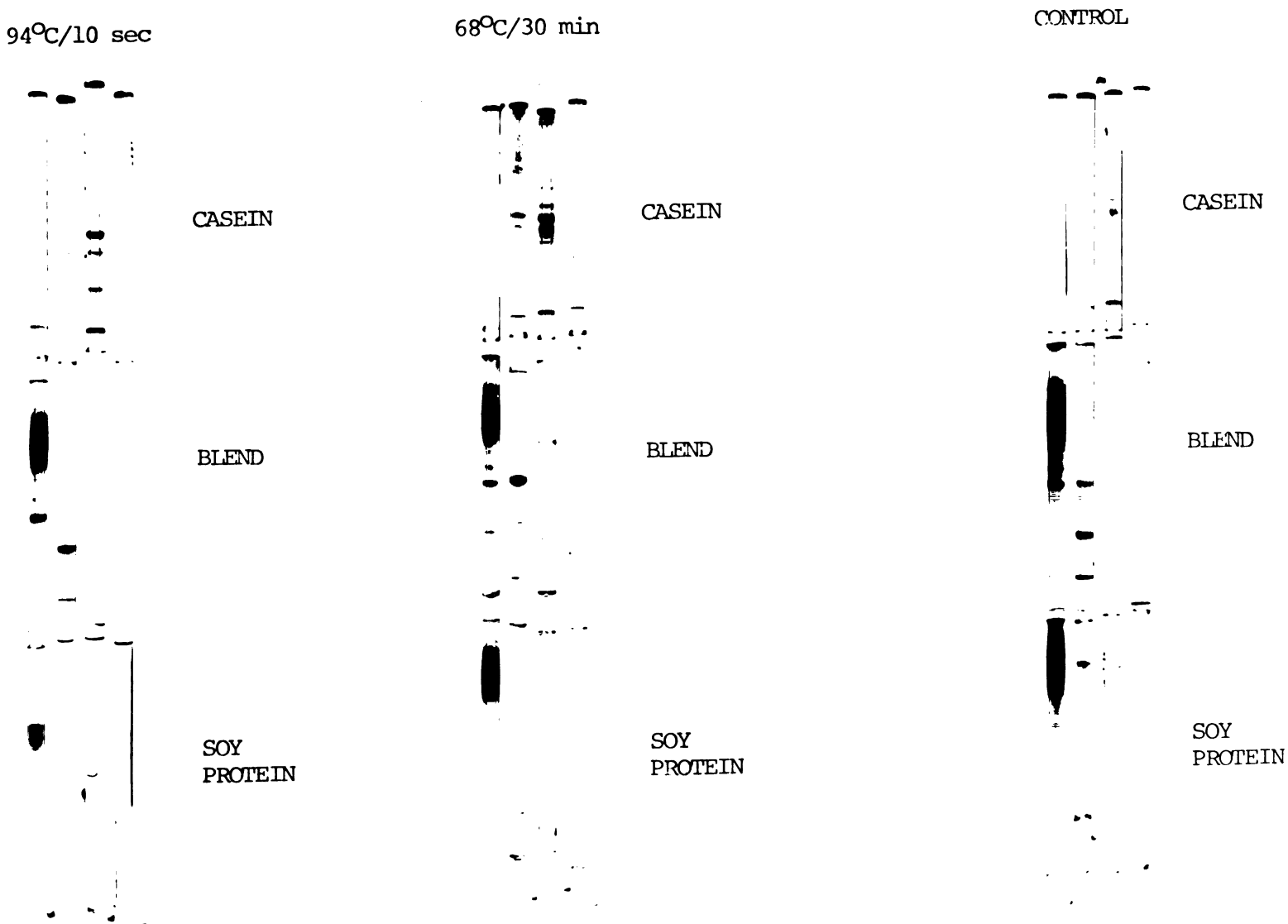



Figure 17. Gel diagrams of whole casein (WC), soy protein (SP) and their blend (CS) heated at 94°C/10 sec. The gel filtration fraction is denoted by the number in the upper right hand corner.

Figure 18. Disc gels of whole casein, soy protein and their blend, heated at: unheated-control, 68 C/30 min and 94°C/10 sec. The gel bands represent protein present in gel filtration fractions 1-4 (from left to right).


94°C/10 sec
CASEIN
BLEND
SOY
PROTEIN
68°C/30 min
CASEIN
BLEND
SOY
PROTEIN
CONTROL
CASEIN
BLEND
SOY
PROTEIN

component. The casein gels remained approximately the same regardless of the heat treatment employed.

Soy protein, fraction 1, was composed of the 7S and 11S fractions primarily. Fraction 2, contained (in addition to residual 7S and 11S proteins) two species which migrated farther down the gels than the 7S and 11S fractions. Fractions 3 and 4 were also composed of faster migrating species. These patterns were similar to the soy nonurea gels. The gel diagrams of sample heated to 68°C/30 min remained about the same. Disc PAGE of the samples heated to 94°C/10 sec demonstrated the heat susceptibility of soy 7S and 11S proteins. The breakdown of these fractions resulted in a greater number of less intense bands.

