PROPERTIES OF PROTEINS AND FOOD PRODUCTS FROM MICRONIZED SOYBEANS

A Thesis by

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Submitted to Faculty of Graduate Studies and Research
in partial fulfillment of the requirements
of the degree of Master of Science

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March, 1990.

Suggested Short Title

PROPERTIES OF MICRONIZED SOYBEANS AND SOY PRODUCTS

ABSTRACT

The effect of infra-red heating (micronization) on the composition and textural properties of full-fat soybeans and its product (soy isolate, soymilk and tofu) were investigated. There was little difference in the overall proximate composition between the micronized and processed soybeans. Yield, protein content and textural properties of tofu made from micronized beans using standard procedures ($70^{o}C$ and $CaSO_4.2H_2O$ as coagulating agent) were lower than those of tofu from unprocessed beans; tofu prepared from micronized beans and coagulated at $90^{o}C$ using a mixture of citric acid (0.01M) and calcium sulphate (0.03M) showed improved characteristics. The microstructure of tofu prepared from micronized beans lacked the regularity of honeycomb-like structure as shown by tofu from unprocessed beans.

Functional, biochemical and nutritional properties of the micronized soybeans, soy isolate, soymilk and tofu were studied. The results indicate the following: the digestibility of micronized soybean (84.3%) was higher compared to the unprocessed soybean (76.5%); the available lysine content of soy isolate, soymilk and tofu from micronized soybeans were higher than the corresponding products derived from unprocessed beans; the unprocessed soybean flour displayed maximum foam capacity at pH 9.0 while the micronized soybean flour showed no foam capacity at pH 3.0 and 5.0; polyacrylamide-disc gel electrophoresis showed that heat treatment by micronization had little effect on the protein constitution of the soybean and on the protein-carbohydrate interaction but induced some interactions of protein and lipid components in the soybeans.

RESUME

L'effet de chauffage à l'infra-rouge (micronisation) sur la composition et les propriétés de la texture du soya entier ainsi que sur ses produits transformés (le soya concentré, le lait de soya et le tofu) a été étudié. Les résultats tendent à montrer qu'il y a peu de différences entre la composition de soya micronisé et celle du soya transformé par d'autres méthodes. Le rendement, le contenu en protéine et les propriétés de la texture du tofu fabriqué à partir du soya micronisé en utilisant les méthodes normales (70°C et le $CaSO_4.2H_2O$, utilisé comme un agent de coagulation) ont été plus faibles que ceux obtenus pour le tofu fabriqué à partir du soya non-traité. Le tofu, préparé à partir du soya micronisé et coagulé à 90°C en utilisant le mélange d'acide citrique (0,01 M) et le sulphate de calcium (0,03 M) a montré une amélioration de ses caractères rhéologiques.

Les propriétés fonctionelles, biochimiques et nutritionelles du soya micronisé, du soya concentré, du lait de soya et du tofu ont été aussi étudiées. Les résultats tendent à montrer que la digestibilité du soya micronisé (84,3%) est supérieure à celle du soya non-traité (76,5%). D'autre part, le contenu en lysine disponible du soya concentré et du tofu (préparé à parir du soya micronisé) a été plus élevé que celui des produits transformés (obtenus à partir du soya non-traité). La farine du soya non-traité a montré une capacité maximale pour la formation d'une mousse à pH 9 tandis que la farine du soya micronisé n'a pas pu montré une telle capacité à pH 3 et à pH 5. L'électrophorèse en gel de polyacrylamide a montré que le traitement thermique du soya à l'infra-rouge a peu d'effet sur la composition des protéines et sur l'intéraction entre les protéines et les glucides. Cependant, un tel traitement peut induire quelques intéractions entre les composants protéiques et lipidiques du soya.

ACKNOWLEDGEMENTS

I would like especially to thank Dr. Inteaz Alli for his guidance and assistance throughout the study. His encouragement, patience and understanding were very much appreciated.

I would like to thank Dr. S. Kermasha for his contribution towards my research.

I wish to thank Manuela Benetti for her assistance and Mrs. Farida Alli for her support.

I would like to thank my fellow graduate students and friends for their constant encouragement and support, there are too many to whom I am grateful, for me to name.

I would like also to thank my family especially both my parents. I am certain that at times they felt my studies would never end; they were always in my thoughts throughout the course of this study.

I would like to acknowledge the financial assistance from the Brunei Government, and the Officials of the Brunei Agricultural Department for granting me study leave.

I wish to convey special thanks to a special friend, Tony DiLollo.

Finally, I would like to dedicate this thesis especially to my late sisters, Anidah and Norainah, and late brother, Rosmadi with warm remembrance always.

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A: INTRODUCTION

Soybean is valued for its high protein and when properly processed, is a source of protein of good quality (Wang and Cavins, 1989). In the orient, soybeans have contributed an important part of the human diet for centuries (Norman, 1978; Coppock, 1974; Wolf and Cowan, 1971), mainly as soymilk and associated products (e.g., curd and cheese) and as fermented products (e.g., soy sauce, miso, natto, and tempeh) (Wolf and Cowan, 1971). Before 1960, China was both a major producer and exporter of soybeans (Schmidt, 1976).

In the Western world, significant soybean production has occurred only since the 1960s (Coppock, 1974; Wolf and Cowan, 1971), and the variety normally cultivated is *Glycine max* (Norman, 1978; Coppock, 1974). Commercial processing of soybeans was established in the U.S. in 1922 (Smith and Circle, 1978), initially as a source of inexpensive edible oil, with lipids being the most important commercial constituents of the bean (Orthoefer, 1978). During these early days, soybean protein was only a byproduct of relatively little economic value, although used as cattle feed and fertilizer (Wolf, 1976; Wolf and Cowan, 1971). The processing of defatted soybean meal into edible protein products for human food came later, and today soybeans are major source of food protein. The demand for oil and meal has increased enormously with increasing world population, and currently the U.S. dominates the soybean and soy oil export trade (Schmidt, 1976).

There was little doubt that widespread utilization of soy protein products offer important nutritional and economic advantages (MacLeod and Ames, 1988) However, there are certain problems associated with the using of soybean as human food. The first of these involves the presence in the bean, of various antinutritive factors, such as phytic acid, protease inhibitors, antivitamin compounds, hemagglutinins, flatus factors, saponins, goitrogens, lysinoalanine, and allergenic

factors (Rackis, 1981a,b, 1978; Jaffe, 1981; Liener, 1980), where some of these are heat-stable, others are heat-labile and can be removed by heat processing (Rackis, 1981a).

Heat treatment has been shown (Wolf and Cowan, 1971) to be effective for the elimination or reduction of antitrypsin factor, "beany flavor" components, and hemagglutinins. However, careful control of the thermal processing conditions is essential to prevent functional and nutritional changes which result from excessive heat treatment of the protein (Kakade *et al.*, 1972). Excessive heat can result in loss of heat-sensitive nutrients such as lysine, cystine, methionine and thiamine (Kouzeh-Kanani *et al.*, 1981). Oxidative stability is impaired due to the heat induced destruction of natural antioxidants present in soybeans. Furthermore, heat treatment results in off-colour and off-flavour (Kouzeh-Kanani *et al.*, 1981).

In addition to the conventional moist heating process (Smith and Circle, 1972), other traditional methods for heat treatment of soybeans include immersion cooking (Albercht *et al.*, 1967), dry heating or roasting (Cowan, 1979), extrusion (Bookwalter *et al.*, 1971), dielectric heating (Borchers *et al.*, 1972), microwave processing (Wing and Alexander, 1975), and infrared (micronization) cooking (Livingston, 1977).

Micronization (infrared heating) is a relatively recent technology that is applied to the processing of full-fat soybeans. The term "micronization" has often been used to refer to a continuous process of heat treatment of cereals, pulses, oilseeds, etc. which is based on relatively short-time processing by infrared radiation (Livingston, 1977). The present study was aimed at evaluating the effects of processing by micronization (infrared heating) on the properties of (a) protein and (b) food products derived from micronized full-fat soybeans. The specific objectives were as follows:

1. To determine the effects of micronization on the composition of soybeans, and on soy isolate, soymilk and tofu prepared from micronized soybean.
2. To determine the effects of micronization on the composition and textural properties of tofu prepared from micronized soybean.
3. To investigate the effects of various coagulation conditions on the composition and textural properties of the tofu prepared from micronized soybean.
4. To study the effects of micronization on the biochemical properties of protein, and on protein-lipid, and protein-carbohydrate interactions.
5. To study the effects of micronization on nutritional and functional properties of soybean, and of soy protein isolate, soymilk and tofu prepared from micronized soybean.

B: LITERATURE REVIEW

1.0: Composition of soybeans

The major components of soybeans are proteins and lipids. Carbohydrates, ash and fibre also make up a significant proportion of the beans. Table 1 gives the chemical composition of soybeans.

Genetic factors and climate have been shown to influence the chemical composition of soybeans. DeMan *et al.* (1975) in a study of the proximate composition of 55 soybean varieties grown in Ontario, reported that protein contents in the samples tested ranged from 30.3% to 46.13% with a mean of 40.84%; Fat contents ranged from 14.46% to 21.26% with a mean of 17.18%; and the moisture content ranged from 4.21% to 7.8% with a mean value of 5.0%. Smith *et al.* (1960) in a comparative study, found that Japanese beans contained protein contents higher than that of the American varieties.

Carter and Hopper (1942) reported that, in addition to varietal differences, geographical location also affected the protein, lipid, and mineral contents. These workers also reported a significant climate effect and noted that climate was more important than location in determining some aspects of soybean composition. Other variables which have been shown to influence soybean composition are agricultural practices, crop year, and planting date (Beatty *et al.*, 1982; Krivoruchco *et al.*, 1979; Taira *et al.*, 1977).

2.0: Traditional and current ways of utilization and processing soybeans.

Soybeans was first cultivated in China, where, it has been suggested (Watanabe and Kishi, 1984) it has been employed as a food for more than five thousand years (Watanabe and Kishi, 1984). The Chinese have developed a wide variety of processed soybean foods which continue to be important in their diets today. These foods can be divided into two broad classes: (1) foods originating with

soymilk but are not fermented (eg., tofu and processed versions of it); and (2) fermented foods (eg., miso, soy sauce, and fermented tofu).

In Japan, in addition to these traditional ways of preparation, soybeans are used in kinako (roasted soy flour), dried frozen tofu, and natto, which are distinctively Japanese products (Watanabe and Kishi, 1984). Previously, in both Japan and China, defatted soybeans remaining after oil extraction were used as fertilizer and animal feed and later in the production of soy sauce (Watanabe and Kishi, 1984). In addition, defatted soybeans are employed in the production of miso, tofu, and amino acid mixtures (Wolf and Cowan, 1971). More recently, defatted soybeans and derived proteins have been used widely in the manufacture of simulated processed meat and fish products (Watanabe and Kishi, 1984).

Indonesians, like the Chinese, Japanese, and Koreans, traditionally eat soybean foods, especially tempeh and Chinese-type soybean foods (Watanabe and Kishi, 1984).

In the Western world, food uses of soybeans have followed a different pattern from those of the orient. Watanabe and Kishi (1984) reported that of the 62 million tons of soybeans grown in the United States in 1982, 40 million was used for the production of edible oil. A large proportion of the remainder was exported. Only a relatively small proportion was used domestically in the whole bean form for foods. This report also indicated that more than 80% of the 32 million tons of defatted soybean remaining after oil production was used domestically. Although most of the defatted beans was used for animal feed, a small quantity was used as a source of protein food ingredients.

The traditional soy protein foods are divided into two groups: fermented and nonfermented. Fermented foods are soy sauce, miso, natto, sufu, and tempeh; nonfermented products are soymilk, tofu and its derived products, kori-tofu, yuba, kinako and moyushi.

2.1: Nonfermented foods

Bean curd or fresh tofu is the major soybean food in Japan. It is prepared by water soaking, grinding, and cooking beans and filtering off the insoluble residue to obtain soymilk. A protein-oil curd is then precipitated by adding c gulant such as calcium sulfate to the warm milk. After the supernatant or whey is removed the curd is carefully washed and sliced (Wolf and Cowan, 1971). In general, tofu contains 88% moisture, 6% protein and 3.5% oil and its curd is very fragile and perishable (Fukushima, 1981).

Dried (Kori) tofu is made from soymilk by precipitating the curd with calcium chloride to form a hard and grainy tofu. After removing the whey, the curd is ground, shaped, washed and sliced. The slices are rapidly frozen at -10^{O}C and then aged at -1^{O} to -2^{O}C for 2 to 3 weeks. During aging the tofu is thawed, dried and packaged (Wolf and Cowan, 1971). Some types of tofu are treated with gaseous ammonia before packaging (Fukushima, 1981; Wolf and Cowan, 1971). Ammonia treatment improves the textural characteristics when the product is subsequently cooked (Wolf and Cowan, 1971).

Soymilk a popular drink in China is not widely used in Japan because of the presence of eg., green beany, rancid and/or throat-catching chalky flavor (Fukushima, 1981). The techniques to produce soymilk have improved and packaged soymilk has appeared on the market in Japan (Fukushima, 1981). A hot grinding method (Wilkens *et al.*, 1967) for soymilk manufacturing has also been introduced.

"Yuba" is another unique, traditional soymilk product. In yuba-making, soymilk is heated at a temperature just below the boiling point in a flat pan. The coagulated film formed on the surface of soymilk is scooped up successively by a fine stick and dried at room temperature. The resulting yuba contains 52-53%

protein, 24% fat, 12% soluble carbohydrate, 8-9% moisture and 3% ash (Fukushima, 1981).

Kinako is a nonfermented soy product made by roasting soybeans, dehulling, and then grinding. It is used as a cake base, and when mixed with sugar it is used on baked rice cakes (Wolf and Cowan, 1971).

2.2: Fermented foods

The three most important fermented soybean products are miso and soy sauce which originated in China, and natto, which originated in Japan (Watanabe and Kishi, 1984). These products result from metabolic and fermentative activity of microorganisms (Watanabe and Kishi, 1984).

Soy sauce made by fermentation and acid hydrolysis is well known in the U.S., however, the traditional Japanese product is made by fermentation and has the characteristic flavor associated with fermented soy sauce (Wolf and Cowan, 1971). Cooked defatted soybean flakes are mixed with roasted wheat and inoculated with *Aspergillus oryzae*. After an initial growth phase of 45 to 65 h, salt solution is added and the fermentation proceeds for 8 to 12 months. The liquid phase is then separated from the insoluble residue, pasteurized, filtered, and bottled (Wolf and Cowan, 1971).

Miso is also a fermentation product of soybeans and cereals in the presence of salt (Fukushima, 1981). It is made by cooking soybeans followed by blending with koji (steamed rice covered with a growth of *Aspergillus oryzae*), salt, and water and then inoculating with a yeast. The mixture is then fermented for several months to a paste-like consistency (Fukushima, 1981; Wolf and Cowan, 1971). Miso is used to flavor soups and vegetables (Wolf and Cowan, 1971).

Natto is another popular fermented soybean product in Japan and is made by inoculating cooked soybeans with *Bacillus natto* followed by incubation for 14 to 40

h at 40°C (Wolf and Cowan, 1971). Fermentation covers the cooked beans with viscous substances which forms long threads when the product is pulled apart (Fukushima, 1981; Wolf and Cowan, 1971). Natto is eaten with cooked rice seasoned with soy sauce or salt.

Another important fermented soy protein food is tempeh. It is used in Indonesia rather than in Japan (Wolf and Cowan, 1971). Soybeans are soaked overnight, dehulled, cooked, and mixed with a previous batch of tempeh. After an incubation period of 24 h or less, *Rhizopus oligosporous* and related organisms grow and bind the fermented beans into a cake like mass in which individual soybean cotyledons can still be readily detected (Watanabe and Kishi, 1984; Fukushima, 1981; Wolf and Cowan, 1971). The final product is sliced and then fried. It serves as a main dish rather than as a flavoring agent for other foods (Wolf and Cowan, 1971).

3.0: Properties and utilization of soy protein

Technological advances have made it possible to have soybean protein available for use in various forms: as whole seeds and flours, as protein concentrates and as protein isolates. These products differ in composition as well as in functional properties (Table 1). However, amino acid patterns on a protein content basis are essentially the same (Bressani, 1981).

Table 1. Chemical composition of soy protein products (g/100g)[a]

Product	Protein[b]	Fat	Fiber	Ash	Carbohydrate (Total)
Whole soybeans	41.0	20.0	2.3	5.4	31.3
Soy flours (defatted)	50.5	1.5	3.2	5.8	34.2
Soy flours (full-fat)	41.0	20.5	2.8	5.3	25.2
Concentrates	70.0	1.0	4.5	5.0	19.5
Isolates	96.0	0.1	0.1	3.5	0.3

[a]Source: Bressani (1981)
[b]Protein (%N x 6.25)

3.1: Processing of soybeans

The production of flours from soybeans involves the removal of hulls to produce a full-fat soy flour (~41% protein) or removal of both hull and oil fractions to produce a defatted flour (~50% protein) (Mustakas, 1971). Steps in the manufacture of both full-fat and defatted soy flours by procedures conventionally used in oil extraction plants, are shown in Figure 1. Precise control of the degree of heat treatment of the defatted soy flakes during the desolventizing process and during subsequent steps is critical to both the nutritive value and the functionality of the defatted meal (Kellor, 1974).

Soy concentrates (~70% protein) are generally prepared from defatted soy flakes or flour by removing the oligosaccharides, part of the ash and some of the minor components in one of the following three initial precipitation processes (Figure 2): (a) use of a 60% to 80% aqueous alcohol leach, (b) use of a dilute acid leach, or (c) use of moist heat followed by a water leach (Wolf, 1970). The concentrate is dried at pH 7 in case of a precipitation with alcohol or use of the

water leach, or neutralized before drying if acid precipitation is employed. The manufacture of isolated soy protein (~90% protein) requires a separate procedure which involves removing the water insoluble polysaccharides as well as the oligosaccharides and other low molecular weight components. The protein can be neutralized before drying to yield a proteinate which is water soluble, unlike isoelectric protein (Barraquio and van de Voort, 1988).

Numerous workers have reported on procedures for heating full-fat soybeans for use in chick diets. The methods include steam cooking (Rogler and Carrick, 1961), extruding (White *et al.*, 1967), infrared cooking (Featherston and Rogler, 1966) and microwave radiations (Wing and Alexander, 1975). White *et al.* (1967) reported that extrusion process produced beans with nutritional values approaching those of commercially processed soybean meal. Hull *et al.* (1968) found that extruded soybeans supported chick growth equal or superior to rations containing extracted soybean meal with soybean oil added, while infrared processing was found to produce inferior results when compared to the commercial meal. Arnold *et al.* (1971) using a still air oven, produced soybean that promoted weight gains and feed efficiencies when compared to commercial soybean meal. Wing and Alexander (1975) reported that microwave processing yielded beans of high nutritive quality, which compared favorably with beans processed by conventional heating techniques (direct steam-heat treatment).