Visual examination and calculation of RF values (comparing the blended and unblended systems) were used to investigate the possibility of casein:soy protein interaction. Soy 7S and 11S proteins did correspond to zones found on gels of the CS blend, fraction 1. Several minor soy protein bands also had the same RF values. In the blended system the major caseins were eluted in the first two fractions. The casein gels from all three fractions were examined and compared primarily against CS gels 1 and 2. The large number of minor soy and casein bands made it extremely difficult to relate all of the bands. In addition, the smearing of soy 7S and 11S proteins (caused by heating at the higher temperatures) complicated the situation. A heat treatment of 68°C/30 min did not substantially

alter those results recorded for the unheated samples. Heating the sols at 94°C/10 sec resulted in the partial breakdown of soy 7S and 11S proteins. These same proteins were not as dissociated when heated in the presence of casein at 94°C/10 sec. The bands retained greater intensity. There appeared to be some mechanism protecting the soy protein from heat dissociation. The casein portion of the blend gels remained approximately the same. The major casein and soy protein bands were accounted for by visual examination and calculation of the RF values. It was difficult to relate all of the minor bands because of their number and location.

A densitometric scan was performed upon gels of WC, SP and the CS blend which had been subjected to heat treatments of: 1) unheated-control and 2) 94°C/10 sec. Migration distances and traces were obtained. The densitometric traces of the gels (unheated-control) of WC, SP and CS are shown in Figure 19. Soy and casein had many minor components. By combining the traces obtained from soy and casein it was observed that its pattern was quite close to that found for CS. Retention times were very similar. This work confirmed the data gathered by visual examination of the protein systems.

The gel filtration chromatogram for casein (void volume from the sephacryl column concentrated 10 to 1) reapplied over sepharose is shown in Figure 20. Only patterns for samples heated at: 1) unheated-control, 2) 68°C/30 min and