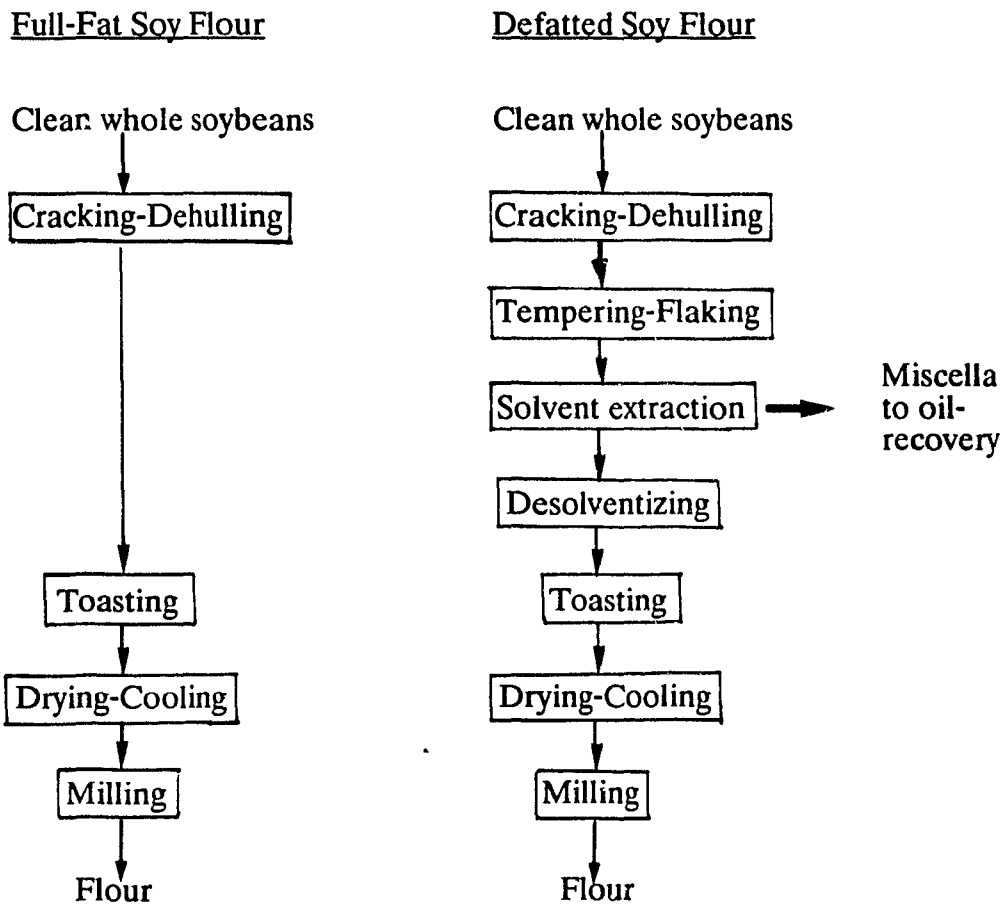


Figure 1. **Production of full-fat and defatted soy flours by conventional processes. (Mustakas, 1971).**

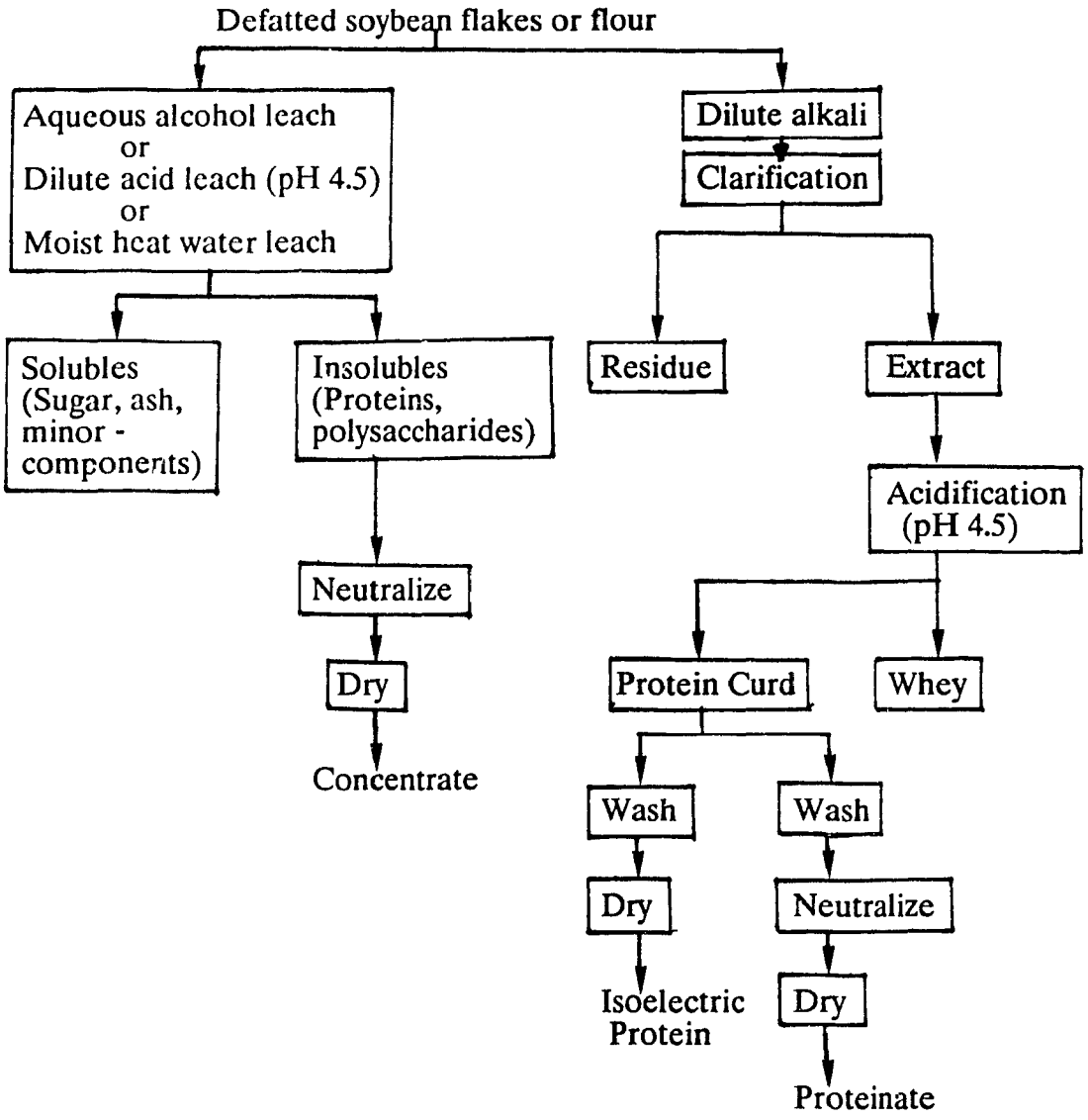


Figure 2. Scheme for soy concentrate and isolate production. (Wolf, 1970).

3.2: Utilization

Waggle and Kollar (1979) and Dobois and Hoover (1981) have reviewed the use of soy protein in cereal grain products. According to the latter authors the greatest usage of soy protein by the bakery industry is as components of other ingredients, such as milk replacers. Defatted soy flours are commonly used as ingredients in the replacers but concentrates and isolates may also be used (Pearson, 1983).

Soy protein is also widely used in dairy whiteners and artificial creams, either sour or sweet (Pearson, 1983). Isolated soy proteins are used in yogurt, artificial cheese and cheese dips (Pearson, 1983). Isolated soy proteins and soy concentrates are also used in snack foods and cereals (Waggle and Kollar. 1979); their use in beverages, soups and a variety of other products has been discussed by a number of workers (Morales *et al.*, 1981; Pereira and De Campos, 1981; Waggle and Kollar, 1979).

Incorporation of soy proteins in ground meat and sausage products has been singularly successful in expanding consumption of soy proteins without any serious effects upon acceptability (Pearson, 1983). Waggle and Kollar (1979) and Waggle *et al.* (1981) have reviewed the functional and nutritional advantages of adding soy proteins to ground meat and sausages but have indicated the importance of limiting the levels used so as not to impair the acceptability of the extended meat, poultry and fish products.

3.3: Physico-chemical properties

On the basis of sedimentation rates, soy proteins can be fractionated into 2s, 7s, 11s, and 15s fractions (Table 2), with the major fractions being the 7s globulin (β- andγ-conglycinin) and the 11s globulin (glycinin). The 2s fraction of soybean protein (α- conglycinin) includes cytochrome C and the group of trypsin inhibitors (Wolf, 1969). Catsimpoolas and Eckenstain (1969) have shown thatα-,β-, andγ-conglycinin

and glycinin are antigenically distinct proteins. The 7s globulin fraction accounts for 50% of the total 7s protein or about 18% of the total soy proteins (Wolf and Sly, 1967). Koshiyama and Fukushima (1976) identified β-conglycinin (27.9%) as the major component while γ-conglycinin was only 3% of 7s globulin. Approximately 80% of soy proteins have molecular weight greater than 100,000 implying a high degree of structural order.

Table 2. Proportions and components of ultracentrifuge fractions of water extractable soy proteins[a].

Soy protein fraction	% of total protein	Protein component	Molecular Weight
2s	22	Trypsin inhibitors Cytochrome C	8,000 - 21,500 12,000
7s	37	Hemaglutinin lipoxygenase Beta amylase 7s globulin	110,000 102,000 61,700 180,000 - 210,000
11s	31	11s globulin	350,000
15s	11		600,000

[a]Source: Wolf, 1970

β-conglycinin, a major 7s protein, exists in monomeric (7s) and dimeric (9s) forms at 0.5M and 0.1M ionic strength, respectively (Ibuchi and Imahori, 1978a,b). The original 7s fraction is a glycoprotein (Koshiyama, 1969) and contains carbohydrate with one unit attached to the aspartic acid residue at the N-terminal end of the molecule (Yamauchi *et al.*,1975). The carbohydrate moiety consists of 38 mannose and 12 glucosamine residues per molecule of protein. The molecular weight of the 7s form is in the range of 150,000 to 175,000 daltons and that of 9s

weight of the 7s form is in the range of 150,000 to 175,000 daltons and that of 9s form is approximately 370,000 daltons (Thanh and Shibasaki, 1978). The 7s form is composed of three subunits (α,α' andβ) which interact to produce six isomeric forms (B_1 to B_6) with varying properties.

The 7s protein fraction is especially low in sulfur-containing amino acids. It contributes only 1/7 of the total methionine of soy protein (Roberts and Briggs, 1965). It also contains relatively few sulfhydryl groups. This scarcity of sulfhydryl groups probably accounts for its greater heat sensitivity compared to the 11s fraction (Hashizume *et al.*, 1975). No intra-chain disulfide bonds were found in 7s globulin (Thanh and Shibasaki, 1977). Hoshi *et al.* (1982) reported the presence of sulfhydryl groups in 7s globulin but noted that they were located at the interior of the molecule with limited potential for disulfide interchange reactions.

The 11s globulin (glycinin) is made up of 6 acidic and 6 basic subunits, and has a molecular weight of 302,000 to 375,000 daltons (Utsumi *et al.*, 1981; Kitamura *et al.*, 1976; Badley *et al.*, 1975). Kitamura *et al.* (1976) reported that the acidic and basic subunits are linked together in specific combinations through disulfide bridges resulting in the formation of intermediary subunits.

Glycinin contains more than twice the quantity of methionine in conglycinin although the amount relative to proteins from other sources is still low (Fukushima, 1968). Similarly, the number of half cystine residues found in glycinin is more than twice that found in the 7s globulin (Fukushima, 1968). Catsimpoolas *et al.* (1969) found 48 moles of half cystine residues per mole of protein in the 11s globulin. Moreira *et al.* (1979) also found large amounts of glutamate and aspartate in glycinin.

Protein-protein interactions occur in soy proteins with both the 7s and 11s proteins capable of forming intermolecular disulfide bonds leading to insolubility, turbidity, and increased viscosity (Kilara and Sharkasi, 1986; Nash and Wolf, 1967;

Circle *et al.*, 1964; Briggs and Wolf, 1957). Both 7s and 11s proteins have complex quarternary structure and exhibit different degrees of ionic strength-dependent association-dissociation reactions (Kilara and Sharkasi, 1986). They have low content of α-helix (Hermansson, 1978) and are primarily made up of antiparallel β-structure and disordered regions (Fukushima, 1968). A 15s protein often accompanies purified 11s as a minor component of low electrophoretic mobility (Derbyshire *et al.*, 1976). The association of glycinin via disulfide bridges during isolation may explain the occurrence of the 15s form and the presence of the 18s fraction could be due to the association of a 7s globulin, which usually a major constituent of species having the 18s component (Kilara and Sharkasi, 1986).

4.0: Preparation and properties of soymilk and tofu

4.1: Soymilk preparation

Preparation of tofu involves first, the preparation of soymilk followed by the coagulation of proteins in the soymilk by adding the coagulant. A traditional method of tofu preparation is described by Shurtleff and Aoyagi (1984) and is outlined in Figure 3.

Dry soybean is rinsed and soaked overnight in water. The soaking process not only facilitates grinding but enables better dispersion and suspension of soybean solids during subsequent extraction. The rate of water absorption by soybean is related to the temperature of the water. Wang *et al.* (1979) found that the rate of water absorption increased with increasing hydration temperature. As the temperature increased from 20^{0} to 37^{O}C, the time required for maximum hydration decreased from 5.5 h to 2.5 h. Johnson and Snyder (1978) reported a time of 14 h for maximum water absorption at 4^{O}C compared to 9 and 2 h for absorption at 21^{O} and 100^{O}C respectively. Watanabe *et al.* (1964) found that soaking time at 70^{O}F and at 50^{O}F were 10 h and 18 h, respectively.

Loss of solids is associated with the soaking step in soymilk preparation. A 5% loss of solids occurred after 24 h at 1°C, the loss increased to 10% after 72 h (Lo *et al.*, 1968a). The solids lost were carbohydrate (76.4%) and protein (23.6%), the loss of protein was reported to occur during the initial 24 h period. Wang *et al.* (1979) found increased losses at elevated hydration temperatures and increase in the ratio of protein lost to total solids lost with increasing temperature.

After an overnight soaking period the soybean is drained and ground with water to extract proteins. Water is added to hydrated beans to give a water to dry bean ratio of 10:1 (Wang, 1981; Bourne *et al.*, 1976; Lo *et al.*, 1968b; Smith and Nash, 1961). Beddows and Wang (1987a) reported that this traditional 10:1 water:bean ratio gave not only the best protein yield but also a tofu that has a lower bulk due to less water being held.

A decrease in protein extractability is associated with high temperatures of grinding (Johnson and Snyder, 1978). Yoshino *et al.* (1982) found a decrease of water soluble nitrogen with long storage times. Saio and Arisaka (1978) reported similar results and also found a higher ratio of 7s to 11s protein fractions with longer storage times.

Wilkens *et al.* (1967b) found that a "hot grind" technique produced soymilk having a more bland flavor than that produced by some other traditional methods. Lo *et al.* (1968b) also reported improved flavor with the use of high grinding temperatures but noted substantial reductions in yield at temperatures greater than 85°C. Johnson and Snyder (1978) compared the effectiveness of a 30 min blanch at 100°C before a 20° to 25°C grind, with no blanching step. The blanching process resulted in a very low protein recovery although the product had a very bland flavor. Disruption of protein bodies before extensive heating allowed greater release of protein.

The grinding step is followed by heating to a boil then holding at this temperature for 10 to 20 min (Schaefer, 1986). Cooking of the slurry using proper temperature/time combination inactivates trypsin inhibitors and could facilitate extraction of soymilk from the slurry (Sing-Wood, 1984). Watanabe *et al.* (1964) reported that cooking of the slurry at 100^{o}C for about 7 to 14 min resulted in the desired recovery of soymilk solids, soymilk protein, and tofu solids. Saio *et al.* (1973) found that heating the slurry at 60^{o}-80^{o}C for 30 min resulted in higher solids recovery in soymilk than heating at 100^{o}C for 30 min or without heating. Watanabe *et al.* (1964) reported that soymilk made from a slurry cooked at 120^{o}C required more coagulant for protein precipitation than that cooked at 100^{o}C. It was also reported that tofu made from slurry cooked at 120^{o}C lacked cohesiveness. Heating above 130^{o}C caused off-flavor in soymilk and tofu, and resulted in undesirably soft curds (Watanabe *et al.*, 1964).

After cooking, the hot slurry is filtered to remove the insoluble material (okara) from the proteinaceous liquid which is the soymilk.

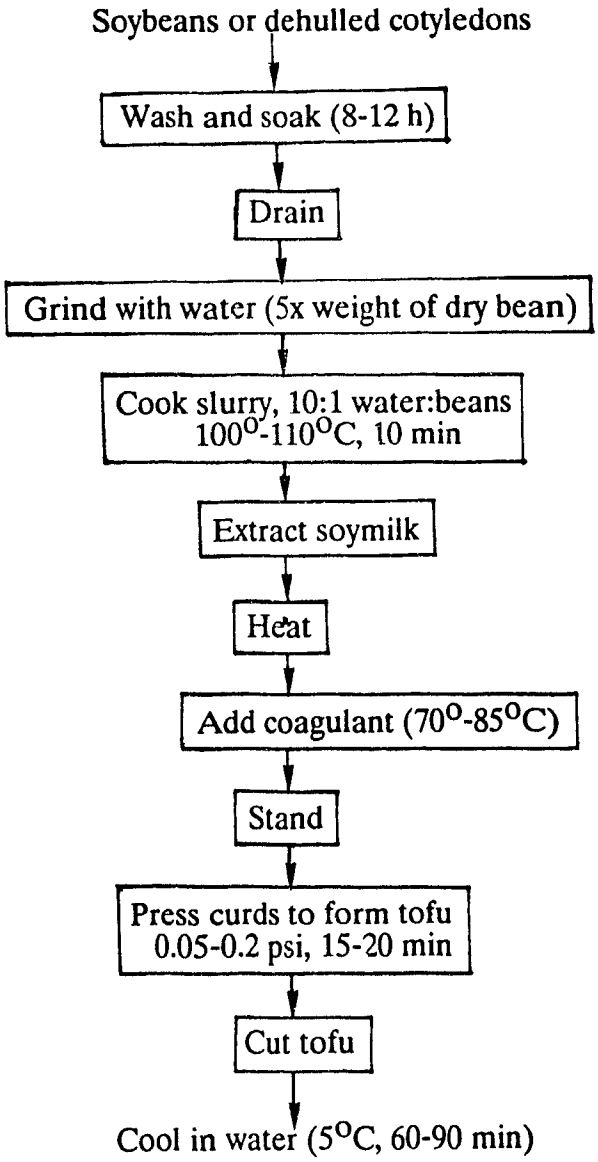


Figure 3. **Procedure of traditional regular tofu manufacturing (Japanese). (Shurtleff and Aoyagi, 1984).**

4.2: Tofu preparation

Proteins are precipitated from hot soymilk by the addition of a coagulant. Watanabe *et al.* (1964) investigated the effect of temperature of soymilk on solids yield, volume yield and firmness of tofu and showed that the solids and volume yield of tofu increased slightly when cooking temperatures was increased from 60° to 100°C. The tofu firmness was low at cooking temperatures ranging from 60° to 80°C, but was increased markedly at temperatures from 80° to 100°C.

Various coagulants have been used and may be classified into three general types: 1) chloride or nigari types, 2) sulfate types, and 3) acid types. Chloride or nigari coagulants included sea water, natural nigari, calcium chloride and ferric chloride. Nigari, the most traditional coagulant, is prepared from dehydrated sea water with sodium chloride removed and is composed primarily of magnesium chloride. Chloride types seem to be faster reacting than sulfate coagulants (Saio, 1979), sulfate type coagulants include magnesium sulfate and calcium sulfate which is widely used commercially (Lu *et al.*, 1980). The primary acid type of coagulant is glucono delta lactone (GDL) used in the manufacture of silken tofu, a very soft tofu product which is not pressed. Acetic acid (Lu *et al.*, 1980; Kawaguchi, 1979; Chiu, 1960) and lemon juice (Pontecorvo and Bourne, 1978) may also be used in tofu preparation at the domestic scale.

Several other coagulants have been used with varying degrees of success. Tsai *et al.* (1981) reported that calcium acetate and calcium gluconate failed to produce a curd. Kamel and DeMan (1982) obtained a curd with calcium acetate, but found no coagulation using calcium citrate, calcium hydroxide, calcium carbonate and calcium hydrogen phosphate.

The concentration of coagulant affects curd formation. DeMan *et al.* (1986) reported that the minimum concentration required for coagulation were 0.5% calcium sulphate, 0.15% calcium chloride, 0.3% magnesium sulphate, 0.2%

magnesium chloride and 0.37% GDL. Wang (1981) obtained curd formation using 0.008M to 0.080M calcium sulphate, calcium chloride, magnesium sulfate, and magnesium chloride. No curd was obtained at concentration below 0.006M and above 0.1M. Saio (1979) reported that the optimum calcium concentration for coagulation was 0.02N. Beddows and Wong (1987b) found that 9-10mM calcium sulphate gave tofu with smooth texture and maximum contents of protein and solids.