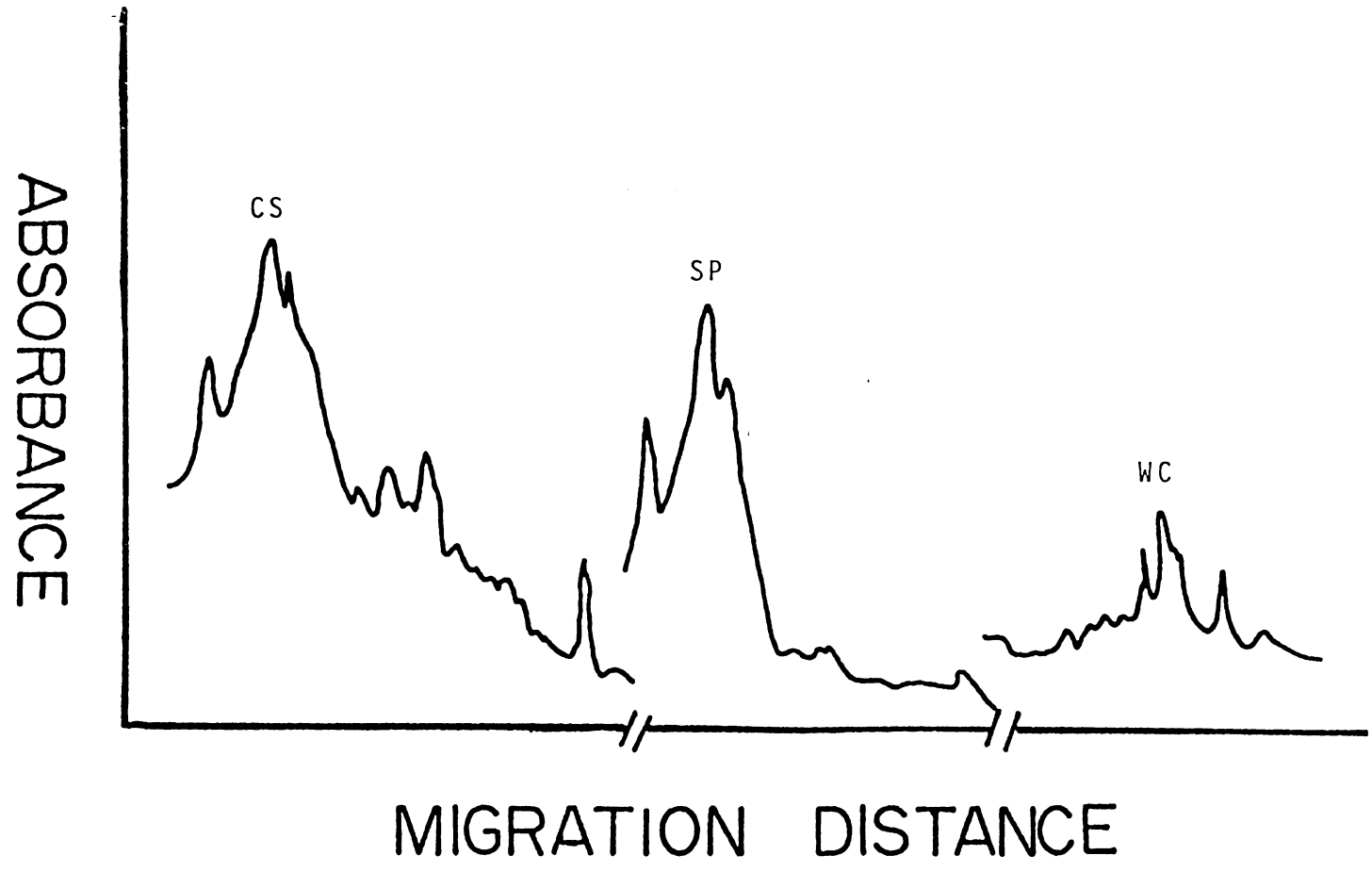
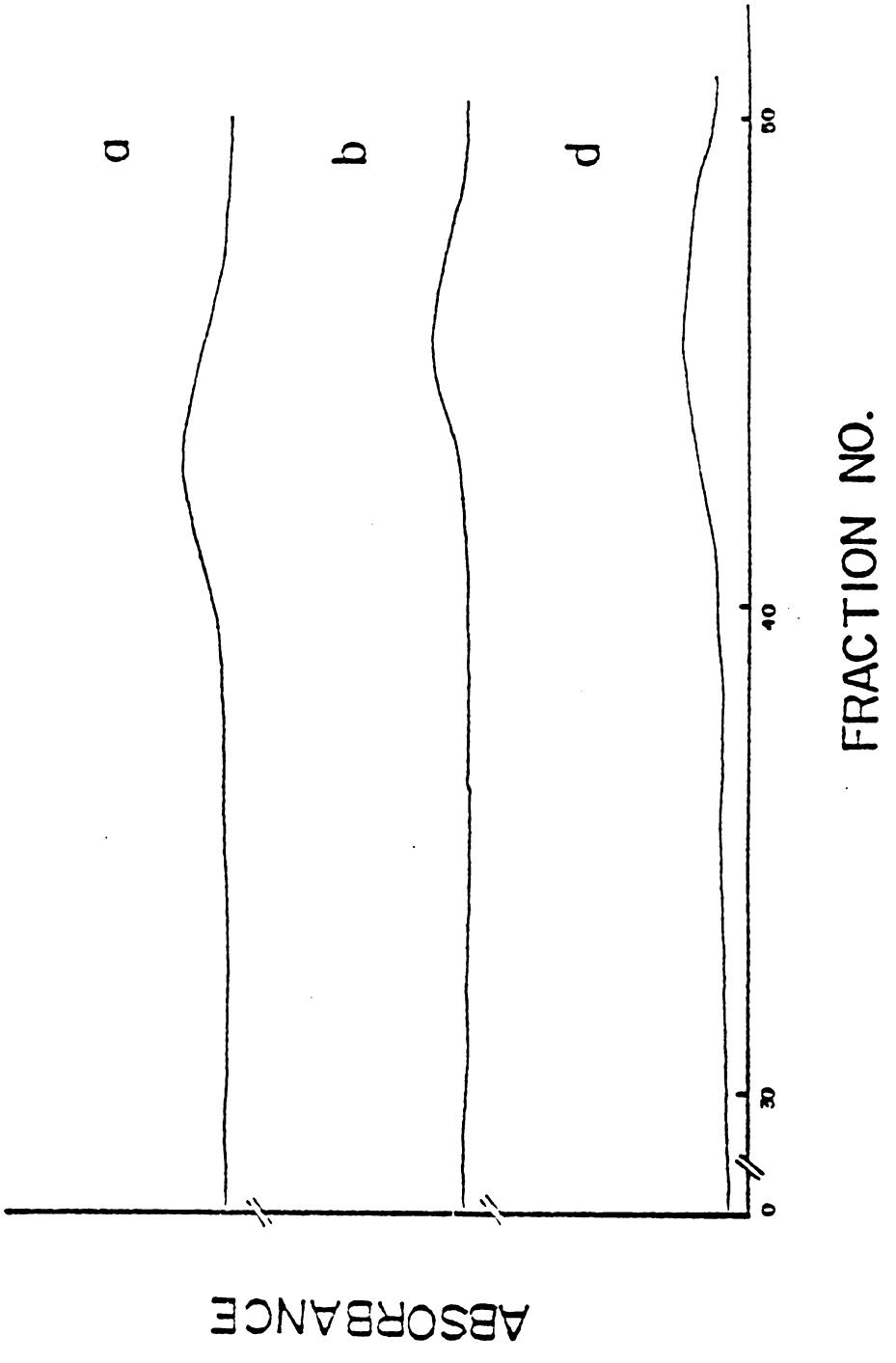



Figure 19. Densitometric trace of the gels (unheated-control) of soy protein (SP), whole casein (WC) and their blend (CS).

Figure 20. Gel filtration chromatograms of whole casein concentrated and reapplied over Sepharose 4B. The heat treatments included: unheated-control (a), 68°C/30 min (b) and 94°C/10 sec (d).


a
b
d
0
30
40
60
FRACTION NO.
ABSORBANCE

3) 94°C/10 sec are shown because samples heated at 77°C/ 20 sec and 121°C/5 sec did not show appreciable differences from those presented. Gel filtration of casein sols resulted in the resolution of only one fraction. This pattern changed very little as the heat treatment varied. The gel filtration chromatogram of the CS blend is presented in Figure 21. Gel filtration resulted in the separation of two fractions. The first, eluting near the total volume of the column was composed of both casein and soy protein while the other was principally soy protein. The nitrogen distribution (Table 42) shifted towards the second peak as the heating temperature rose. This shift followed that observed for soy protein.