The quantity of coagulant required is related to the type of coagulant (DeMan *et al.*, 1986; Kamel and DeMan, 1982; Wang and Hesseltine. 1982). Minimum coagulants levels necessary for coagulation were 0.10% for a chloride coagulant, 0.25% for sulphate coagulants, and 0.20% for an acetate coagulant (Kamel and DeMan, 1982).

Lu *et al.* (1980) compared the uses of calcium chloride, calcium acetate, acetic acid, calcium lactate, calcium sulfate and glucono delta lactone and showed that soybean curds prepared with calcium salts had a higher weight yield than those with non-calcium compounds. In addition curds prepared with calcium chloride, calcium acetate and acetic acid were softer than those from the remaining coagulants. These researchers pointed out that the reason for the softer curds was related to the smaller quantity of coagulant used.

Lu *et al.* (1980) also investigated the effect of pH on coagulation of soymilk by acetic acid, GDL, and the calcium salts of the following anions: sulfate, carbonate, acetate, gluconate, chloride, and lactate. Precipitation occurred when a sufficient amount of coagulant was added to cause a decrease in pH to approximately 6.0 from an original soymilk pH of 6.4. These authors concluded that pH, and not coagulant concentration, was the important consideration in soy curd formation.

Soymilk can be coagulated over a range of temperatures. Wang (1981) reported that the optimum temperature for tofu curd formation is between 60^{o} and

70°C. A change in coagulation temperature affects moisture content and texture of tofu; higher temperatures result in a decrease in moisture and a firmer product (Wang and Hesseltine, 1982). Beddows and Wong (1987b) reported that the net yield of tofu decreased with increase in temperature over the range 50°-90°C and that tofu produced below 70°C was soft and watery. In the range 70°-80°C, the texture was very desirable; above this temperature the texture was unacceptable.

After the addition of coagulant to form the tofu curds, a short settling period is required for the completion of curd formation. The curd is then transferred to a cloth-lined perforated pressing box, pressed and the whey is allowed to drain, usually for a period lasting from 20 min to 1 h. The weight used for pressing and the time of pressing affects the texture of tofu (Schaefer, 1986). Schroeder and Jackson (1972) found that a pressure of 1 psi resulted in a smooth, rubbery texture while lower pressures resulted in a pastelike consistency.

4.3: Soymilk composition

The composition of soymilk has been determined by several workers. DeMan *et al.* (1975) reported on the composition of soymilk prepared from 55 soybean varieties. Average moisture, protein and lipid contents were 94.02%, 2.71% and 1.24%, respectively. Wang and Cavins (1989) reported that protein contents of the soymilk from three varieties of beans samples tested ranged from 3.1% to 3.5%, fat contents ranged from 1.5% to 1.8%, and the moisture contents ranged from 93.2% to 93.4%. Shurtleff and Aoyagi (1975) reported the following values for moisture, protein, fat, and ash contents, respectively: 90.8%, 3.6%, 2.0%, and 0.5%.

Investigations on the effect of various lengths of soybean hydration on soymilk composition showed that soaking beans for up to 72 hours did not have a significant effect on the gross composition of the soymilk (Lo *et al.* 1968a).

There are relatively few reports on the mineral composition of soymilk (Schaefer, 1986). Chang and Murray (1949) found that on a dry basis, calcium, iron, and phosphorus in soymilk were 0.195%, 0.0072%, and 0.65%, respectively; on a moist basis (5.98% moisture), calcium, phosphoric, and magnesium concentrations in soymilk were 0.10, 0.53 and 0.20 mg/g, respectively. The recoveries of the bean calcium, phosphorus and magnesium in the soymilk were 62%, 82% and 72%, respectively (DeMan *et al.*, 1975).

Soymilk has been shown (Del Valle, 1981) to contain higher levels of lysine and aromatic amino acids but lower levels of threonine, tryptophan, leucine, and valine than the soybeans from which it is prepared. Wang and Cavins (1989) found that the sulfur amino acids were the most limiting amino acids in soymilk. Miller *et al.* (1952) reported that 50% to 90% of the thiamine, 90% of the riboflavin, and 60% to 80% of the niacin found in soybeans was retained in soymilk.

4.4: Tofu composition

Proximate analysis has shown that tofu contains 88% moisture, 6% protein, and 3 to 3.5% lipid (Fukushima, 1981; Kinsella, 1978). Shurtleff and Aoyagi (1975) reported values of 84.9%, 7.8%, 4.3%, and 0.7% for moisture, protein, lipid, and ash, respectively. Chang and Murray (1949) reported the following composition of tofu: 76.5% moisture, 15.0% protein, 7.1% lipid, and 0.9% ash. More recently, Wang and Cavins (1989) showed that the composition of tofu prepared from three soybean varieties were 84.9-87.3% moisture, 6.5-8.8% protein, and 4.1-4.6% lipid.

Tseng *et al.* (1977) reported calcium levels on a wet basis in tofu of approximately 0.2%. Chang and Murray (1949) found calcium concentrations 0.24% and 0.69% (DM basis) when magnesium chloride and calcium chloride, respectively, were used as coagulants. Kantha *et al.* (1983) found 1.64% calcium in tofu (DM basis). Phosphorus levels in tofu have been reported as 0.80% (DM

basis) (Chang and Murray, 1949) and approximately 0.08% (on a wet basis) (Tseng *et al.*, 1977).

Chang and Murray (1949) found 105 ppm iron in tofu (DM basis), while Kantha *et al.* (1983) found a value of 70 ppm. Miller *et al.* (1952) noted that tofu retained from 50% to 60% of the iron found in the soybean. The zinc content of tofu was reported to be 60 ppm (Kantha *et al.*, 1983).

5.0: Nutritional properties of soy proteins

There are numerous reports in the literature dealing with the potential of soybeans for meeting the protein needs of the world (Scrimshaw, 1981; Young *et al.*, 1979; Bressani *et al.*, 1974; Graham and Baertl, 1974).

Soybeans contain approximately 30-45% protein depending on the variety (Smith and Circle, 1977). Soy proteins for human food have been shown to be of relatively high nutritional value when compared to well known plant proteins (Pearson, 1983). Soy proteins contain relatively high levels of lysine (Table 3) which is the most limiting amino acid in cereal-based diets (Liener, 1978).

Table 3. Typical essential amino acid composition of soy protein products (g/100g protein, DM basis)[a]

Amino acid	Soy flour	Soy protein concentrate	Soy protein isolate
Isoleucine	4.7	4.8	4.9
Leucine	7.9	7.8	7.8
Lysine	6.3	6.3	6.4
Methionine	1.4	1.4	1.3
Cystine	1.6	1.6	1.3
Phenylalanine	5.3	5.2	5.4
Tyrosine	3.8	3.9	4.3
Threonine	3.9	4.2	3.6
Tryptophan	1.3	1.5	1.4
Valine	5.1	4.9	4.7

[a]Source: Pearson (1983)

In summarizing the nutritional value of soy proteins for human food, Williams (1970) stated that the most limiting amino acid is methionine, followed by threonine. Table 3 presents the amino acid composition of soy flour, soy protein concentrates and soy protein isolates. Williams (1970) indicated that supplementation of soy isolates with 1.5% of DL-methionine increased the PER from 1.75 to 2.00-2.45. Bradford and Orthoefer (1983) reported that when antinutritional factors such as trypsin inhibitors and lectins in soybeans are reduced or eliminated by heat denaturation during processing of soy meal and supplemented with methionine, the PER value of soy protein concentrates reached values of 2.5 or greater. Similar results were obtained by Meyer (1967) on work on three commercial soy protein concentrates. This researcher reported that when supplemented with 0.15% methionine, the soy protein concentrates gave PER values higher than casein (Table 4).

Table 4. Protein efficiency ratios of commercial soy protein concentrates and casein[a]

Concentrate	PER Methionine Supplementation None	0.15%
A	2.29	3.00
B	2.16	2.88
C	2.36	3.06
Casein	2.50	

[a]Source: Meyer (1967)

The nutritional value of soy proteins has been improved by the formation of mixed disulfide bonds resulting from heating soy flour in the presence of cysteine or

N-acetylcysteine (Friedman *et al.*, 1984). Conventional processing of soy isolate is known to result in the loss of methionine and cysteine/cystine by their transformation to unavailable forms (Chang *et al.*, 1985), up to 10% of the methionine present is oxidized to methionine sulfone resulting in low PER.

The use of PER assays can lead to underestimation of protein quality since less protein is required for growth in rats than maintenance in man (Barraquio and van de Voort, 1988). Furthermore, the rat's requirement for sulphur-containing amino acids and lysine are higher than for humans, suggesting that the PER method is more directly proportional to the quality of the protein (Walker, 1983); therefore caution should be exercised in extrapolating animal data to humans.

The use of soy proteins in combination with other proteins to meet the nutritional needs of human is well documented (Hopkins and Steinke, 1981; Bressani *et al.*, 1979; Bressani, 1977). It has been shown that soy protein can be used to supplement and/or extend meat and fish proteins and to supplement single or mixed vegetable-based protein diets (Hopkins and Steinke, 1981; Bressani, 1981, 1977). Soy protein fortified foods have been shown to be useful in relieving malnutrition among specific segments of the population, such as infants and children (Chavez, 1981; Sgarbieri *et al.*, 1981; Aguilera and Lusas, 1981; Torun, 1981). Mixtures of soy proteins with other food proteins can therefore make a significant contribution to the quality of human diets (Bressani, 1981).

It has been suggested that the essential amino acid requirements in human could be met with soybeans as the only source of proteins (Wilcke *et al.*, 1979; Young *et al.*, 1979; Bressani *et al.*, 1974). The amino acid content of soybeans in comparison to other foods is shown in Table 5.

Table 5. Essential amino acid content (g/16g N) of soybeans, maize, meat and milk[a]

Amino acid	Soybean	Maize	Meat	Milk
Isoleucine	5.37	4.62	5.23	6.51
Leucine	7.71	12.96	8.19	10.02
Lysine	6.32	2.88	8.74	7.94
Phenylalanine	4.94	4.54	4.11	4.94
Tyrosine	3.18	6.11	3.39	5.20
Cystine	1.78	1.29	1.26	0.91
Methionine	1.34	1.85	2.48	2.50
Threonine	3.94	3.98	4.42	4.70
Tryptophan	1.38	0.61	1.17	1.44
Valine	5.25	5.10	5.55	7.01

[a]Source: Bressani (1981)

Bressani (1981) reported that the digestibility of soybean protein by humans is lower than that of animal protein (Table 6). In addition protein isolates and concentrates have a higher digestibility than soybean flour (Bressani, 1981). Hopkins (1980) suggested that with soy flours, the processing conditions or other compounds, which were not present in concentrates or isolates, could be factors responsible for reducing the digestibility.

The beneficial effect of heat treatment on the nutritive value of soy proteins has been the subject of numerous studies. Bressani (1981) pointed out that it is generally believed that the improvement in nutritive value of soy proteins results from inactivation or destruction of antinutritional factors. Smith *et al.* (1964) reported that moist heat is essential for marked improvement in nutritive value of soy proteins, and that steaming under pressure produced this effect at a faster rate than steaming under atmospheric conditions.

Table 6. Protein digestibility of various foods[a]

Protein Source	Digestibility (%)	
	Apparent	True
Whole egg	73-86	93-100
Milk	69-77	90-98
Beef	73-82	91-99
Casein	71-78	94-97
Soy flour	70	75-92
Soy flour (Extruded)	66-79	84-90
Soy protein (Isolated)	81-82	93-97
Soy protein (Spun)	83-88	101-107

[a]Source: Bressani (1981)

In the preparation of soymilk as well as tofu by the traditional method, proteins are separated from the beans. In the first case, water soluble or dispersible proteins are separated from insoluble or nondispersible proteins, which remains in the residue. In the second case, proteins which are precipitated by acid, calcium or magnesium ions are separated. According to Del Valle (1981) this fractionation of proteins resulted in a fractionation of amino acid and this change modified the nutritional value of the proteins separated. The extent of amino acid composition of soybeans and some derived products is shown in Table 7.

Del Valle (1981) showed that the amino acid content of soymilk and curd proteins are similar with soymilk proteins containing somewhat higher levels of lysine, sulfur-containing amino acids, threonine, tryptophan and valine (Table 7).

Table 7. Average amino acid composition (g/16g N) of soybeans and some derived products[a]

Amino Acid	Soybeans	Residue	Soymilk	Tofu	Whey
Aspartic acid	12.61	11.63	11.91	11.70	12.48
Threonine	4.11	4.42	4.01	4.00	4.52
Serine	5.74	5.47	5.19	5.32	4.15
Glutamic acid	19.76	17.71	19.61	19.26	23.62
Proline	5.53	5.66	5.33	5.47	4.87
Glycine	4.46	4.61	4.16	4.14	4.87
Alanine	4.49	4.36	4.14	4.11	4.42
Valine	3.73	5.28	4.88	4.99	2.65
Cystine	0.78	trace	0.03	trace	2.40
Methionine	1.34	1.67	1.59	1.43	2.61
Isoleucine	3.46	4.50	4.66	4.85	2.92
Leucine	7.90	8.31	7.94	8.32	3.89
Tyrosine	3.90	3.74	3.91	3.99	3.39
Phenylalanine	4.85	5.20	5.15	5.41	2.52
Lysine	6.19	6.36	6.08	6.14	8.56
Histidine	2.60	3.07	2.64	2.64	3.21
Arginine	8.64	8.61	8.65	8.52	9.69

[a]Source: Wang and Cavins (1989)

6.0: Functional properties of proteins from soybeans

With increasing world population and increasing demand for food, more use will be made of plant proteins. These proteins must retain esthetic, organoleptic and other functional properties to be useful (Bressani, 1981).

An important attribute of soybean protein is related to the fact that, by controlling processing conditions, soybean products with different functional properties can be prepared for a variety of food applications. These functional properties which include emulsification, fat and water absorption, texture, dough and film formation, adhesion, cohesion and elasticity, foaming, color and flavor control, have been well described (Kinsella, 1979; Wolf, 1970). Some functional properties of different soy protein preparations are shown in Table 8.

The ability of soy proteins to bind off-flavors (Kinsella and Damodaran, 1980) and the marked thermal stability of glycinin (German *et al.*,1982) limit their use in many food systems. The high heat stability is generally believed to be the result of extensive disulfide bridge formation within the major 11s globulin (Kinsella, 1979) and results in its ability to form gels; this is an important functional property for many textural foods such as tofu.

Table 8. Some functional capacities of different soy protein preparations[a]

Soy products	Solubility (%)	Water-holding (%)	Fat capacity (%)	Emulsification capacity (%)
Flour	21	130	84	18
Concentrate A	2.3	227	133	3
Concentrate B	6.0	196	92	19
Isolate C	17.4	447	154	25
Isolate D	71.1	416	119	22

[a]Source: Kinsella (1979)

6.1: Gelation

Dispersions of soy protein form true gels when heated and cooled (Circle *et al.*,1964) and by the addition of calcium salts (Catsimpoolas and Meyer, 1971a,b; 1970). A minimum protein concentration of 8% is required for gelation; disulfide cleaving agents (i.e. sulfite, mercaptoethanol, cysteine) impair gelation (Catsimpoolas and Meyer, 1970). Aoki and Sakurai (1969) attributed the anti-gelation effect of sulfites and thiols to the involvement of disulfide bonds in the gelation phenomena. However, hydrophobic interactions are considered to play a role since lipid materials enhance gelation (Catsimpoolas and Meyer, 1970; Circle *et al.*, 1964).

Gels formed from 11s globulins are firmer and more resilient than those formed from 7s globulins (Hashizume *et al.*, 1975; Saio *et al.*, 1975a). Both hydrogen and disulfide bonds have been implicated in the development and maintenance of gel structures (Utsumi and KInsella, 1985). The differences in thermal denaturation of 11s and 7s globulins, especially in the presence of varying salt concentrations (Hashizume *et al.*, 1975), may be exploited to prepare gels with different physical characteristics.

Saio *et al.* (1974) reported that gels which formed after autoclaving of tofu curds made from 11s globulin showed the greater expansion and a softer elastic texture than 7s proteins (Table 9). Kinsella (1979) pointed out that disulfide bonds played a role in the formation and expansion of calcium gels. This explained the fact that the 11s proteins formed superior gels.

Table 9. Thermal expansion of calcium-precipitated gels from 7s protein, 11s protein, and soy isolate[a]

	Average expansion ratio		
	7s protein	11s protein	Soy isolate
pH 6.7	2.46	2.69	3.26
pH 7.3	2.74	4.27	3.36
pH 8.0	3.14	5.70	5.11
pH 8.4	2.66	4.72	4.46

[a]Source: Saio *et al.* (1974)

Babajimopoulas *et al.* (1983) suggested that both van der Waals interaction and hydrogen bonding are involved in the gelation of soy protein with negligible contribution from hydrophobic and electrostatic interactions.

6.2: Water holding capacity

Soy protein gels have considerably higher water holding capacities than gels formed from milk proteins (Barraquio and van de Voort, 1988). For example, tofu has a moisture content greater than 80% (Smith *et al.*,1960) while rennet casein curd has a moisture content of approximately 50% (Lee and Marshall, 1979). The water holding capacity (WHC) of acid precipitated soy proteins can be doubled by heat treatment while the WHC of the 11s protein is increased in the presence of protease bromelain (Mohri and Matsushita, 1984). The high WHC of soy protein gels has limited their incorporation in dairy products where syneresis is desirable (i.e. hard cheeses), however these gels may be used in systems where syneresis is not required, eg., in yoghurt (Schmidt and Morris, 1984; Kollar *et al.*, 1979).

6.3: Solubility

The solubility of soy protein is an important functional property for use in beverages. Treatment of soy protein products with combinations of heat and organic solvent improves flavor (Honig *et al.*, 1976) but reduces solubility. Anderson (1974) demonstrated that the acid precipitation process used for isolating soy proteins results in "acid denaturation", which adversely alters the solubility of the proteins. Solubility is affected by the degree of agglomeration which occurs during the centrifugation and/or drying steps during isolation.

6.4: Emulsifying

Many important properties of foods involve the interaction(s) of proteins and lipids, e.g., emulsions, fat entrapment in meats, flavor absorption. The capacity of soy protein to interact with lipid materials is important in food formulation and processing (Kinsella, 1979). Kamat *et al.* (1978) reported that native soy proteins interact to a negligible extent with lecithin; however, following dissociation of soy proteins into subunits (possibly unfolded polypeptides), lipoprotein formation with

phospholipids and triglycerides can occur. Lipoproteins of denatured soy proteins with polar lipids can be used as emulsifiers in cake mixes and as substitutes for egg yolk (Kinsella, 1979).

Soy isolate shows greater (six-fold) emulsifying capacity than soy protein concentrate (Hutton and Campbell, 1977); other researchers have shown that this is related to method for preparation of the protein (Shemer *et al.*, 1978; Lin and Humbert, 1974).

6.5: Foaming

Soy protein preparations exhibit good foaming properties (Table 10) with isolates having superior foaming characteristics than concentrates (Fleming *et al.*, 1974; Lin and Humbert, 1974). However, the presence of residual lipid material in soy preparations can destabilize the protein foams. Hexane and aqueous alcohol treatment of soy proteins which remove neutral and bound polar lipids, respectively, markedly enhance foaming properties (Glabe *et al.*, 1956). Partial proteolysis or heating to 70-80^{o}C improved the foaming properties of soy protein (Horiuchi *et al.*, 1978); the heat applied increased the tendency of polypeptides to unfold at the interface and facilitated hydrophobic associations, thereby increasing film thickness, foam stability, viscosity and reducing air leakage.

Table 10. Foam formation and stability of soy protein preparations[a]

	Volume increase (%)	Volume (mL/6g) after time (min)				
		1	10	30	60	120
Flour	70	160	131	108	61	20
Concentrate A	170	400	28	13	8	5
Concentrate B	135	370	265	142	30	24
Isolate C	235	670	620	572	545	532
Isolate D	230	660	603	564	535	515

[a]Source: Kinsella (1979).