The disc PAGE gels (5% in 7M urea) of the material reapplied over sepharose are shown in Figure 12. The casein gels contained several bands, many of which had RF values close to those observed for bands found in the CS gels. The soy 7S and 11S proteins were also observed on the gels of the CS blend.

There was no evidence that any interaction (disulfide) had occurred between casein and soy proteins. There was no indication that new bands had been formed or that old bands had disappeared. The possibility remains that there may be substantial hydrophobic interaction between casein and soy proteins. Aldrich (1977) demonstrated that soy 7S and 11S proteins owe much of their stability to intramolecular hydrophobic bonding. Ribadeau-Dumas et al. (1972) and

Figure 21. Gel filtration chromatograms of the casein: soy protein blend fraction 1, concentrated and reapplied over Sepharose 4B. The heat treatments included: unheated-control (a), 68°C/30 min (b) and 94°C/10 sec (d).

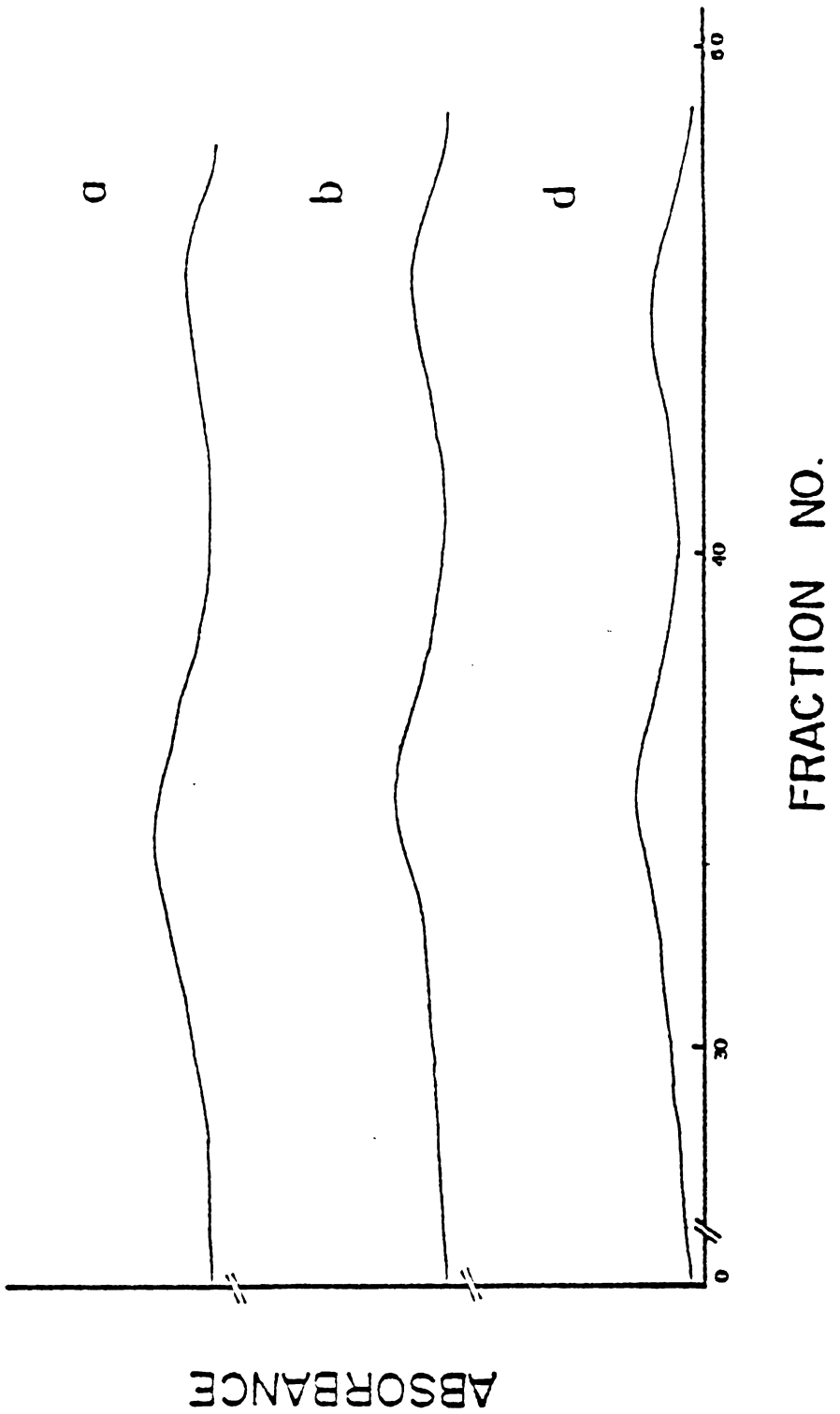

a
b
d
0
30
40
60
FRACTION NO.
ABSORBANCE

Table 41. Nonprotein nitrogen of samples of AC, AS, SP, CS and WC heated at 94°C/10 sec and 121°C/5 sec

| Sample | Treatment | |
|---|---|---|
| | 94°C/10 sec | 121°C/5 sec |
| AC[a] | 5.0 | 6.3 |
| AS[b] | 4.3 | 6.0 |
| SP[c] | 4.4 | 6.6 |
| CS[d] | 2.8 | 3.0 |
| WC[e] | 0.9 | 1.3 |

a = whey protein from acid whey

b = whey:soy protein combination

c = soy protein

d = casein:soy protein combination

e = whole casein

Table 42. Percent nitrogen in each fraction of whole casein and the casein:soy protein blend separated by gel filtration with sepharose 4B

| Treatment | Fraction No. | |
|---|---|---|
| | Whole casein | |
| | 1 | 2 |
| Control | 100.0 | - |
| 68°C/30' | 100.0 | - |
| 94°C/10" | 100.0 | - |
| | Casein:soy protein blend | |
| Control | 38.2 | 61.8 |
| 68°C/30' | 37.1 | 62.9 |
| 94°C/10" | 17.2 | 82.8 |

Mercier et al. (1973) reported that the casein constituents possess higher than average hydrophobicity. The stability of soy protein in the presence of casein may be due to this bonding.

## In Vitro Estimation of Protein Quality

In vitro enzymatic digestions are rapid, sensitive tools which have importance in the nutritional evaluation of protein quality. Many researchers have used these procedures and concluded that the results observed were comparable to animal feeding studies (Sheffner et al., 1956; Akesson and Stahmann, 1964; Stahmann and Woldegiorgis, 1975; Satterlee, 1977; and McCune, 1977). In vitro enzymatic hydrolyses have been used to estimate the differences in quality between proteins and between treatments affecting one or more proteins. Various techniques (including quantitation of amino acids) have been employed for the purpose of measuring and relating enzymatic hydrolyses to protein quality. Melnick (1964) used a modified formal titration to estimate the extent of hydrolysis. Van Buren et al. (1964) found significant correlation between free amino groups and PER (at the 99% level). Yamashita et al. (1970) calculated the pepsin-pancreatin digestibility of soy plastein while Saunders et al. (1973) estimated the digestibility of alfalfa protein by determination of the residue protein. Ford and Salter (1966) used both static

and dynamic digestions to estimate protein quality. Digests were analyzed for soluble protein, peptide content and free amino acids. Broadly similar results were obtained for all the methods. Maga et al. (1973) measured the initial rate of proteolysis and was able to correlate this to protein quality. Hsu et al. (1977) used a multienzyme technique to estimate protein digestibility. This method was sensitive enough to detect processing and protease inhibitor effects.

In this research, nonfat dry milk, soy isolate and their blend were reconstituted in distilled water and subjected to 24 different treatments. Portions of each sample were then exposed to a sequential pepsin-pancreatin digestion. Following precipitation and removal of the residue protein, the amino nitrogen was determined in the supernatant of each sample. The purpose of this study was twofold: firstly, was there significant interaction between proteins which affected liberation of amino nitrogen and secondly, did any of the treatments increase or decrease the amount of nitrogen released. The statistical procedures employed (appendix) consisted of 2 factor analysis, Dunnett's t test for comparison of treatment affects and a f test for contrast among materials. The amino nitrogen of the in vitro digested samples were compared to values from acid hydrolyses of the same untreated materials. A percent of the total (acid hydrolysis) was then computed for each sample.

The data in Table 43 are representative of the values gathered in this study. Two controls were used to compare

treatment effects. No treatment (NT) samples were employed as controls against the samples not heated to 68°C/30 min. The samples heated at 68°C/30 min were used as controls for all samples employing this heating process as part of their treatment. All samples were prepared and analyzed in triplicate. In vitro enzymatic hydrolysis released approximately 59% of the amino nitrogen from each of the untreated samples. The values were very close regardless of the protein source.

None of the heat treatments employed (68°C/30 min, 77°C/30 min, 94°C/4 sec, 121°C/4 sec, 113°C/15 min, microwave 77°C/flash) significantly altered the release of amino nitrogen from the samples. The amount liberated from soy increased slightly, though the values were not significant. Taira et al. (1965), Hackler et al. (1965) and Fritz et al. (1947) reported that heating soybean products at high temperatures (100-115°C) for one hr or less had no detrimental effect on the growth rates of animals fed these products. Hankes et al. (1948), Menden and Creamer (1966) and Mauron et al. (1955) found that autoclaving had no effect on the BV of casein. Cook et al. (1951) reported that there was no change in the PER of a commercially prepared lactalbumin heated at 100°C. Normal heat processing does little nutritional damage to the protein in milk products and often enhances the quality of legume proteins (Bender, 1972).

Addition of either glycan or CMC prior to heating at 68°C/30 min did not significantly alter release of amino

nitrogen. Addition of reducing and oxidizing agents likewise did not substantially modify liberation of nitrogen. Patton (1954) suggested that the sunlight flavor in milk products might be caused by degradation of methionine to methional. In this study samples were exposed to sunlight sufficient to cause this flavor problem. However, no significant decrease was noted in the amount of released nitrogen. Addition of soybean oil to the samples prior to heating did not significantly alter liberation of amino nitrogen. Hsu (1977) found that the presence of fat had no effect on the proteolytic digestion of several samples.