7.0: Biochemical properties of proteins from soybeans

Initially, the globulin fraction of soybean has been loosely referred to as soybean globulins (Nash and wolf, 1967; Roberts and Briggs, 1965; Hasegawa *et al.*, 1963), soybean caseins (Koshiyama and Iguchi, 1965), acid percipitable soybean proteins (Smith *et al.*, 1955), or glycinin (Rackis *et al*, 1957; Osborne and Campbell, 1898).

Early workers relied almost solely on the solubility characteristics of proteins to effect fractionation. Osborne and Campbell (1898) and later others (Jones and Csonka, 1932; Hartman and Cheng, 1936; Smiley and Smith, 1946) isolated the principal soybean protein which was named glycinin. However, all glycinin preparations were later found to be heterogenous (Briggs and Mann, 1950). Smith and Rackis (1957) reported that some of the heterogeneity of glycinin was due to the formation of dissociable protein-phytate complexes. Briggs and Mann (1950) also showed that a "cold- insoluble" (CI) fraction was a constituent of glycinin.

Ultracentrifugal data showed that reduced soybean globulin in alkaline buffer manifest four resolvable constituents corresponding to 2s, 7s, 11s, and 15s (Naismith, 1955; Wolf and Briggs, 1956), and an unresolvable fraction with values

greater than 15s (Wolf and Briggs, 1956). The ultracentrifugal pattern was influenced by ionic strength, neutral salts, urea, and pH (Kelley and Pressey, 1966; Wolf *et al.*, 1958; Rackis *et al.*, 1957; Wolf and Briggs, 1956; Naismith, 1955). In acidic media three resolvable constituents (2s, 7s and 13s) were found (Rackis *et al.*, 1957), whereas in highly alkaline media, 3s to 5s components were observed (Kelley and Pressey, 1966). In 6M urea there was a propenderance of material in the 1s to 2s region and a small amount of 4s component (Kelley and Pressey, 1966). Some of these constituents were isolated by gel filtration on Sephadex (Koshiyama and Iguchi, 1965; Hasegawa *et al.*, 1963) and calcium phosphate gel columns (Wolf *et al.*, 1962).

Knox *et al.* (1965) used DEAE-cellulose column chromatography to study the protein content of five varieties of soybeans and found that protein patterns of "Harosoy", "Lindarin" and "Hawkeye" were somewhat similar, whereas the patterns for the whey proteins of "Adams" and "L59g-3R" differed substantially, these authors concluded that the Adams variety contained proteins not found in other varieties. Morrison (1962) found that paper and starch-gel electrophoresis were not effective in establishing differences among the five varieties and that DEAE-cellulose chromatography was a better analytical tool for this purpose.

Larsen (1967) analyzed seed proteins of 61 soybean varieties by disc electrophoresis to determine whether differences in behaviour could be used to supplement morphological characters for identifying soybean varieties. In each gel, approximately 21 bands were observed and the general intensity of staining and spacial arrangement of the bands were similar for the proteins of all varieties. Larsen (1967) also reported that on the basis of polyacrylamide electrophoresis, the varieties could be separated into two major groups: component "A" was present in 13 varieties, and component "B" was present in 48 varieties. In no instance were A and B observed in a single variety.

Catsimpoolas *et al.* (1971) reported that the 11s globulin is composed of an acidic and a basic subunit having molecular weights of approximately 22,300 and 37,000, Daltons, respectively. Saio *et al.* (1975b) reported that unheated cold insoluble fraction (CIF) showed two principal bands and a minor one, derived from 11s globulin and a few minor bands derived from 7s globulin.

Thanh and Shibasaki (1976) developed an effective procedure for fractionating 7s and 11s proteins the two major storage soybean proteins (Damodaran and Kinsella, 1982; German *et al.*, 1982; Gayler and Sykes, 1981; Meinke *et al.*, 1981; Utsumi *et al.*, 1981). Brooks and Morr (1984) modified the fractionation procedure of Thanh and Shibasaki (1976) to determine the P and phytate contents of the resulting soy protein fractions by gel electrophoresis, the authors concluded that most of the P of 11s protein is non-phytate and phytate accounts for a major portion of the P content of 7s and soy whey fractions.

8.0: Texture profile analysis

Textural properties of food may be measured either by sensory tests or by objective measures of mass, time, and distance. Szczesniak (1972) cautions that texture measurement devices do not measure texture but measure some physical property which can be related to textural attributes. Textural attributes may be detected only by sensory systems. Objective methods of measurement may be divided into three groups: 1) fundamental tests, 2) empirical tests, and 3) imitative tests (Bourne, 1982).

Fundamental tests measure fundamental rheological properties, such as viscosities and elastic moduli. However, fundamental tests usually correlate poorly with sensory evaluation of the textural properties of foods. Empirical tests, such as punctures, shear and extrusion, can be correlated with textural qualities of foods. However, these tests are often poorly defined. Imitative tests, such as texture profile

analysis (TPA), attempt to imitate, with the instruments, the conditions to which the food is subjected in the mouth or on the plate.

The General Foods Texturometer and the General Foods Texture Profile Analysis are developed to describe the range of textural characteristics of foods. Texture profile consists of parameters of hardness, cohesiveness, elasticity, adhesiveness, brittleness or fracturability, chewiness, gumminess, and viscosity which are measured in two compressions of the food sample (Friedman *et al.*, 1963). The texture profile system was later adapted for use with an Instron Universal Testing Machine (Bourne, 1968). A sample tracing from an Instron is shown in Figure 4, the parameters measured are described in Table 11.

The General Foods Texturometer, the General Foods Texture Profile Analysis and the Instron Universal Testing Machine have been extensively used in studies of tofu texture. Saio *et al.* (1969a) measured hardness, springiness, adhesiveness, chewiness and gumminess of tofu using the texture profile analysis. Other work by this group also utilized the texturometer for evaluation of tofu texture (Saio, 1979; Saio *et al.*, 1971). Furukawa *et al.* (1979) and Furukawa and Ohta (1982) studied the texture of thermally induced soy protein gels using the General Foods Texturometer.

The Instron Universal Testing Machine is the most commonly used tool for the textural evaluation of tofu. Lu *et al.* (1980) measured tofu hardness with an Instron using 75% compression. Similar procedures have been used by other researchers (DeMan *et al.*, 1986; Schaefer, 1986; Wang *et al.*, 1983; Kamel and DeMan, 1982; Wang and Hesseltine, 1982). Skurray *et al.* (1980) measured hardness and cohesiveness with an Instron at 90% compression. Johnson *et al.* (1983) used an Instron at 75% deformation to measure a number of texture profile analysis parameters including hardness, brittleness, elasticity, and gumminess.

A number of other devices have also been used in the measurement of the textural characteristics of tofu. Hashizume *et al.* (1975) studied tofu texture using a curdmeter. Breshnan *et al.* (1981) used the Warner-Bratzler Shear and the Ottawa Texture Test to evaluate integrity of a tofu-like product. Tsai *et al.* (1981) measured tofu gel strength, softness, and chewiness using a rheometer.

Table 1. Texture profile parameters[a]

Term	Texture profile definition	Represented in Figure 4
Hardness	Height of first compression	b
Cohesiveness	Ratio of 2nd peak area to 1st peak area	A_2/A_1
Elasticity	Distance food recovers between 1st and 2nd compressions	c-d
Adhesiveness	Area under curve on upward stroke	A_3
Brittleness	Height of 1st significant break in the 1st compression	a
Chewiness	Product of hardness, cohesiveness and elasticity	$b*(A_2/A_1)*(c-d)$
Gumminess	Product of hardness and cohesiveness	$b*(A_2/A_1)$

[a]Szczesniak (1963), Friedman *et al.* (1963) and Bourne (1968)

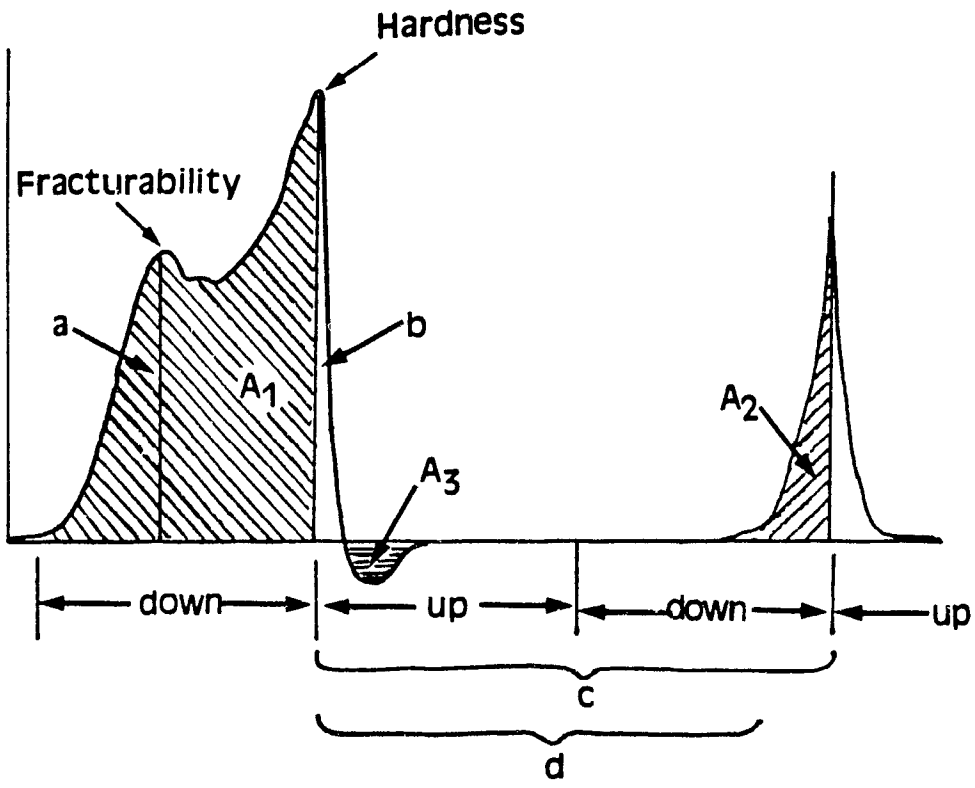


Figure 4. Tracing of force/distance curve obtained using an Instron Universal Testing Machine. Modified from Bourne (1968).

8.1: Tofu texture

Tofu texture is affected by many factors, several of which have already been mentioned. Another factor influencing tofu texture is the coagulant chosen. Calcium and magnesium chloride produced firmer tofu than either calcium or magnesium sulfate, when tofu was tested using the Instron Universal Testing Machine (Wang and Hesseltine, 1982). Saio (1979) found that tofu produced by GDL required more force to penetrate than a calcium sulfate coagulated product but that the interior was softer. Lu *et al.* (1980) reported that tofu prepared using calcium chloride was firmer than tofu using other coagulants, while that prepared with GDL was the softest. When cohesiveness and elasticity were measured no difference was found among the coagulants tested. The coagulants included calcium and magnesium sulfate and calcium and magnesium chloride. The chloride type coagulants resulted in a more brittle product than the sulfate type coagulants (Wang and Hesseltine, 1982)

Coagulant concentration also influences tofu texture (Schaefer, 1986). Increasing coagulant concentration increases the firmness of the product (Schaefer, 1986). Because of the limited solubility of calcium sulfate, increasing the concentration of this coagulant does not produce as marked an increase in tofu firmness as is seen with other coagulants (Wang and Hesseltine, 1982). At coagulant levels greater than 0.03N, Tsai *et al.* (1981) found that the texture of the tofu became more coarse and hard.

Saio (1979) reported greater hardness of tofu with higher coagulation temperatures. Wang and Hesseltine (1982) confirmed this phenomenon noting greater elasticity with higher coagulation temperatures; no effect on tofu cohesiveness was seen. Agitation of the coagulated product increased tofu firmness by disrupting the gel network, the subsequent release of whey produces a less moist, firmer curd (Wang and Hesseltine, 1982; Saio, 1979).

Hashizume *et al.* (1978) reported that tofu hardness decreased with increased heating time for soymilk. This effect was particularly noticeable in the first 15 to 20 min of heating. Escueta (1979) found increases in hardness and gumminess of tofu which were not significant when soymilk was boiled for up to 12 min, this worker reported a significant decrease in hardness and gumminess after 30 min, no effect on cohesiveness of tofu after boiling for 30 min but decreased cohesiveness after 60 min and no effect on elasticity after boiling for 60 min.

Using a General Foods Texturometer, Saio *et al.* (1969b) studied the texture of tofu prepared from the 7s and 11s fractions. Tofu prepared from the 11s fractions was firmer, more gummy, and more chewy than that made using 7s protein. Tofu made from the 7s protein was softer and more adhesive than the 11s product. On the otherhand tofu prepared from 7s and 11s fractions mixed in varying proportions, hardness decreased with increasing proportions of 7s protein. These authors concluded that the 11s fraction was primarily responsible for a firm tofu texture while the 7s protein softened the gel. Skurray *et al.* (1980) found no relationship between the 7s to 11s ratio and tofu quality.

Kantha *et al.* (1983) prepared a tofu-like product by the coagulation of winged bean protein, the very soft curd produced was attributed to lack of the 11s component. Murphy and Resurreccion (1984) found high correlation between glycinin content and hardness, brittleness, elasticity, and gumminess of tofu, no relationship between conglycinin content and textural characteristics of tofu was observed.

C: MATERIALS AND METHODS

1: Materials

Unprocessed and micronized (infrared heated) full-fat soybeans were obtained from a soybean processing plant, Micrograin Inc., St-Robert, Quebec, Canada. The micronization process for the full-fat soybeans involved infrared dry heating for 90 seconds during which the soybeans reached an average temperature of 110^{O}-115^{O}C. The unprocessed and micronized soybeans were ground to a full-fat flour (100-200 mesh) using a Wiley mill, and stored in Mason jars at room temperature.

Methods:

2: Preparation of protein isolate

Protein isolate was prepared from full-fat soybeans using the procedure of Fan and Sosulski (1974). Figure 5 shows a flow diagram of the procedure. Freshly ground soybeans (10 g) were mixed with NaOH solution (100 mL, 0.02%) and allowed to stand for 1 h with intermittent stirring. The mixture was centrifuged (4000xg) for 10 min. The extract was filtered (glass wool) and the residue was discarded. The pH of the extract was adjusted to 4.5 by dropwise addition of HCl (2N) with continuous stirring. The precipitated proteins were recovered by centrifugation (4000xg, 10 min) and freeze dried.

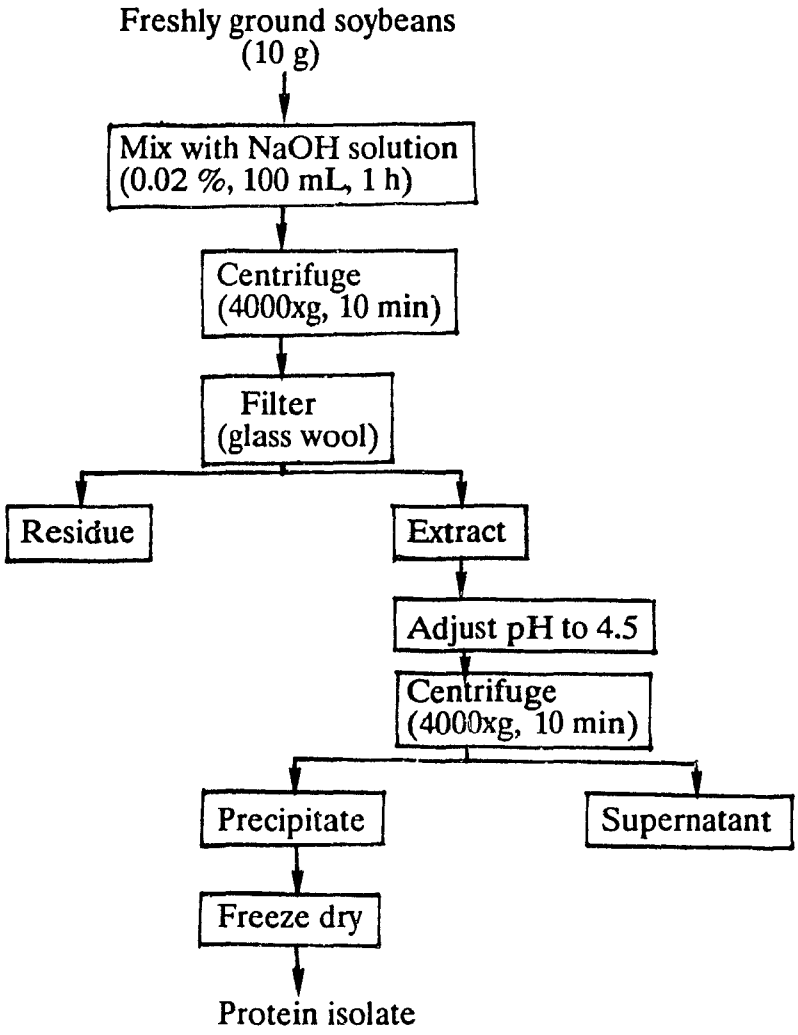


Figure 5. **Flow diagram of procedure for preparation of protein isolate by sodium hydroxide extraction of unprocessed and micronized soybeans.**

3a: Preparation of soymilk and tofu

The laboratory procedure for preparation of soymilk and tofu from unprocessed and micronized soybeans is summarized in Figure 6 (Shurtleff and Aoyagi, 1984).

Soybeans (250 g) were rinsed, soaked in water (1,500 mL) overnight (10-12 h) at 5°C, then homogenized with water in a Waring blender (2 min). The soybean slurry was heated to boiling, simmered for 10 min then filtered hot through 2 layers of cheesecloth to separate the whole soluble components (soymilk) from the insoluble material (okara).

The soymilk (1, 500 mL) was heated to boiling, then cooled to 70°C. Calcium sulphate solution (7.5g $CaSO_4.2H_2O$ in 250 mL hot water) was added to the soymilk at 70°C (coagulating temperature) with stirring until the proteins coagulated; the mixture was allowed to stand for a further 10 min without agitation in order to achieve complete coagulation. The coagulated soymilk was poured gently in a perforated plastic mold (15x12x5.5 cm) lined with 3 layers of cheesecloth. The curd which remained in the cheesecloth was pressed with a weight of 0.5 kg for 30 min. The resultant tofu cake was weighed and refrigerated (5°C) until analyzed further Samples of soymilk and tofu were freeze dried and stored at 5°C for subsequent analysis.

The pH and transparency of the whey obtained during pressing of the curd, were measured using a pH meter (Fisher, Model 610) and a LKB Biochrom Novaspec 4049 Spectrophotometer (400 nm), respectively.

3b: Effect of coagulating temperature

Tofu was prepared from micronized soybeans at coagulating temperatures of 70°, 80° and 90°C by using the procedure described above (section 3a); calcium sulphate (reagent grade, Anachemia LTD, Montreal, Canada) was used as coagulating agent.

3c: Effect of coagulating concentration

Tofu was prepared from micronized soybeans at coagulating temperature of 90^{O}C by using the procedure described above (section 3a); calcium sulphate at three different concentrations (0.02M; 0.03M; 0.04M) was used as coagulating agents.

3d: Effect of coagulating agents

Tofu was prepared from micronized soybeans at coagulating temperature of 90^{O}C by using the procedure described above (section 3a); calcium sulphate (0.03M), citric acid (0.01M) (reagent grade, Anachemia LTD, Montreal, Canada) and a mixture of citric acid and calcium sulphate (0.01M citrate/0.03M sulphate) were investigated as coagulating agents.

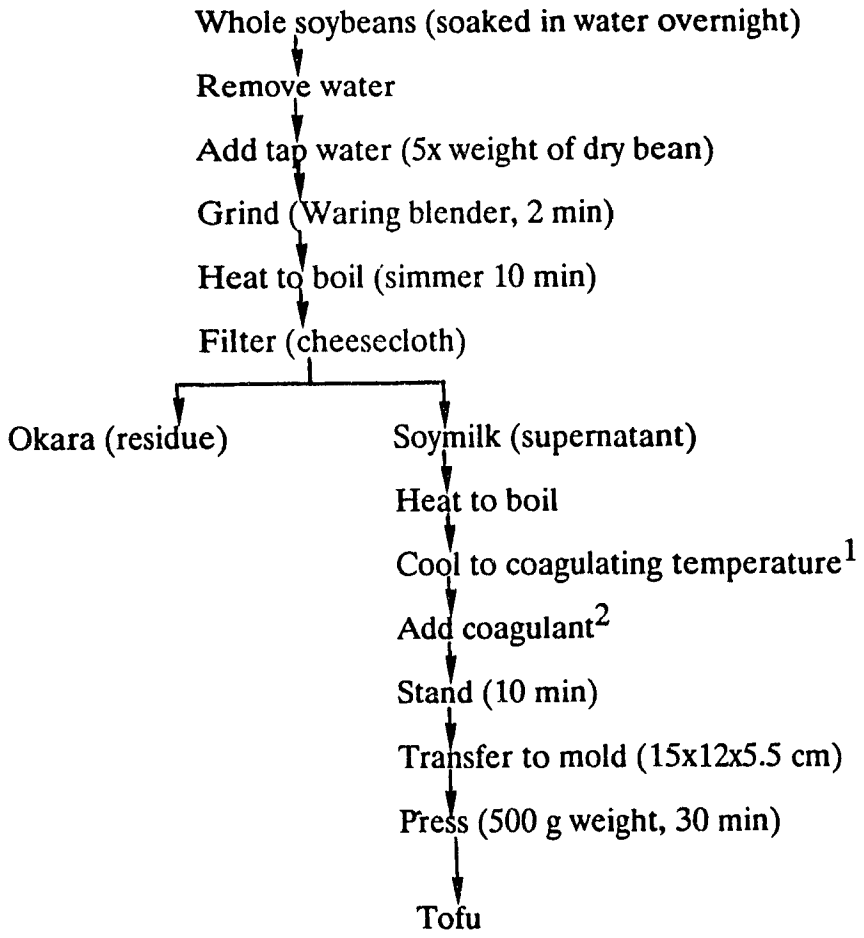


Figure 6. Diagram of procedure for preparation of tofu and soymilk.

[1] 70^0, 80^0, 90^0C (as described in section 3b).

[2] $CaSO_4.2H_2O$ (0.02M, 0.03M, 0.04M) (as described in section 3b); $CaSO_4.2H_2O$, citric acid, sulphate/citrate mixture (as described in section 3c).

4: Proximate analysis of soybeans, soy protein isolate, soymilk and tofu

4a: Moisture

Moisture content was determined using the procedure of the AOAC (1984). Weighed quantities (2 to 3 g) of full-fat soybean, soy isolate and soymilk were oven dried at 110^{o}C until constant weight was obtained. Weighed quantity (approximately 3.5 g) of tofu was dried on a steam bath for 15 min then by forced air oven drying at 110^{o}C for 12 h.

4b: Ash

Ash content was determined by procedure described in the AOAC (1984). Weighed quantities (approximately 2 g) of full-fat soybean, soy isolate, tofu and soymilk were preashed before placing into a preheated (600^{o}C) muffle furnace for 2 h.

4c: Crude protein

Crude protein content was determined by the Kjeldahl method (AOAC, 1984) using a Labconco Rapid Still III (Labconco Corporation, Kansas City). A factor of 6.25 was used to convert % nitrogen to % crude protein.

4d: Crude fat

Crude fat was determined on oven dried samples using the Soxhlet fat extractor and petroleum ether (35^{o}-60^{o}C boiling range) as the solvent (AOAC, 1984). Weighed quantities (approximately 2 g) of dried materials were extracted for 16 h. The residues remaining after removal of residual solvent were weighed and converted to percent crude fat.

5: Mineral analysis

Calcium, phosphorus, magnesium, copper, zinc and iron were determined using the procedure of the AOAC (1984). Weighed quantities (0.5g) of full-fat soybean, soy isolate, soymilk and tofu were placed in Kjeldahl flasks (100 mL) and digested with a mixture of concentrated HNO_3 (10 mL) and $HClO_4$ (4 mL, 70%). The digests were transferred to volumetric flasks (100 mL) and made to volume with deionized water.

Standard solutions with the concentration ranges shown in Table 12 were prepared. The minerals were analysed using an automated atomic absorption spectrophotometer (Perkin Elmer Model 2380). The conditions used for the mineral analysis are shown in Table 12. The concentration of the various minerals in the samples was read directly from the instrument after applying the appropriate standards.

Table 12. Conditions used in mineral analysis[1]

Mineral	Stock solution	Concentration ranges of the standard solution (ug/mL)	Wavelength (nm)
Calcium	500 mg/L: 1.249g $CaCO_3$ + 10 mL HCl, made to volume in deionized water.	0-50	422.7
Copper	1000 mg/L: 1g Cu metal in 5 mL (1+1) HNO_3,made to volume with 1% (v/v) HNO_3.	0-50	324.8
Iron	1000 mg/L: 1 g Fe wire in 5 mL (1+1) HNO_3, made to volume with deionized water.	0-50	287.4
Magnesium	1000 mg/L: 1 g Mn metal in (1+1) HCl, made to volume with 1% (v/v) HCl.	0-5	313.0
Phosphorus	2000 mg/L: 8.79g KH_2PO_4 in 1 L deionized water.	0-15	400.0
Zinc	500 mg/L: 0.5 g Zn metal in 5 mL (1+1) HCl, made to volume with 1% (v/v) HCl.	0-10	213.9

[1]Flame used in atomic absorption spectrophotometer: Air-acetylene

6: Texture and microstructure of tofu

Textural characteristics of tofu samples were measured using the Instron Universal Testing Machine. Cubes (2 cm^3) of tofu were cut from the interior of prepared tofu cake (15x12x5.5 cm). The cubes were compressed to 25% of the original height (5 mm) at a probe speed of 30 mm/min. The compression force was recorded at a chart speed of 200 mm/min. The full scale load used was 1 kg. Figure 7 shows a typical force/distance curve obtained for tofu samples using an Instron Universal Testing Machine. Firmness and cohesiveness were calculated using the following equations:

$$\text{Firmness (N/mm)} = \frac{\text{peak height}}{\text{peak distance}} \times \frac{\text{full scale (kg)} \times 0.098}{\text{Factor}} \qquad \text{(Eqn. 1)}$$

$$\text{Cohesiveness (N)} = \text{peak height} \times \frac{\text{full scale (kg)} \times 0.098}{\text{Factor}} \qquad \text{(Eqn. 2)}$$

$$\text{where, Factor} = \frac{\text{Instron speed}}{\text{chart speed}} \times 0.98 \qquad \text{(Eqn. 3)}$$

Instron speed = 30 mm/min

Chart speed = 200 mm/min

Microstructure of tofu was determined using a Stereoscan 600 Electron Miscroscope (Cambridge Scientific Instruments Limited, Cambridge, England). The tofu samples were fixed in glutaraldehyde solution (2%, pH 7.2) for 2 h at room temperature then rinsed 3 times with water. The samples were postfixed in osmium tetroxide (1%) for 1 h at room temperature then washed 3 times with water. The fixed samples were frozen in liquid nitrogen and freeze dried. The dried samples were mounted on aluminum stub and coated with gold. The specimens were observed and photographed with scanning electron microscope.

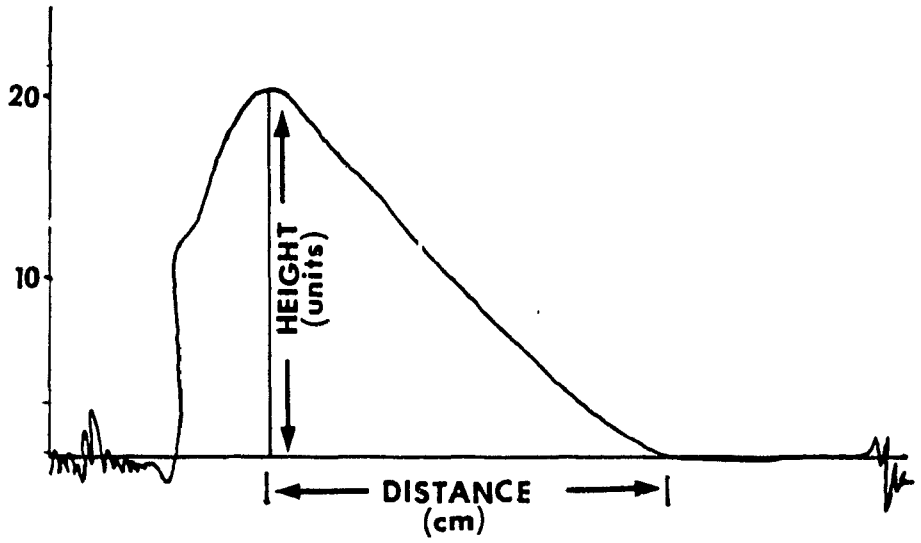


Figure 7. Typical force/distance curve obtained for tofu samples using Instron Universal Testing Machine.

7: Nutritional quality

7a: In vitro digestibility

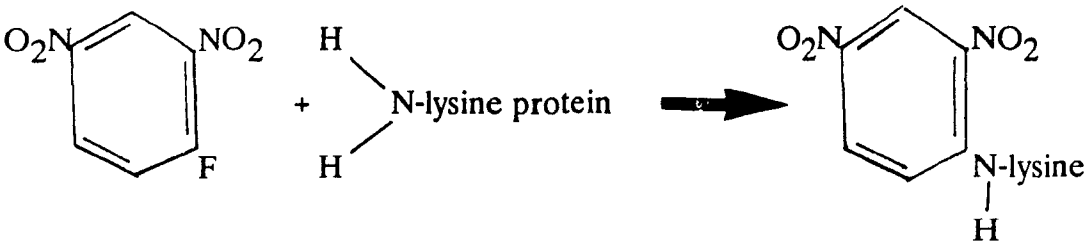
A multi-enzyme technique of Hsu *et al.* (1977) was used to measure the *in vitro* protein digestibility of full-fat soybeans, soy isolate, soymilk and tofu. Sodium caseinate and the following enzymes were obtained from Sigma Chemicals (St. Louis, Missouri): pancreatic trypsin (type 11), bovine pancreatic chymotrypsin (type 11) and porcine intestinal peptidase (Grade 111). A suspension consisting of trypsin (1.6 mg/mL), chymotrypsin (3.1 mg/mL) and peptidase (1.3 mg/mL) was prepared using deionised water. An aqueous suspension (50 mL) of each sample containing 6.25 mg protein/mL was prepared; the actual weight of each sample used was calculated on the basis of its protein content (%N x 6.25). The suspension were adjusted to pH 8.0 by addition of HCL (0.1N) and/or NaOH (0.1N). The multienzyme solution (5 mL) was added to the suspension of each sample and the mixture was incubated (20 min, 37^{o}C). The changes in pH, at 5 min intervals during the 20-min incubation was measured (Fisher accumet selective Ion Analyser Model 750 fitted with a pH combination electrode). This procedure was performed in triplicate for each sample. The *in vitro* digestibility of each sample was calculated according to the following equation (Hsu *et al.* 1977):

$$Y = 210.46 - 18.10X \qquad \text{(Eqn. 4)}$$

where
Y is the *% in vitro* digestibility
X is the pH recorded after 10 min of *in vitro* digestion

7b: Determination of available lysine

Available lysine was determined by a procedure described by Carpenter (1960) using modifications suggested by Booth (1971). This method is based on the formation of a yellow complex [dinitro-phenyl-lysine, (DNP-lysine)] when 1-fluoro-2,4-dinitrobenzene (FDNB) reacts with epsilon-amino groups of lysine as shown in the equation below:



(FDNB) (protein-bound lysine) (DNP-lysine) (yellow complex)

Reagents:

i) Lysine standard solution: Mono-ξ-N-dinitro-phenyl hydrochloride (0.314 g; Sigma Chemicals, St. Louis, Missouri) was dissolved in HCl (8.1M). A quantity (10 mL) of this solution was diluted to 100 mL with distilled water.

ii) 1-fluoro-2,4-dinitrobenzene (FDNB) solution: 2.7% (v/v) FDNB in methanol (80%).

iii) Methyl chloroformate (Anachemia Ltd., Montreal, Canada).

iv) Sodium bicarbonate solution: 80 g $NaHCO_3$/L distilled water.

v) Peroxide-free diethyl ether.

vi) Phenolphthalein solution: 400 mg phenolphthalein/L ethanol (60%).

vii) Sodium hydroxide solution: 120 g NaOH/L distilled water.

viii) Buffer solution: pH 8.5; (19.5 g $NaHCO_3$ and 1 g Na_2CO_3 dissolved in 250 mL distilled water).

Procedure

Samples (1-1.5 g) of full-fat soybean flour, soy isolate, soymilk and tofu were placed in round bottomed flasks (100 mL) fitted with condensers. Sodium bicarbonate solution (8%, 10 mL) was added to each flask and the flask was agitated. Fifteen millilitres of FDNB solution was added and the mixture stirred on a magnetic stirrer (2 h). The ethanol in the FDNB solution was evaporated over a water bath (75^{O}-85^{O}C) and the mixture cooled to room temperature. Hydrochloric acid (30 mL; 8.1 M) was added followed by refluxing (16 h) using a heating mantle. The mixture was filtered hot (Whatman No.1); the filtrate allowed to cool and the volume adjusted to 250 mL.

Aliquots (2 mL) of the filtrate were pipetted into two stoppered graduated test tubes labelled A and B. The contents of tube B were extracted once with peroxide free ether (5 mL); the ether layer was removed with a dropping pipet. The tube was placed in a water bath (75^{O}-85^{O}C) to remove the residual ether. After cooling, phenolphthalein solution was added dropwise until the first pink colour was observed. Sodium carbonate buffer solution (2 mL; pH 8.5) was added, followed by methyl chloroformate (5 drops; fume hood). The tube was stoppered and the mixture shaken vigorously with occasional release of the pressure. After 8 min, concentrated HCl (0.75 mL) was added slowly, the mixture was agitated intermittently, extracted four times with ether. The residual ether was removed over a water bath (75^{O}-85^{O}C). The volume of the solution was adjusted to 10 mL with distilled water.

The contents of tube A were extracted with the peroxide-free ether (3x5 mL). After removing the residual ether, the contents were made up to 10 mL with HCl (1M). The absorbance (435 nm) was then read on a Perkin-Elmer Lambda 2 Spectrophotometer. The absorbance of A minus that of B is the absorbance due to the DNP-lysine. The hydrolysis procedure was replicated four times for each sample

and each hydrolysate was analysed in duplicate. Available lysine was calculated using the following equation:

$$\text{Available lysine} = \frac{W_S \times A_u \times V \times 10^4}{W_u \times A_S \times a \times cp} \qquad \text{(Eqn.5)}$$

where
W_S = weight of standard (mg in a 2 mL aliquot)
W_u = weight of unknown sample (mg)
A_S = absorbance of the standard
A_u = absorbance of the sample
V = total volume of the filtered hydrolysate
a = aliquot of filtrate taken
cp = crude protein content of the sample (%N x 6.25)

Booth (1971) suggested that a correction be applied for the destruction of the 2,4-dinitrophenyl-lysine, consequently all calculated values obtained in this study were divided by the correction factor 0.89.

8: Functional properties of proteins

8a: Foaming capacity and foaming stability

Foam capacity and foam stability were determined using the procedures described by Satterlee *et al.*, (1975) and Gierhart and Potter (1978). Dispersions (2%) of the full-fat soybean flour, soy isolate, tofu and commercial soy protein (Ardex D Dispersible, Frank E. Dempsey & sons Limited, Lachine, Quebec) were prepared. The pH of the dispersions were adjusted to 3.0, 5.0, 7.0 and 9.0 by dropwise addition of HCl (2N) or NaOH (2N) with continuous stirring. The volume of the dispersions were adjusted to 20 mL using distilled water after the pH adjustment.

The protein dispersions were whipped for 2 min at room temperature using a Virtis homogenizer (Model 45) at a speed of 6000 rpm. The whipped slurries were

immediately transferred to graduated cylinders (100 mL) and allowed to stand for 2 min. Foam volume was recorded at 2 min and 30 min after whipping.

Foam capacity was calculated using Equation 6 (Satterlee *et al.*, 1975). Foam stability was calculated using Equation 7 (Gierhart and Potter, 1978).

$$\text{Foam Capacity} = \frac{\text{Foam volume after standing (2 min)}}{\text{volume of slurry before whipping}} \times 100 \qquad \text{(Eqn. 6)}$$

$$\text{Foam Stability} = \frac{\text{Foam volume after standing (30 min)}}{\text{Initial foam volume}} \times 100 \qquad \text{(Eqn. 7)}$$

8b: Water absorption

The water absorption characteristics of the full-fat soybean flour, soy isolate, tofu and commercial soy isolate (Ardex D) were determined using the method of Sosulski (1962). Excess water (20-30 mL) was added to the freeze dried sample (1.5 g) in weighed centrifuge tubes (50 mL, 28.5x103 mm). The suspension was mixed vigorously 4 times with a 10 min rest period between each mixing. The supernatant was decanted; the tube air-dried (10 min) until no residual liquid could be seen and the precipitate was weighed. Water absorption was expressed in percentage as the amount of water absorbed by 100 g sample (Equation 8).

$$\text{Water absorption} = \frac{\text{Weight (g) of water held}}{\text{Weight (g) of dry sample}} \times 100 \qquad \text{(Eqn. 8)}$$

8c: Fat absorption

Fat absorption of the full-fat soybean flour, soy isolate, tofu and commercial soy protein (Ardex D) were determined using the method of Lin and Humbert (1974). The sample (0.5 g) and corn oil (3.0 mL) were placed in centrifuge tube (50 mL, 28.5x103 mm). The contents were stirred for 1 min with a thin brass wire to disperse the sample in the oil. The mixture was allowed to stand for 30 min then centrifuged (3200 rpm, 25 min). The oil layer was poured into a graduated cylinder (15.0 mL) and the volume of oil was measured. Fat absorption was expressed in percentage as the amount of corn oil bound by 100 g (Equation 9).

$$\text{Fat absorption} = \frac{\text{Volume (mL) of oil bound}}{\text{Weight (g) of dry sample}} \times 100 \qquad \text{(Eqn. 9)}$$

9: Electrophoretic analysis

Full-fat soybean, soy isolate, soymilk and tofu were subjected to polyacrylamide-disc gel electrophoresis (PAGE) and sodium dodecyl sulphate (SDS)-PAGE.

9a: Polyacrylamide-disc gel electrophoresis

Procedure of Mauer (1971) was used for PAGE. The solutions used for the preparation of the gels are given in Table 13, the mixing ratios of these solutions are given in Table 14. The solutions used in the electrophoretic analysis are given in Table 15.

i: Preparation of gels

Glass tubes (length = 100 mm; external diameter = 7 mm; internal diameter = 5 mm) were cleaned (chromic acid cleaning solution) and dried. One end of each tube was sealed securely with parafilm. The tubes were placed vertically on a rack with the sealed end at the bottom. Separation gel solution (1.5 mL) was placed in each tube with the aid of a syringe. Water was placed on the top of the separation gel to give a layer of approximately 2 mm, care was taken to avoid mixing of the gel solution and the water. The gels were left to polymerize for 1 h.

The water layer was removed by means of small filter paper wicks. Spacer gel solution (0.2 mL) was placed on top of the separation gel with the aid of a syringe. The tubes were placed under a fluorescent lamp for 20 min to allow photo-polymerization of the spacer gel to take place. A sample gel solution was prepared in exactly the same manner as the spacer gel solution. A quantity (100 uL) of this solution was placed on top of the spacer gel. The tubes were placed under a fluorescent lamp for 20 min for photopolymerization of the sample gel to take place.