Addition of glucose prior to heating did not result in decreased liberation of amino nitrogen. Various researchers have shown that autoclaving casein in the presence of glucose resulted in an appreciable loss of available lysine. Dimler (1975) observed that the total amino acids remained approximately the same (when casein was autoclaved in the presence of glucose) even though the levels of certain amino acids decreased. In this study the material was not autoclaved but heated at the much lower temperature of 68$^{o}$C. Thus the temperature enhancing effect may have been less. Secondly, this technique may not be sensitive enough to compensate for partial loss of one amino acid relative to the total.

Of the 24 treatments examined only 4 were found to significantly affect the liberation of amino nitrogen from intact proteins (Table 44). Utilizing the Dunnet t test

these 4 treatments were found to have significance at the 99% level. Adjustment of the pH to 11.5 substantially reduced the liberation of amino nitrogen. Badenhop (1970) reported that high pH reduced the PER of soy protein, which was mainly due to the destruction of cystine. Degroot and Slump (1969) evaluated the treatment of food proteins at pH 12.2. The levels of cystine, lysine, serine and arginine decreased which correlated to the reduction in NPU of the protein. Osner and Johnson observed that high pH treatment resulted in the destruction and availability of amino acids. The loss of amino nitrogen liberated by in vitro digestion was partially due to the decomposition of sulfur containing amino acids.

The succinylation of the samples resulted in markedly lower amino nitrogen values. Succinic anhydride reacts with the $\epsilon$ amino group of lysine and renders this bond unavailable to tryptic digestion. With tryptic hydrolysis being limited to those bonds involving arginine, the total amino nitrogen released could conceivably be reduced. Maleylation with maleic anhydride did not result in the same reduction. This is possibly due to the fact that maleic anhydride is deblocked when held at low pH for several hrs (conditions encountered in the digestion process) whereas succinic anhydride is more difficult to deblock. Succinylation to improve the functionality of proteins could depress the nutritive value of the protein.

The incubation of oxidizing safflower oil with protein significantly lowered the amount of released amino nitrogen. The liberated nitrogen was reduced by about 45%. Osner and Johnson (1968), Horigome and Miura (1974) and Yanagita and Sugano (1973) reported that oxidized fats lowered both the digestibility and biological value of the protein. Lewis and Wills (1962) found that exposing protein to oil undergoing oxidation resulted in rapid destruction of the SH groups associated with the amino acids. Roubal and Tappel (1966) described the damage to proteins by peroxidizing lipids. Transient free radicals were produced in the protein-lipid system. The damage to proteins included loss of solubility and destruction of such amino acids as methionine, histidine, cystine and lysine. McCune (1977) reported that oxidizing lipid reduced the PER of proteins incubated in its presence.

Homogenization of the samples significantly increased the liberation of amino nitrogen. This may have in part been due to the increased solubility of the samples (see Functionality page 87). This treatment may have physically disrupted the structure of the proteins, possibly increasing their vulnerability to enzymatic digestion.

The examination for material interactions was conducted using a f test for contrast among materials. Analysis of the data revealed that there were no material interactions which resulted in significantly higher or lower amounts of released amino nitrogen. No values were obtained from

hydrolysis of the blend samples (regardless of the treatment) which could not be accounted for by examination of the measurements from the unblended samples. Material interactions were not significant at the 95% level.

Processing damage can result from four different types of reaction, namely: 1) destruction of amino acids by oxidation, 2) loss of palatability, 3) modification of linkages or functional groups and 4) formation of linkages that are not biological available. Loss of availability can occur through: 1) reaction between the amino group of the amino acid and a reducing substance, 2) reaction between the terminal group of lysine and the carbonyl secondary decomposition products of autoxidizing lipids and 3) protein-protein interaction independent of the presence of reducing substances (Bender, 1973). In this study there were no interactions between the protein systems that were responsible for significantly different levels of liberated amino nitrogen. Four of the treatments tested significantly affected the release of amino nitrogen from intact proteins. In general, most of the processing treatments employed had little effect.

Table 43. The percent of amino nitrogen released by in vitro hydrolysis of NFDM, soy isolate and their blend as affected by various treatments

| Treatment | Sample* | | |
|---|---|---|---|
| | NFDM | NFDM:Soy Isolate | Soy Isolate |
| NT | 59.8 | 58.3 | 59.1 |
| 68°C/30 min | 58.4 | 57.3 | 59.0 |
| 121°C/4 sec | 56.7 | 60.4 | 60.2 |
| CMC-68°C/30 min | 55.4 | 63.0 | 60.9 |
| glycan-68°C/30 min | 62.3 | 59.7 | 60.3 |
| Cys.-$H_2O_2$-Cat. | 61.0 | 62.6 | 58.1 |
| glucose-68°C/30 min | 60.1 | 60.2 | 58.5 |
| Sunlight oxid. | 58.4 | 59.8 | 57.1 |

*Means of triplicates

Table 44. The percent of amino nitrogen released by in vitro hydrolysis of NFDM, soy isolate and their blend as affected by various treatments

| Treatment | Sample* | | |
|---|---|---|---|
| | NFDM | NFDM:Soy Isolate | Soy Isolate |
| pH 11.5 | 45.3 | 41.3 | 33.7 |
| Succinic Anhyd. | 37.7 | 40.1 | 41.4 |
| Oxid. Oil | 32.7 | 32.4 | 32.4 |
| Homog.-68$^{o}$C/30 min | 59.1 | 63.2 | 66.0 |

*Means of triplicates

# SUMMARY

The functional properties of milk products, soy isolate and their blends were examined at three protein concentrations and several blend ratios. The samples were subjected to forty five different treatments prior to evaluation of their functionality.

Milk products were the most soluble of the materials studied. Sodium caseinate, WPC and electrodialyzed whey had solubilities greater than 90%. Soy isolate had the lowest solubility of any of the materials examined. Milk product:soy isolate blends were found to have appreciably higher solubilities than soy isolate. Specific treatments resulted in widely varying solubilities which made it possible to achieve greater solubility by selecting the appropriate treatment for the protein.

At 3.2% protein the viscosities of the milk products, soy isolate and their blends were similar regardless of the treatment. The viscosities of either sodium caseinate or soy isolate increased with increasing concentration. At 8.0% protein the viscosities of reconstituted electrodialyzed and sweet whey powders were very high due to the high total solids content. Specific treatments resulted in higher (or lower) viscosities but the majority had little

effect.

Very limited success was achieved when the milk products were subjected to a heat process designed to encourage gelation. Only WPC demonstrated appreciable potential. Edi-ProN, the soy isolate used throughout this research, did not gel when subjected to the established conditions. Promine D, a soy isolate, which demonstrated substantial gelation ability was used in lieu of Edi-ProN. WPC:Promine D blends formed firm gels though slight wheying off occurred with partial visual separation of the two protein systems.

Soy isolate had slightly less emulsion capacity than the milk products. Blends of soya and milk components often had emulsion capacities approaching those of pure milk products. Specific treatments improved (or lowered) the emulsion capability of the samples. By selecting the treatment corresponding to a particular protein it was possible to obtain substantially improved emulsion capacity.

Stable foams were produced from sols of NFDM, sodium caseinate, electrodialyzed whey and their soy isolate blends. WPC sols were whipped into stable foams after subjection to the appropriate treatment. Soy isolate sols whipped into stable foams in only a few instances. Milk product:soy isolate foams had approximately the same specific volumes but shorter stability times than the respective milk product. Many treatments improved the whipping properties of the samples.

The sensory properties of soy isolate were unacceptable. However, the flavor scores of milk product:soy isolate blends were only slightly less than those recorded for the particular milk product. Many treatments failed to improve the sensory qualities of the samples. Several treatments while markedly improving functionality resulted in unsatisfactory flavor characteristics. This may limit or modify the use of such treatments in actual food products.

Functional testing of proteins provides the technologist with information important to his understanding of food ingredients. The data gathered in this study should not be interpreted as having direct application to food products. The results should be used as guidelines from which to make reasonable decisions. Model systems provide the basic information necessary to incorporate ingredients, in a logical order, into food products.

The second part of this research involved the examination of milk:soy protein blends for heat induced interactions. The milk proteins included whey proteins and caseinates. The soy protein was extracted from soy isolate.

Gel filtration resolved whey protein into five distinct fractions none of which were homogenous but all of which were composed of one major species. Gel electrophoresis identified these proteins as immunoglobulins, serum albumin, β-lactoglobulin and α-lactalbumin. These proteins were stable to heat treatment through 77$^{o}$C/20 sec. Heating

at greater temperatures resulted in substantial denaturation. Serum albumin, the immunoglobulins and ß-lactoglobulin were the most heat sensitive while α-lactalbumin was the most heat resistant.

Gel filtration resolved casein into three major and one minor fraction. The fractions were heterogenous. All of the casein components were stable to the heat treatments employed in this study.

Gel filtration resolved soy protein into four fractions. Fraction 1 was composed of the soy 7S and 11S proteins. These proteins were stable to the low temperature pasteurization processes normally encountered during the heating of fluid milk products. Heating soy protein at UHT temperatures resulted in partial denaturation and loss of solubility. The 7S and 11S proteins underwent substantial dissociation into other components. At 94°C/10 sec and 121°C/5 sec the 7S and 11S proteins nearly disappeared.

Utilizing gel electrophoresis, no interaction could be detected between the major whey and soy proteins. The proteins of both systems moved independently of each other. The addition of urea to samples containing casein precluded the possibility of detecting other than disulfide interchange between soy and casein. There did not appear to be any interaction of this type taking place. The possibility exists that hydrophobic interaction between the two systems may have occurred. Interaction between proteins from different sources could conceivably affect the functionality of protein

blends. Examination of these interactions is of importance because of the possible benefit derived from the modification or manipulation of them.

The final segment of this research was concerned with examining the protein quality of products used in this study. Samples of NFDM, soy isolate and their blend were exposed to 24 different treatments prior to in vitro enzymatic hydrolysis. The amino nitrogen liberated by this digestion was quantitated and compared to the amino nitrogen liberated by acid hydrolysis. Two factor analysis, Dunnett's t test and a f test were employed to determine if material interactions had significantly affected the release of amino nitrogen and to determine whether or not the processes had altered liberation of amino nitrogen. Of the treatments examined only four (succinic anhydride, oxidized oil, pH 11.5 and homogenization) significantly modified the liberation of amino nitrogen. No material interactions took place which significantly affected the release of amino nitrogen. Most of the treatments employed in this study did not result in nutritional damage to the proteins.