Table 13. Materials used in the preparation of polyacrylamide gels (basic conditions)

Separation gel	Spacer gel
Solution No.1 (pH 8.9) 1N HCl 48.0 mL TRIS[a] 36.6 g TEMED[b] 0.23 mL Distilled water added to to adjust volume to 100 mL.	Solution No.4 (pH 6.7) 1N HCl 48.0 mL TRIS[a] 5.98 g TEMED[b] 0.46 mL Distilled water added to adjust volume to 100 mL.
Solution No.2 Acrylamide 30.0 g BIS[c] 0.8 g Distilled water added to adjust volume to 100 mL.	SOLUTION No.5 Acrylamide 12.0 g BIS[c] 3.0 g Distilled water added to adjust volume to 100 mL.
Solution No.3[d] Ammonium persulphate (0.14 g) in 100 mL (aqueous) solution.	Solution No.6 Riboflavin (4.0 mg) in 100 mL (aqueous) solution.
	Solution No.7 Sucrose (40 g) in 100 mL (aqueous) solution.

[a] TRIS: tris (hydroxymethyl) aminomethane
[b] TEMED: N, N, N', N'-tetramethylethylenediamine
[c] BIS: N, N-methylenebisacrylamide
[d] Solution No.3: prepared immediately prior to use.

Table 14. Mixing ratios for preparation of gels (basic conditions)

Separation gel	Spacer gel
1[a] part Solution No.1 2 parts Solution No.2 1 part distilled water 4 parts Solution No.3	1 part Solution No.4 2 parts Solution No.5 1 part Solution No.6 4 parts Solution No.7

[a] parts by volume

Table 15. Solutions prepared for electrophoresis and staining of gels

Solution	Composition
Electrode buffer	Tris[a] (0.60 g), glycine (2.88 g), in 1 L (aqueous) solution.
Indicator solution	Bromophenol Blue (1 mg), in 1 L (aqueous) solution.
Fixative solution	Trichloroacetic acid (12.5 g) in 100 mL (aqueous) solution.
Staining solution	(a) Coomassie Brilliant Blue (1 g), in 100 mL (aqueous) solution. (b) Trichloroacetic acid (12.5 g), in 100 mL (aqueous) solution. Mixture of 1 volume of solution (a) and 19 volumes of solution (b).
Destaining solution	Trichloroacetic acid (10 g), in 100 mL (aqueous) solution.
Storage solution	Glacial acetic acid (7 mL), in 100 mL (aqueous) solution.

[a] TRIS: tris (hydroxymethyl) aminomethane

ii: Electrophoresis

The parafilm was carefully removed from the end of the tubes which were inserted into the openings on the manifold of the upper buffer reservoir of the electrophoretic apparatus (Figure 8). Buffer solution was added to the upper and lower reservoirs to completely cover the electrodes. Bromophenol blue solution (1 mL, 0.001%) was added to the buffer solution contained in the upper reservoir. The electrodes were connected to the power supply (anode, lower reservoir; cathode, upper reservoir) and the current (D.C.) was turned on. The current was adjusted to 1 ma per tube for the first 2 min and then increased to 4 ma per tube for the remainder of the electrophoretic run. Electrophoresis was stopped when the bromophenol blue indicator reached the bottom of the tube; this took approximately 1 h.

The gels were removed from each tube with the aid of a flexible needle which was passed between the gel and the inner wall of the tube.

iii: Protein Staining

The gels were placed in test tubes (13x100 mm) containing fixative solution (trichloroacetic acid, 10 mL, 12.5%) for 0.5 h. The fixative solution was replaced with staining solution (Coomassie Brilliant Blue, 10 mL, 1% in 12.5% trichloroacetic acid) for about 18 h. The staining solution was replaced with destaining solution (trichloroacetic acid, 10 mL, 10%). The gels were kept in the destaining solution for several days with bi-daily changes of the solution. After destaining, the gels were transferred for storage to test tubes (13x100 mm) containing acetic acid (10 mL, 7%).

iv: Glycoprotein staining

Staining of gels for glycoprotein was done according to the method described by Zacharius *et al.* (1969). The fuchsin-sulphite staining solution was prepared by

dissolving basic fuchsin (2 g, Allied Chemicals, special for Flagella stain) in distilled water (400 mL) with warming and then cooled and filtered. HCL (10 mL, 2N) and sodium metabisulphite (4 g) were added. The solution was placed in a stoppered bottle and kept cool in the dark overnight. A small amount (approximately 1 g) of activated charcoal was added and the mixture was filtered through filter aid. Hydrochloric acid (10 mL or excess of 10 mL, 2N) was then added until a drop dried on a glass slide did not turn red. The resultant clear, colourless solution was stored at $4^{O}C$. The gel was placed in trichloroacetic acid solution (10 mL, 12.5%) for 15 min, rinsed lightly with distilled water, then placed in $HIO_4.2H_2O$ solution (10 mL, 1% in 3% aqueous acetic acid) for 50 min. Excess of the iodate ion was removed by prolonged (overnight) washing of the gel with distilled water until the wash water was free of iodate as indicated by negative reaction to silver nitrate. The gel was immersed in a fuchsin sulphite staining solution (10 mL, 0.5%) for 2 h in the dark and then washed thrice with freshly prepared sodium metabisulphite solution (10 mL, 0.5%).

v: Lipoprotein staining

The gels were stained for lipoprotein using the method described by Swahn (1952). After electrophoresis, the gels were placed in an alcoholic solution of Sudan Black B (10 mL, 0.1% in 60% ethanol) for 6 h then destained with ethanol (10 mL, 50%).

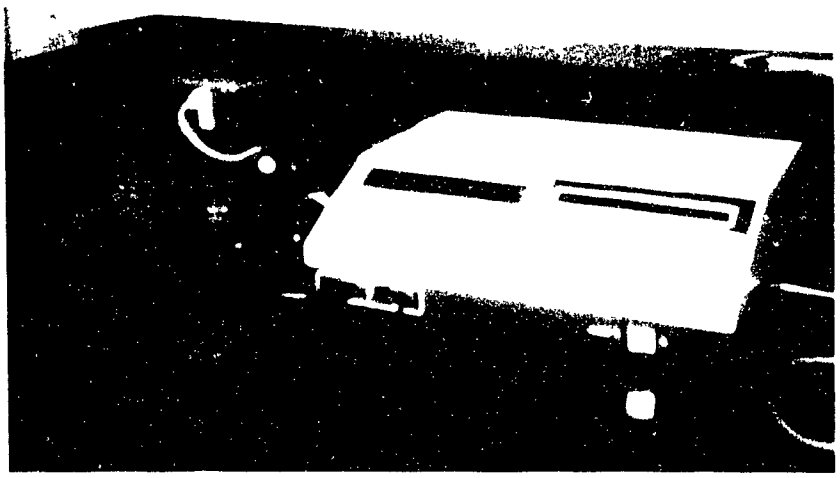


Figure 8. Electrophoresis apparatus.

9b: SDS- Electrophoresis

The procedure described by Weber *et al.* (1972) was used for sodium dodecyl sulfate (SDS) - phosphate polyacrylamide gel electrophoresis.

i: Preparation of gels

Gel solution (22.2 g acrylamide; 0.6 g methylenebisacrylamide in 100 mL aqueous solution) was prepared along with a buffer solution (pH 7.2; 7.8 g sodium dihydrogen phosphate, 38.6 g disodium hydrogen phosphate and 2 g sodium dodecyl sulfate in 1 L aqueous solution). Acrylamide solution (10.0 mL), distilled water (3.4 mL), gel buffer solution (pH 7.2, 15.0 mL) and N, N, N', N' - tetramethylethylenediamine (TEMED) (0.045 mL) were mixed. The resultant solution was refrigerated (5 min). Ammonium persulphate solution (1.5 mL, 1.5%) was added to the cooled solution. The resultant solution was placed in electrophoresis tubes (internal diameter = 5 mm, height 100 mm). A small volume of distilled water was placed on top of the gel solution to ensure a flat gel surface. The solution was allowed to stand (25 min, 25^{o}C) to allow the gels to polymerize.

ii: Sample preparation

A sample (2 mg) of protein and of protein standards [Bio-Rad's SDS-PAGE Molecular Weight standards, containing phosphorylase b (97,400); Bovine serum albumin (66,200); Ovalbumin (42,699); Carbonic anhydrase (31,000); soybean trypsin inhibitor (21.500); lysozyme (14,400); diluted 1:20 in sample buffer] were placed in screw cap test tubes along with sodium dodecyl sulfate/2-mercaptoethanol solution (0.5 mL, 2%) and sodium phosphate buffer solution (0.5 mL, 0.01 M, pH 7.2). The samples and standard proteins were heated in a boiling water bath for 3 min and then at 37^{o}C (2 h). Sucrose (50 mg) was added, followed by bromophenol blue solution (1 drop, 0.05%). The sample solution (100 uL) and the standard

solution (50 uL) were placed on top of the gel. The gels were transferred to the opening on the manifold of the upper buffer reservoir.

iii: Electrophoresis

An initial current of 1 ma per tube was used for the first minute and then increased to 8 ma per tube. Electrophoresis was allowed to continue until the bromophenol tracking dye reached the bottom of the tube.

iv: Protein staining

The gels were removed from the tubes and then immersed in a fixative solution (10 mL, 40% methanol-7% acetic acid solution) for 24 h. The solution was changed twice. The gels were immersed in staining solution (10 mL, 0.025% Coomassie Brilliant Blue in 40% methanol-7% acetic acid) for 3 h, rinsed with water, then allowed to stand in a destaining solution (10 mL, 5% methanol-7.5% acetic acid) until the gel background was clear. The destaining solution was changed twice daily. The gels were stored in the destaining solution.

v: Glycoprotein staining

Staining for glycoprotein was done according to the method described by Neville (1971). The gels were placed in a fixative solution (10 mL, 40% methanol-7% acetic acid) for 24 h which was replaced with $HIO_4.2H_2O$ solution (10 mL, 1% in 3% acetic acid solution). The gels were allowed to stand in this solution (5^oC) for 1 h in the dark, then washed with acetic acid solution (10 mL, 7%) until the wash water was free of iodate as indicated by a negative reaction to silver nitrate test. The gels were immersed in fuchsin-sulphite staining solution for 1 h in the dark and then washed thrice with freshly prepared sodium metabisulphite solution (0.5%).

vi: Lipoprotein staining

The gels were stained for lipoprotein using the method described by Swahn (1952). After electrophoresis, the gels were placed in an alcoholic solution of Sudan Black B (10 mL, 0.01% in 60% ethanol) for 6 h. The gels were destained with ethanol (50%).

10: Statistical analysis

Analysis of variance for the data was carried out using the McGill University System of Interactive Computing (MUSIC). Means were determined by the Duncan's New Multiple Range Test (Steel and Torrie, 1980).

D: RESULTS AND DISCUSSION

1.0: Effect of micronization on composition of soybeans, soy protein isolate, soymilk and tofu

Table 16 gives the proximate composition of the soybean and its product. The infrared heating resulted in a small reduction of water content of the whole beans. In general there was little effect of micronization on the overall composition of the soybeans or on the protein isolated from the beans. The protein content of soymilk prepared from the micronized beans was higher than that of soymilk prepared from the unprocessed beans. This suggests an increase in the water dispersability of the soybean protein as a result of the micronization process. On the other hand, the protein content of tofu from the micronized beans was lower than that from the unprocessed beans. This suggests that micronization affects the coagulation properties of the proteins.

Table 16. Chemical composition of soybean and soy products[1]

Components (%)	Soybean		Soy isolate		Soymilk		Tofu	
	U[2]	M[3]	U[2]	M[3]	U[2]	M[3]	U[2]	M[3]
Moisture	9.83	7.81	6.10	4.38	93.73	93.92	81.37	82.43
Ash	5.16	5.23	2.58	3.32	0.32	0.28	1.46	1.68
Fat	20.02	18.19	11.67	11.71	1.43	1.73	5.43	5.17
Protein[4]	39.10	38.38	79.04	80.88	2.77	3.66	9.56	6.58
Carbohydrate	25.89	30.33	0.61	0.00	1.75	0.41	2.95	4.14

[1]Results are means of triplicate measurements
[2]Unprocessed soybean
[3]Micronized soybean
[4]Protein (%N x 6.25)
[5]Carbohydrate (by subtraction)

Table 16 also shows that there was little effect of micronization on ash content of the whole beans, soy protein isolate, soymilk and tofu. Analysis of content of individual minerals in the soy products (Table 17), indicates that the infrared heating resulted in reduction of phosphorus, copper and zinc contents of the soy protein isolate, soymilk and tofu. This suggest that the heat treatment of micronization might have an effect on the interactions between these minerals and other components in soybean e.g., protein, carbohydrate and lipids; resulting in the alteration of solubility or extractability properties of these minerals.

Table 17. Mineral content of soybean and soy products[1]

Mineral	Soybean		Soy isolate		Soymilk		Tofu	
	U[2]	M[3]	U[2]	M[3]	U[2]	M[3]	U[2]	M[3]
Calcium (%)	0.17	0.20	0.07	0.06	0.14	0.13	1.44	2.06
Phosphorus (%)	0.68	0.62	0.74	0.66	0.78	0.55	0.86	0.60
Magnesium (%)	0.23	0.24	0.08	0.09	0.24	0.15	0.18	0.12
Copper (ppm)	23.3	18.4	24.3	14.5	22.8	17.7	18.5	13.8
Zinc (ppm)	57.0	49.7	37.2	22.6	62.0	53.5	73.4	53.5
Iron (ppm)	114	111	154	183	87.6	74.6	107	114

[1]Results are means of duplicate measurements
[2]Unprocessed soybean
[3]Micronized soybean

Soybean:

The mean protein content in the unprocessed and micronized soybeans tested was 39.10% and 38.38%, respectively. This is within the range of protein content reported for soybean grown in Ontario by DeMan *et al.* (1975), but slightly

lower than the mean value of 40.84% reported by these authors. The lipid content of 20.02% and 18.19% were found in this study for the unprocessed and micronized soybeans, respectively. DeMan *et al.* (1975) reported a mean lipid contents of 17.18% with a range of 14.46% to 21.20%. Krivoricho *et al.* (1979) found 14.8% to 22.0% lipid in soybeans with a mean value of 19.51%.

The mean calcium contents in unprocessed and micronized soybeans tested were 0.17% and 0.20%, respectively. These values are lower than the values of 0.26% to 0.47% reported by Carter and Hopper (1942). The copper contents of unprocessed and micronized soybeans were 23.3 ppm and 18.4 ppm, respectively (Table 17). Freeland *et al.* (1977) reported 24.6 ppm of copper while Pennington and Calloway (1973) reported a value of 13 ppm.

Soy protein isolate:

The fat and protein contents of the protein isolated from the unprocessed and micronized soybeans were 11.67%, 11.71% and 79.04%, 80.88%, respectively (Table 16). Bressani (1981) reported that the lipid and protein content of a soy isolate from defatted soy meal was of 0.1% and 96.0%, respectively. The relatively high lipid content in the isolate prepared in our laboratory might be related to the fact that full-fat soybeans was used and the method of preparation might be different.

The calcium, magnesium and phosphorus contents of the soy protein isolate from unprocessed beans were 0.07%, 0.08% and 0.74%, respectively, and from the micronized beans were 0.06%, 0.09% and 0.66%, respectively. The calcium contents are lower than that found by Pearson (1983) who reported calcium, magnesium and phosphorus contents of 0.18%, 0.03% and 0.76%, respectively. The contents of iron, copper and zinc from unprocessed and micronized soybeans are

similar to those found by Pearson (1983) who reported the mean iron content of 160 ppm; zinc content of 40 ppm; and copper content of 12 ppm.

Soymilk:

The protein contents of soymilk from the unprocessed and micronized beans were 2.77% and 3.66%, respectively. Chang and Murray (1949) found 2.90% protein in soymilk on a 6% solids basis. DeMan *et al.* (1975) found 2.72% protein (6% solids basis) in soymilk prepared from 55 soybean varieties grown in Ontario. Lo *et al.* (1968a) reported that soymilk prepared from Clark variety of soybeans contained 3.12% protein. Del Valle (1981) also found 3.12% protein in soymilk, while Wang *et al.* (1983) found the average soymilk protein content of 10 soybean varieties was 3.2%.

The lipid content of soymilk from unprocessed and micronized beans were 1.43% and 1.73%, respectively. This is similar to values reported by Wang *et al.* (1983) who found 1.53% lipid in soymilk from 10 soybean varieties. Chang and Murray (1949) found 1.14% lipid in soymilk (6% solids basis) while DeMan *et al.* (1975) found a mean lipid content of 1.24% in soymilk prepared from 55 soybean varieties.

The calcium content in soymilk from unprocessed and micronized beans were 0.14% and 0.13%, respectively. These values are similar to that reported by DeMan *et al.* (1975), but lower than the 0.195% reported by Chang and Murray (1949). The latter authors reported soymilk phosphorus content of 0.65% which is similar to the phosphorus content of the soymilk prepared from the unprocessed (0.78%) and micronized (0.55%) soybeans. Soymilk from unprocessed and micronized beans showed iron contents of 87.6 ppm and 74.6 ppm, respectively; Chang and Murray (1949) reported a value of 72 ppm.

Tofu:

The protein content of tofu prepared from the micronized beans was 6.58% and that prepared from the unprocessed beans was 9.56%. Adams (1975) found 7.83% protein while Shurtleff and Aoyagi (1975) reported 7.80% protein, Del Valle (1981) found 50.79% protein on a moisture-free basis.

The lipid content of tofu prepared from the unprocessed beans and micronized beans were 5.43% and 5.17%, respectively. Adams (1975), Shurtleff and Aoyagi (1975) and Chang and Murray (1949) reported 4.2%, 4.3%, and 7.1% lipid, respectively.

The calcium contents of tofu from unprocessed and micronized beans tested were 1.44% and 2.06%, respectively. Kantha *et al.* (1983) reported 1.638% calcium in tofu on a dry basis. Phosphorus contents of tofu from unprocessed and micronized beans were 0.86% and 0.60%, respectively; Chang and Murray (1949) reported a phosphorus content of 0.80% (DM basis) while Tseng *et al.* (1977) reported a value of 0.08% (FW basis). The iron content in tofu from unprocessed beans was 107 ppm and from micronized beans was 114 ppm. Chang and Murray (1949) reported an iron content of 105 ppm, while Kantha *et al.* (1983) reported an iron content of 70 ppm. The zinc content in tofu from unprocessed beans was 73.4 ppm and from micronized beans was 53.5 ppm. Kantha *et al.* (1983) reported a zinc content of 60 ppm.

2.0: Effect of coagulation conditions on composition and texture of tofu

A study was conducted to determine the effects of micronization on the composition and textural properties of tofu, and to investigate the effects of various coagulation conditions on the composition and textural properties of the tofu prepared from micronized beans.

Table 18 shows that the yield of the tofu from the micronized beans was lower than that from unprocessed beans. This suggests that the micronization

process reduced the coagulation properties of the proteins. The results also indicate that the light transmission (%T) of the whey from micronized soybeans was considerably lower than that of the unprocessed beans. This suggests that in case of the micronized beans, a greater quantity of proteins remained in the whey and therefore a smaller quantity coagulated.

The results in Table 19 show that tofu from the micronized beans was lower in firmness and cohesiveness when compared to tofu from unprocessed beans. This suggests that in the case of the micronized beans, the proteins which coagulated had lower inter-molecular binding properties when compared with the coagulated proteins of unprocessed beans. This could explain the relatively inferior textural properties of the tofu from the micronized beans.

Table 18. Composition of tofu prepared from unprocessed and micronized soybeans[1]

	Whey			Tofu[2]		
	Volume (mL)	pH	T[3] (%)	Yield (g)	Protein (%)	Solid (%)
U[4]	1167[a]	5.73(0.1)[a]	4o.8(2.6)[a]	253.6(3.9)[a]	9.56(0.9)[a]	17.4(1.1)[a]
M[5]	1260[b]	5.90(0.1)[a]	19.1(0.1)[b]	179.7(6.8)[b]	6.58(0.5)[b]	17.9((.5)[a]

[1]Results are means (standard deviations) of triplicate analyses
[2]Coagulated with $CaSO_4 \cdot 2H_2O$ at 70°C
[3]Percent transmission
[4]Unprocessed soybean
[5]Micronized soybean
[a,b]Means in the same column with the same letter superscript are not significantly different ($p<0.05$)

Table 19. Textural properties of tofu prepared from unprocessed and micronized soybeans[1,2]

Soybean	Firmness (N/mm)	Cohesiveness (N)
Unprocessed	0.108(0.01)[a]	0.67(0.08)[a]
Micronized	0.058(0.02)[b]	0.29(0.10)[b]

[1]Results are means (standard deviations) of triplicate analyses
[2]Tofu coagulated with $CaSO_4.2H_2O$ at 70°C
[a,b]Means in the same column with the same letter superscript are not significantly different ($p<0.05$)

2.1: Effects of coagulating temperature

The results in Table 20 indicate that micronized bean tofu obtained by coagulation at 90°C, gave maximum yield and protein content, however these characteristics were lower than those of unprocessed bean tofu coagulated at 70°C.