# APPENDIX

# APPENDIX

## Lowry Procedure for Soluble Protein

Reagent A was 2% $Na_2CO_3$ and 0.02% sodium tartrate in a 0.1N NaOH solution. Reagent B was a 0.5% $CuSO_4 \cdot 5\ H_2O$ solution, Reagent C was prepared from 50 parts A + 1 part B. To samples containing 50-500 µg of protein per ml, 5.0 ml of reagent D was added. Following 10 min of incubation, 0.5 ml of phenol reagent (Folin-Ciocalteau reagent-Fischer) was added in less than 2 sec with a blow out pipet. Color was allowed to develop 30 min before absorbance was read.

## Lowry Procedure for Insoluble Protein

Reagent D was 2% $Na_2CO_3$ and 0.02% sodium tartrate in $H_2O$. Reagent B was discussed previously. Reagent E was prepared from 50 parts D and 1 part B. The samples containing 50-500 µg/ml were made up in 1.0N NaOH and placed in a boiling water bath for 10 min. The standards were treated in an identical manner. After cooling the samples were handed in the same way as described previously for Lowry soluble protein.

## Kjeldahl Nitrogen

The digestion mixture was prepared by adding 5.0 g $CuSO_4 \cdot 5\ H_2O$ and 5.0 g $SeO_2$ in 500 ml of concentrated $H_2SO_4$.

The indicator consisted of 400 mg bromocresol green and 40 mg of methyl red in 100 ml of 95% ethanol.

PAGE Solutions

For systems not containing milk caseins as a component, a stock solution of 10% Cyanogum 41 (Fischer) in Tris-HCl buffer, pH 8.9 (4.6 g tris/100 ml made to pH 8.9 with HCl) was utilized. The gels, 5 and 7% were made by taking the proper amount of stock cyanogum 41 solution and adding to it additional Tris-HCl buffer, pH 8.9 to a total of 16 ml. To this was added 20 µl of TEMED, (N, N, N', N' tetramethyl-enediamine, Bio-Rad Laboratories). To initiate polymerization, 0.1 ml of a 5% solution of ammonium persulfate was added. The samples containing casein were examined in 7 M urea gels. The gel buffer (Tris-HCl, mentioned previously) was made to contain 7 M urea (42 g/100 ml of stock solution). The gels, 5 and 7% were prepared and run as before. The running buffer was prepared by adding 11.2 g tris and 57.6 g glycine to 500 ml of deionized distilled water and adjusting the pH to 8.3 with 2M glycine.

Malik-Berrie Staining Procedure

Gels stained with this procedure did not require any destaining. The position of the tracking dye remained visible after staining. Equal volumes of 0.2% Coomassie blue G 250 and 2N $H_2SO_4$ were mixed together. After standing overnight in the dark the solution was filtered. Ten normal KOH was added until the clear brown solution turned dark

purple-blue. This required approximately 10 ml of 10 N KOH per 90 ml of clear brown solution. The purple-blue solution was then made 12% in TCA.

Ninhydrin Solution

Four hundred mg of stannous chloride dehydrate were dissolved in 250 ml of 0.2M citrate buffer at pH 5.0. The above solution was added to 250 ml methyl cellosolve (ethylene glycol monomethyl ether) containing 10 g of dissolved ninhydrin (Calbiochem). The solution was flushed with nitrogen and stored in a brown glass bottle at $4^{o}C$.

Acid Hydrolysis

Acid hydrolysis was performed by adding 5 ml of 6 N HCl to the dried sample and freezing the mixture in a dry ice-ethanol bath. The ampoules were evacuated, allowed to melt under vacuum to remove gasses, refrozen, sealed with a propane torch and placed in an oil bath maintained at $110^{o}C$. After 22 hours the ampoules were removed and allowed to cool to room temperature.

Statistical Procedures Used in the Evaluation of Protein Quality

2-factor analysis of variance

| | degrees of freedom (df) | Summ. |
|---|---|---|
| Treatments | 23 | $SS_t = (\text{trt. tot.})^2/9 - CF$ |
| Materials | 2 | $SS_m = (\text{mat. tot.})^2/2 - CF$ |
| T X M | 46 | $SS_{tm} = (\text{comb. tot.})^2/3 - CF$ |
| Error | 144 | $SS_e = SS_y - (SS_t + SS_{tm})$ |

$CF - (\text{grand total})^2/216 \quad SS_y = (216\ \text{obsv})^2 - CF$

$MS_t = SS_t/23,\ MS_m = SS_m/2,\ MS_{tm} = 46,\ SS_e/144$

$f = MS_t/MS_e,\ f = MS_m/MS_e,\ f = MS_{tm}/MS_e$

f test for contrast among materials

$f = (\text{mean}_{NFDM} + \text{Mean}_{SI} - 2\ \text{Mean}_{Comb.})^2/6\ (MS_e/3)$

Dunnett's t test for treatment effects

$t = \text{treatment mean}_x - \text{treatment mean}_{control} / \ 2MS_e/3$

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