Micronized bean tofu obtained by coagulation at 90°C, also showed maximum firmness and cohesiveness; however the values were lower than those of unprocessed bean tofu coagulated at 70°C (Table 21). Wang and Hesseltine (1982) reported decreased yield and increased % solids as the coagulation temperature for tofu increased from 60° to 80°C. This is different from the results obtained in this study which shows an increase in yield and a decrease in % solids as the coagulation temperature for micronized tofu increased from 70° to 90°C. It is likely that the decrease in % solids is related to the ability of the curd obtained at higher temperatures to form a network which traps liquid efficiently.

Wang and Hesseltine (1982) also reported increased hardness and elasticity of tofu with increased coagulation temperatures. Similar results were obtained for the micronized bean tofu in the present study; improved textural characteristics were obtained as coagulation temperature increased from 70° to 90°C. Hashizume

et al. (1975) found that maximum hardness of tofu coagulated with glucono delta lactone resulted from coagulation at 80°C, with a decrease in hardness at higher temperatures. Saio (1979) reported that both hardness and yield of tofu increased as coagulation temperature increased from 60° to 100°C. Protein coagulation was probably not complete at the lower temperature used in their study. As coagulation temperature increased, more protein may have been precipitated, resulting in both increased yields and firmer texture.

Table 20. Effect of coagulation temperature on properties of tofu[1]

	Whey			Tofu[2]		
T[3] (°C)	Volume (mL)	pH	T[4] (%)	Yield (g)	Protein (%)	Solid (%)
M70[5]	1260[a]	5.90(0.1)[ab]	19.1(0.1)[b]	179.7(6.8)[b]	6.58(0.5)[b]	17.9(0.5)[c]
M80	1240[a]	5.96(0.1)[a]	12.2(0.1)[c]	180.5(2.9)[b]	6.86(0.3)[b]	14.1(0.8)[a]
M90	1223[a]	5.96(0.1)[a]	10.4(0.2)[c]	219.1(2.3)[ab]	8.72(0.6)[a]	15.6(0.6)[b]
U70	1167[a]	5.73((0.1)[b]	46.8(2.6)[a]	253.6(3.88)[a]	9.56(0.8)[a]	17.4(1.1)[c]

[1]Results are means (standard deviations) of triplicate analyses
[2]Coagulated with $CaSO_4.2H_2O$
[3]Coagulation temperature
[4]Percent transmission
[5]M - micronized beans, U - unprocessed beans
[a,b,c]Means in the same column with the same letter superscript are not significantly different ($p<0.05$)

Whey clarity is considered to be an indication of the extent of precipitation from soymilk. Tsai *et al.* (1981) used % transmittance of whey at 440 nm to indicate the amount of coagulant needed in tofu preparation. Johnson (1984) employed a similar procedure to determine the degree to which soymilk protein had been

precipitated. Based on these reports, whey clarity would be expected to increase with increasing coagulation temperature. However, the results (Table 20) show that whey clarity was lowest in the sample coagulated at the highest temperature (90°C). It is possible that very fine tofu curds were separated with the whey at higher temperatures, leading to lowered whey clarity at these temperatures. Schaefer (1986) pointed out that whey transparency did not accurately reflect completeness of protein separation from whey. This suggests that whey transparency may not be a useful indicator of complete separation of protein.

Table 21. Effect of temperature on textural properties of tofu[1,2]

Temperature[3] (°C)	Firmness (N/mm)	Cohesiveness (N)
M70[4]	0.06(0.02)^{bc}	0.29(0.10)^{bc}
M80	0.04(0.01)^{c}	0.21(0.02)^{c}
M90	0.07(0.01)^{b}	0.35(0.03)^{b}
U70	0.11(0.01)^{a}	0.67(0.08)^{a}

[1]Results are means (standard deviations) of triplicate analyses
[2]Tofu coagulated with $CaSO_4.2H_2O$
[3]Coagulation temperature
[4]M - micronized beans, U - unprocessed beans
[a,b,c]Means in the same column with the same letter superscript are not significantly different ($p<0.05$)

2.2: Effects of coagulant concentration

The effects of coagulant concentration on the composition and textural properties of tofu from micronized beans are shown in Tables 22 and 23, respectively. The results indicate that use of $CaSO_4.2H_2O$ at a concentration of 0.03M gave improved yield and higher protein content of tofu (Table 22); for

textural properties, use of $CaSO_4.2H_2O$ at a concentration of 0.04M gave improved firmness and cohesiveness (Table 23).

Schaefer (1986) reported significantly greater % solids and protein content in tofu prepared using 0.03M $CaSO_4.2H_2O$ as coagulant. Similar results were obtained by Wang and Hesseltine (1982) who reported decreased yield and moisture content of tofu at higher coagulant concentration; these workers noted that this effect was less important when calcium sulphate was the coagulant compared to some other coagulant types eg. chloride salts and they attributed this effect to the relatively low solubility of calcium sulfate.

Saio (1979) reported increased hardness of tofu prepared with the use of high concentration of coagulant; the results from our study also indicate that both firmness and cohesiveness of micronized bean tofu increased at higher coagulant concentrations (Table 23).

Table 22. Effect of coagulant concentration on properties of tofu prepared from micronized soybeans[1]

	Whey			Tofu[2]		
Conc.[3] (M)	Volume (mL)	pH	T[4] (%)	Yield (g)	Protein (%)	Solid (%)
0.02	1223[a]	6.03(0.1)[a]	21.3(7.1)[ab]	170.1(4.5)[a]	7.63(1.1)[a]	11.8(2.3)[a]
0.03	1240[a]	5.90(0.0)[b]	14.6(1.4)[b]	172.7(7.4)[a]	8.94(0.8)[a]	16.0(1.0)[a]
0.04	1267[a]	5.90(0.0)[b]	26.7(3.4)[a]	162.8(5.0)[a]	7.96(0.1)[a]	14.7(1.9)[a]

[1]Results are means (standard deviations) of triplicate analyses
[2]Coagulated with $CaSO_4.2H_2O$ at 90°C
[3]Coagulant concentration in Molarity
[4]Percent transmission
[a,b]Means on the same column with the same letter superscript are not significantly different ($p<0.05$)

Whey clarity was lowest with use of 0.03M calcium sulphate as coagulating agent. This supports the suggestion of Schaefer (1986) that whey transparency does not accurately reflect completeness of protein separation from whey since our results (Table 22) demonstrated that a coagulant concentration of 0.03M also gave tofu with the highest protein content. Tsai *et al.* (1981) found no difference in whey clarity using coagulant concentrations of 0.02M and 0.03M. Johnson (1984) reported whey transparency to be at a maximum between 0.012M and 0.013M calcium sulphate with no further change through the maximum tested (0.015M). Saio (1979) reported similar results showing no change in whey transparency between calcium sulphate concentrations of 0.01M to 0.015M.

Table 23. Effect of coagulant concentration on textural properties of tofu prepared from micronized soybeans[1,2]

Coagulant Conc. (M)	Firmness (N/mm)	Cohesiveness (N)
0.02	0.05(0.01)[b]	0.29(0.01)[b]
0.03	0.06(0.01)[b]	0.32(0.07)[b]
0.04	0.09(0.02)[a]	0.47(0.04)[a]

[1]Results are means (standard deviations) of triplicate analyses
[2]Tofu coagulated with $CaSO_4.2H_2O$ at 90°C
[a,b]SMeans in the same column with the same letter superscript are not significantly different ($p<0.05$)

2.3: Effects of coagulating agents

The effects of three coagulating agents on composition and textural properties of micronized bean tofu are shown in Tables 24 and 25, respectively. The results indicate that the use of citric acid (0.01M) and a mixture of citric acid

(0.01M) and calcium sulphate (0.03M) as coagulating agents led to a decrease in yield, had little effect on protein content, but led to improved textural properties of tofu. Similar results were obtained by Lu *et al.* (1980) who investigated calcium chloride, calcium acetate, acetic acid, calcium lactate, calcium sulfate and glucono delta lactone (GDL) as coagulating agents and showed that soybean curds prepared with calcium salts had a higher weight yield than those with non-calcium compounds; in addition curds prepared with calcium chloride, calcium acetate and acetic acid were softer than those from the remaining coagulants. These researchers pointed out that the reason for the softer curds was related to the smaller quantity of coagulant used.

The results from this portion of the study indicate that preparation of tofu from micronized beans using a mixture of citric acid (0.01M) and calcium sulphate (0.03M) as coagulating agent and a coagulating temperature of 90^{o}C gave a product of comparable firmness and cohesiveness to tofu from unprocessed soybeans.

Table 24. Effect of different coagulants on properties of tofu prepared from micronized soybeans[1]

Coagulant	Whey		Tofu[2]		
	pH	T[3] (%)	Yield (g)	Protein (%)	Solids (%)
Calcium sulphate[4]	5.90(0.0)^{a}	14.6(1.4)^{c}	192.9(2.6)^{b}	8.94(0.8)^{a}	16.0(1.0)^{ab}
Citric acid[4]	4.21(0.1)^{d}	16.1(0.2)^{c}	127.9(9.5)^{c}	10.44(1.9)^{a}	16.3(1.3)^{ab}
Citrate-sulphate[4]	33.86(0.1)^{c}	22.9(0.1)^{b}	130.8(0.2)^{c}	8.25(0.8)^{a}	15.3(0.7)^{a}
Calcium sulphate[5]	5.73(0.1)^{b}	46.8(2.6)^{a}	253.6(3.9)^{a}	9.56(0.9)^{a}	17.4(1.1)^{b}

[1]Results are means (standard deviations) of triplicate analyses
[2]Coagulated at 90°C
[3]Percent transmission
[4]Calcium sulphate (0.03M); Citric acid (0.01M); Citrate-Sulphate (0.01M/0.03M)
[5]Unprocessed bean tofu coagulated at 70°C (0.03M)
[a,b,c]Means in the same column with the same letter superscript are not significantly different ($p<0.05$)

Table 25. Effect of different coagulants on textural properties of tofu prepared from micronized soybeans[1,2]

Coagulant	Firmness (N/mm)	Cohesiveness (N)
Calcium sulphate[3]	0.06(0.01)^{b}	0.32(0.07)^{c}
Citric acid[3]	0.11(0.03)^{a}	0.46(0.09)^{bc}
Citrate-sulphate[3]	0.13(0.02)^{a}	0.55(0.12)^{ab}
Calcium sulphate[4]	0.11(0.01)^{a}	0.67(0.08)^{a}

[1]Results are means (standard deviations) of triplicate analyses
[2]Tofu coagulated at 90^{o}C
[3]Calcium sulphate (0.03M); Citric acid (0.01M); Citrate-sulphate (0.01M/0.03M)
[4]Unprocessed bean tofu coagulated at 70^{o}C (0.03M)
[a,b,c]Means in the same column with the same letter superscript are not significantly different ($p<0.05$)

3.0: Scanning electron microscopy of tofu

Over the last decade the electron microscope has become an important tool in the study of the microscopic structure of proteins and of foods in general. Seckinger and Wolf (1973) suggested that an evaluation of protein quality can be made by microscopic examination of the proteins. Lin *et al.* (1976) claimed that the scanning electron microscope is rapidly becoming a useful instrument for examining particulate structures involved in biological and food systems. In this study, scanning electron microscopy (SEM) was used to demonstrate differences in the fine structure of tofu obtained from unprocessed beans and that from micronized beans.

Figures 9a and 9b show the electron photomicrographs of tofu prepared from unprocessed beans at low (x 500) and high (x 2000) magnification, respectively. The structure showed a typical 3-dimensional honeycomb-like network with certain regularity or uniformity. The continousity and uniformity of the honeycomb-like structure is showed clearly at the high magnification (Figure 9b).

The fine structure of tofu obtained from micronized beans lacked the typical 3-dimensional honeycomb-like network demonstrated by the tofu from the unprocessed beans (Figures 10a and 10b). This structure also showed that the protein network was collapsed. In addition the structure of tofu from the micronized beans showed the lack of regularity associated with that of unprocessed beans.

The use of coagulating agents other than calcium sulphate also affected the fine structure of tofu. DeMan *et al.* (1986) examined the fine structure of tofu coagulated with different coagulants using scanning electron microscope and reported that tofu obtained with glucono delta lactone (GDL) showed superior texture on the basis of smoothness, and the structure showed a fine and uniform

honeycomb-like structure. They also reported that tofu coagulated with calcium sulphate gave a structure similar to that obtained with GDL.

Figures 11a and 11b show the SEM micrographs of tofu prepared from micronized beans using citric acid as coagulating agent. The structure is similar to the structure of tofu prepared from unprocessed beans but in this case, it is less uniform and the holes are larger. At higher magnification (x 2000)(Figure 11b), the structure of tofu obtained from micronized beans using citric acid as coagulating agent showed a lack of regularity and continousity associated with that of unprocessed beans. The structure of tofu obtained from micronized beans using a mixture of citric acid and calcium sulphate as coagulating agents showed a "rough-spongy" like structure (Figures 12a and 12b).

The results from SEM investigations show that there are differences in the microstructure of tofu prepared from the unprocessed and the micronized beans.

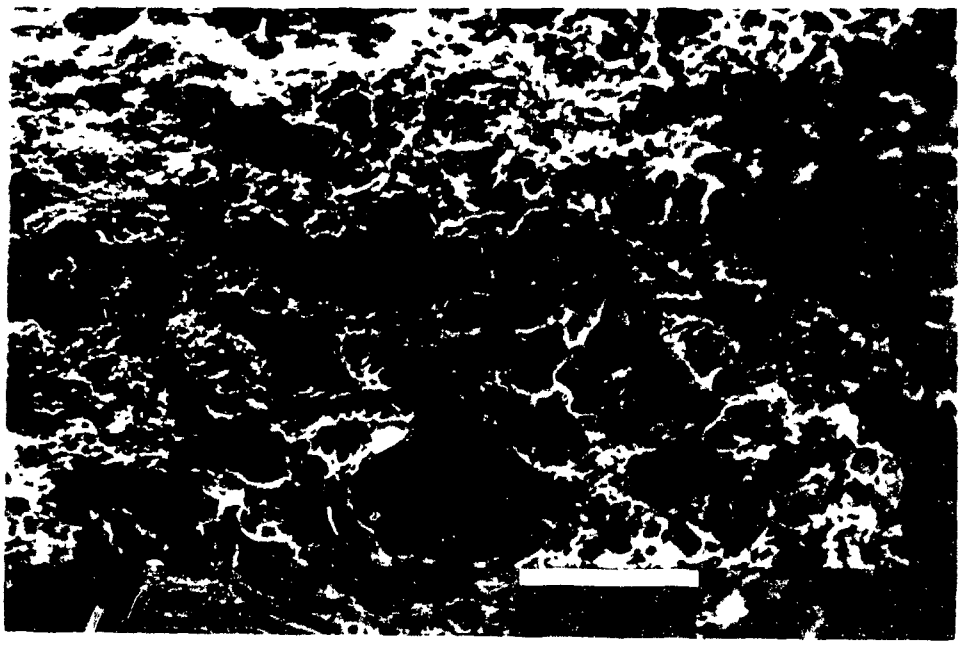


Figure 9a. SEM photomicrograph of tofu obtained from unprocessed beans using calcium sulphate as coagulating agent (Mag. x 500) (White bar = 40 u).

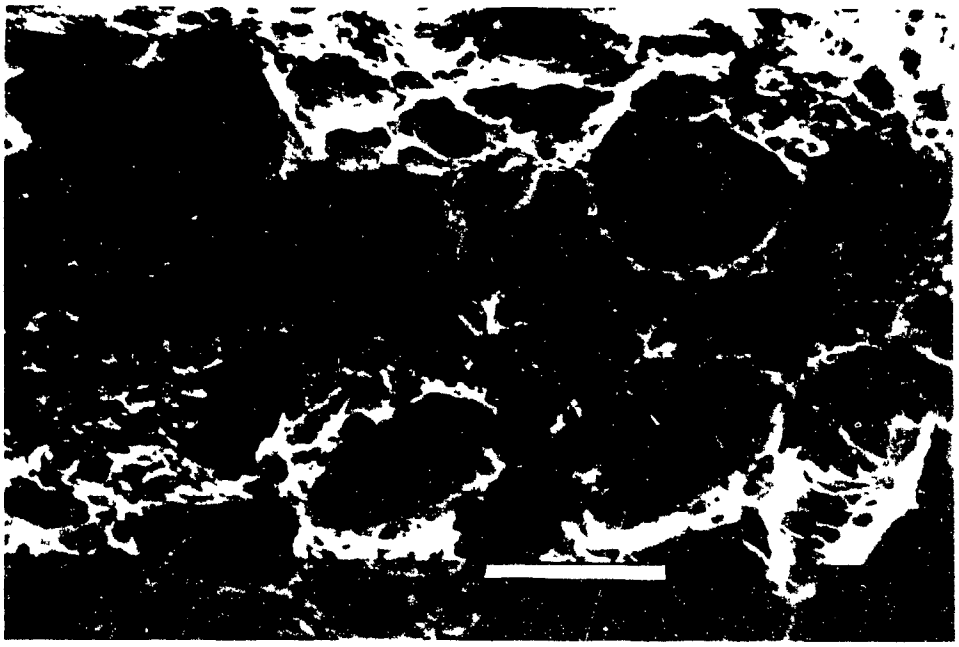


Figure 9b. SEM photomicrograph of tofu obtained from unprocessed beans using calcium sulphate as coagulating agent (Mag. x 2000) (White bar = 10 u).

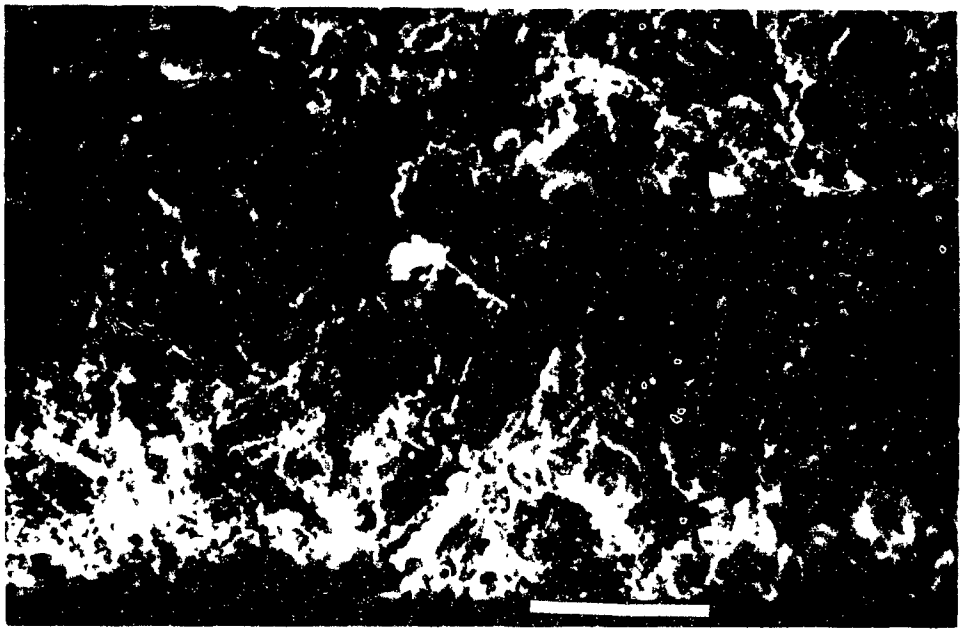


Figure 10a. SEM photomicrograph of tofu obtained from micronized beans using calcium sulphate as coagulating agent (Mag. x 500) (White bar = 40 u).

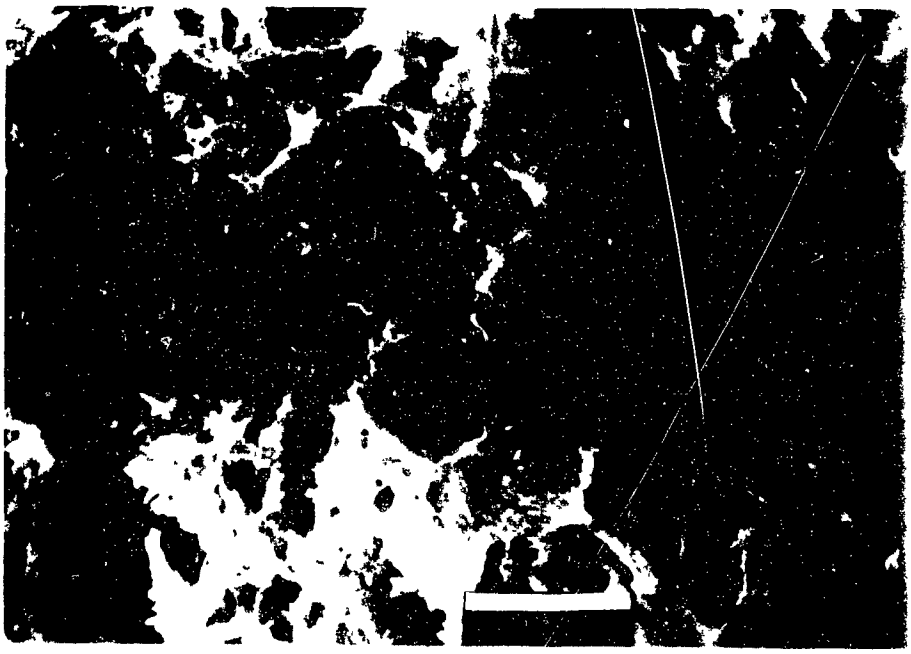


Figure 10b. SEM photomicrograph of tofu obtained from micronized beans using calcium sulphate as coagulating agent (Mag. x 2000) (White bar = 10 u).

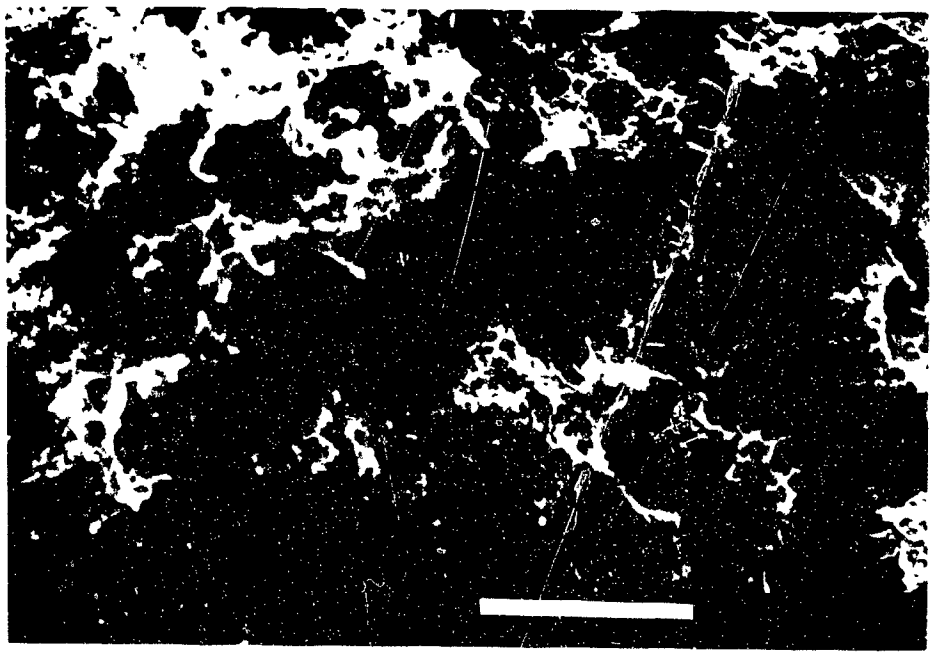


Figure 11a. SEM photomicrograph of tofu obtained from micronized beans using citric acid as coagulating agent (Mag. x 500) (White bar = 40 u).

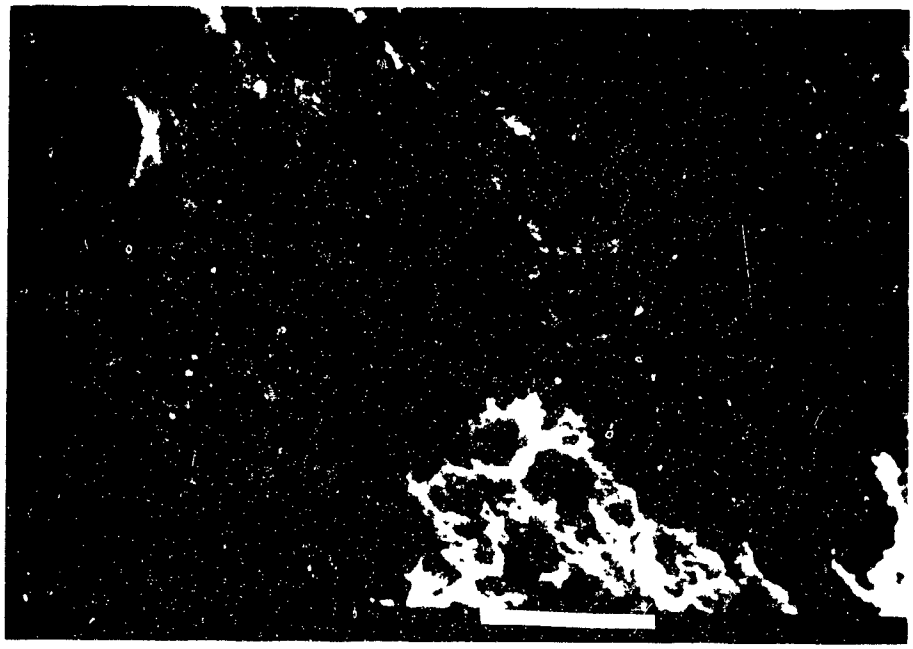


Figure 11b. SEM photomicrograph of tofu obtained from micronized beans using citric acid as coagulating agent (Mag. x 2000) (White bar = 10 u).

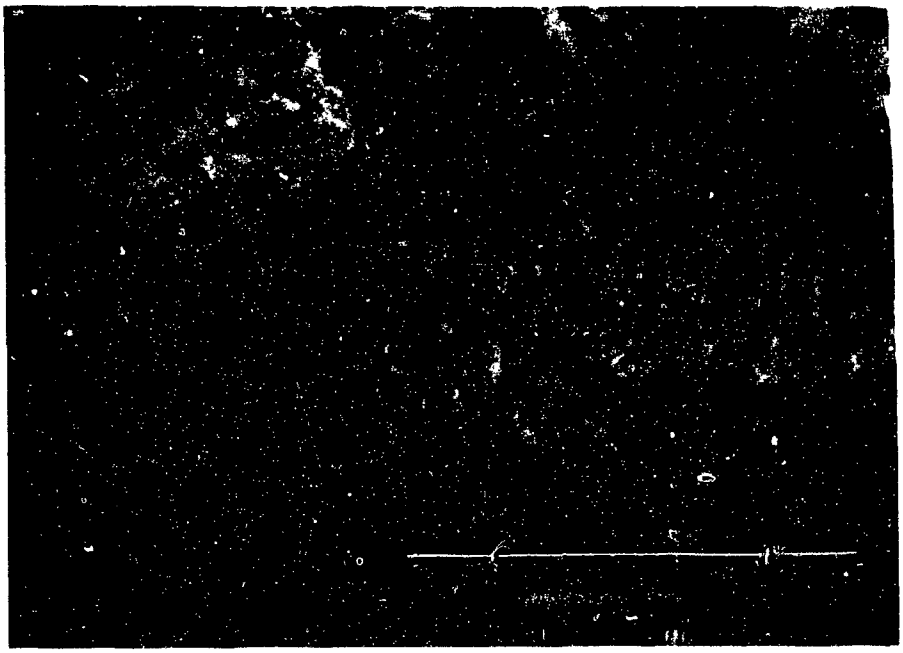


Figure 12a. SEM photomicrograph of tofu obtained from micronized beans using a mixture of citric acid and calcium sulphate as coagulating agent (Mag. x 500) (White bar = 40 u).

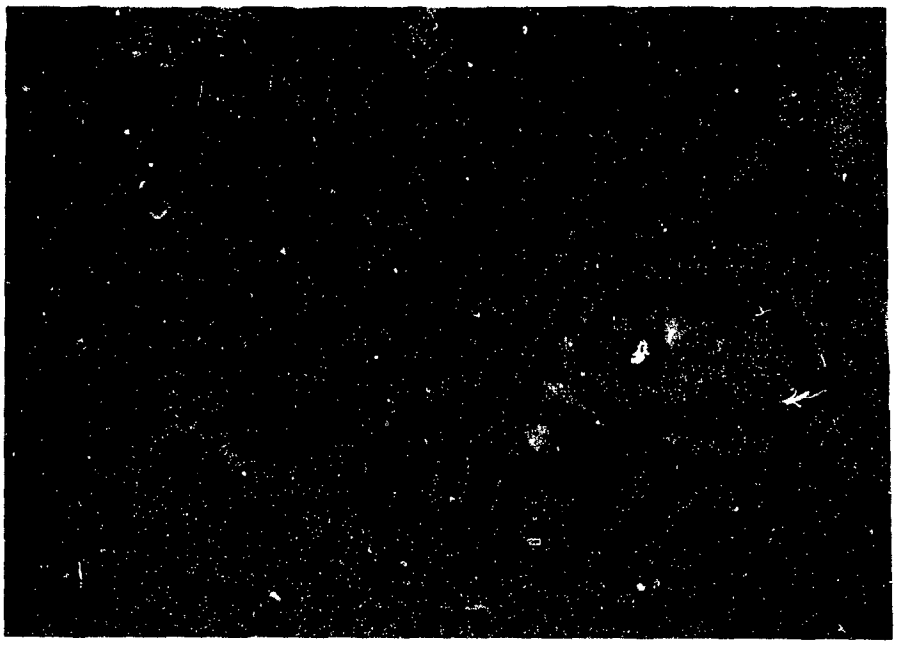


Figure 12b. SEM photomicrograph of tofu obtained from micronized beans using a mixture of citric acid and calcium sulphate as coagulating agent (Mag. x 2000) (White bar = 10 u).

4.0: Effect of micronization on nutritional quality of soybean and derived products

4.1: In vitro digestibility

The effect of micronization on the *in vitro* protein digestibilities of full-fat soybeans, soy isolate, soymilk and tofu are shown in Table 26. The plot of the pH versus time of the *in vitro* digestibility of unprocessed and micronized soybeans and derived products are shown in Figures 13, 14, 15 and 16. The results indicate that the digestibility of micronized soybean was higher than that of the unprocessed soybean. This suggests that the heat treatment of the micronization process resulted in improved digestibility. The lower protein digestibility of the unprocessed soybean when compared to the micronized soybean could be due to the presence of the trypsin inhibitors (Liener, 1981). Heat inactivation of trypsin inhibitor can lead to improved protein digestibility (Nyotu *et al*, 1986). The soy protein isolate, soymilk and tofu prepared from the unprocessed and the micronized soybeans showed somewhat similar digestibilities. Soy protein isolate of both unprocessed and micronized beans showed a higher digestibility than soy bean flour of the unprocessed beans. With soy flours, the processing conditions or the presence of certain compounds, which were not present in soy isolate could be factors responsible for the lower digestibility (Hopkins, 1980).

Kakade (1974) reported that the susceptibility of proteins to proteolytic digestion depends on the availability of amino acid residues which are compatible with enzyme specificity. The digestibilities of the soybean, soy protein isolate and soymilk from unprocessed and micronized soybeans were lower than that of casein (88.2%). However, the digestibilities of tofu obtained from unprocessed (97.2%) and micronized beans (96.7%) were significantly higher ($p<0.05$) than that of casein. Protein digestibility is a measurement of rate of protein hydrolysis by digestive enzyme (Kakade, 1974). Tofu was produced from soymilk which was heated and could result in inactivation of toxic factors (Del Valle, 1981). The high

temperature (100°C) used for heating the soymilk prior to preparation of tofu as well as high temperature (110-115°C) used for the micronization process of the full-fat soybeans may have been factors resulting in improved protein digestibility. Heating of a protein above 50°C results in disruption of secondary and tertiary structure and is termed heat denaturation (Finnigan and Lewis, 1985). A change in the tertiary structure of a protein molecule exposes the enzyme susceptible bonds with a resultant increased rate of protein hydrolysis (Kakade, 1974). In addition, the increase in digestibility observed in the micronized soybeans could have been the result of the action of heat on materials such as carbohydrates and lipids which could be involved in blocking the sites for enzyme attack (Nyotu, 1986).

Table 26. In vitro digestibility of casein, unprocessed and micronized soybeans and soybean derived products

Sample	Digestibility (%)[1]	
	Unprocessed	Micronized
Soybean	76.5 (0.93)	84.3 (0.00)
Soy isolate	89.5 (3.72)	85.3 (0.85)
Soymilk	83.2 (0.27)	86.5 (0.84)
Tofu	97.2 (1.10)	96.7 (1.21)
Casein	88.2 (0.42)	

[1]Results are means (standard deviations) of triplicate determinations

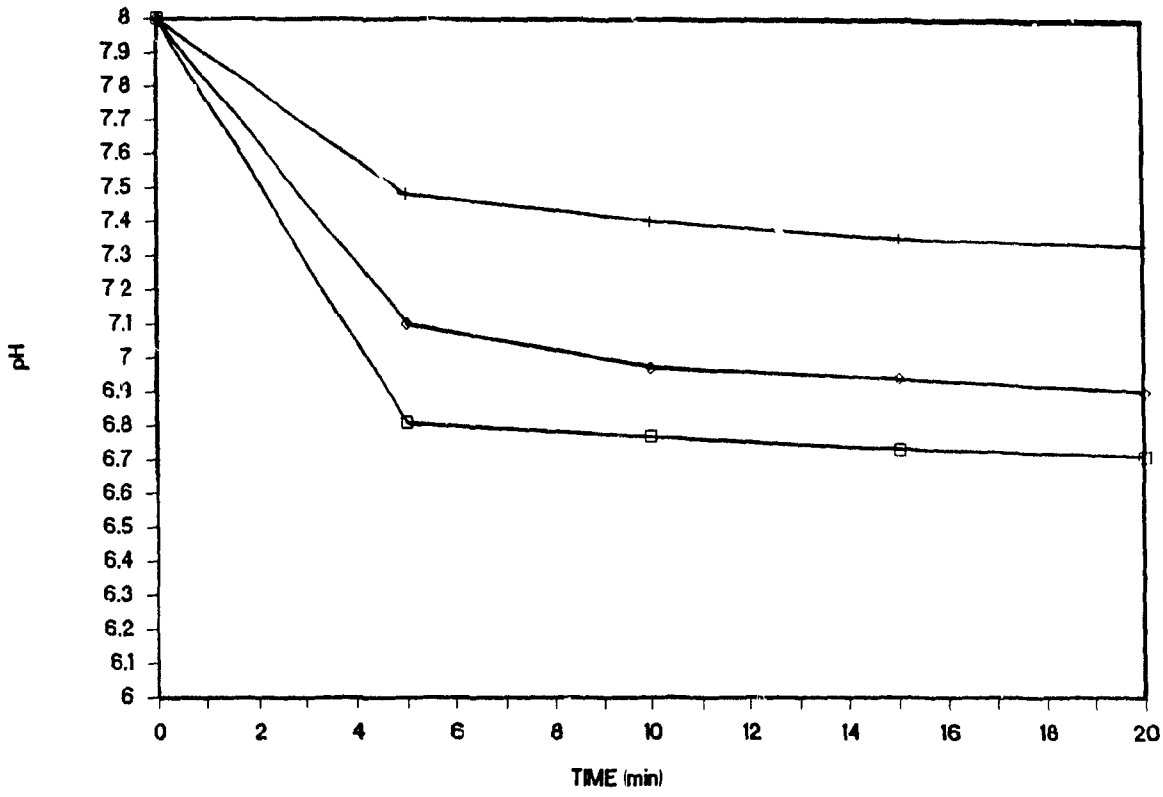


Figure 13. pH vs time curves for determination of *in vitro* protein digestibility of unprocessed and micronized soybeans and casein.

Casein
Unprocessed soybean
Micronized soybean

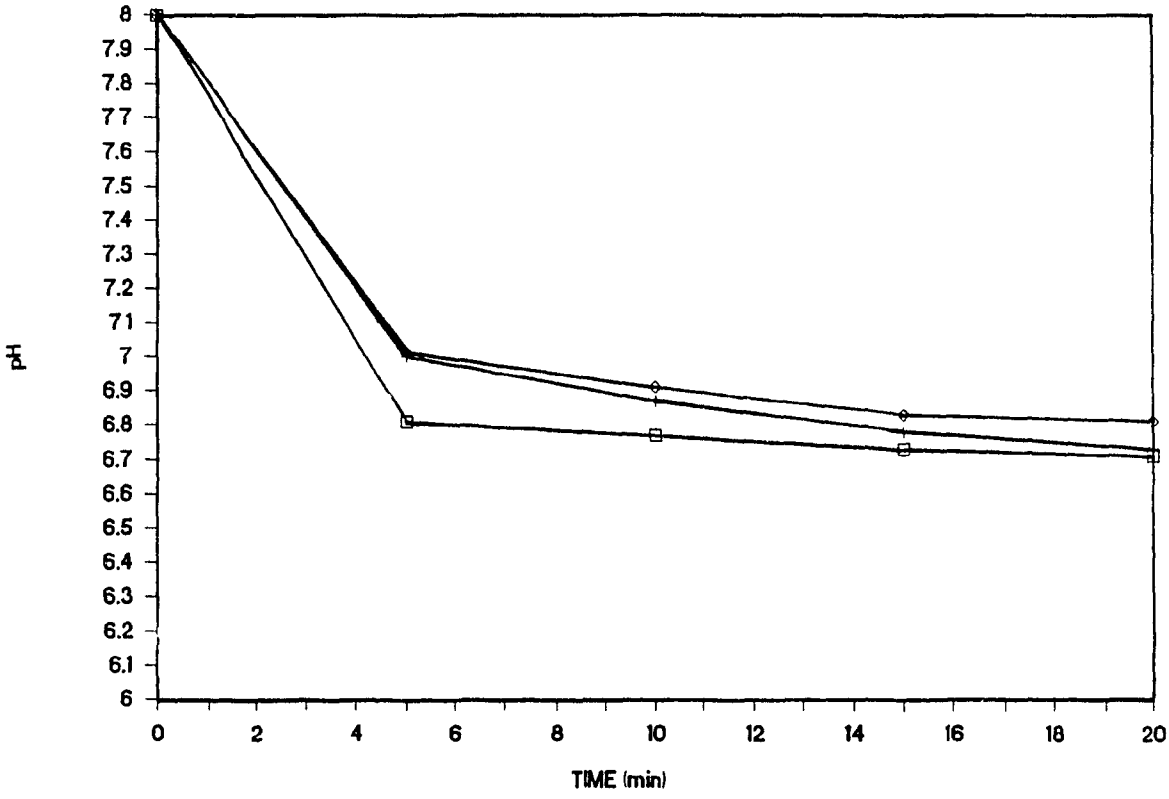


Figure 14. **pH vs time curves for determination of *in vitro* protein digestibility of casein and soy isolate prepared from unprocessed and micronized soybeans.**

- Casein
- Unprocessed
- Micronized

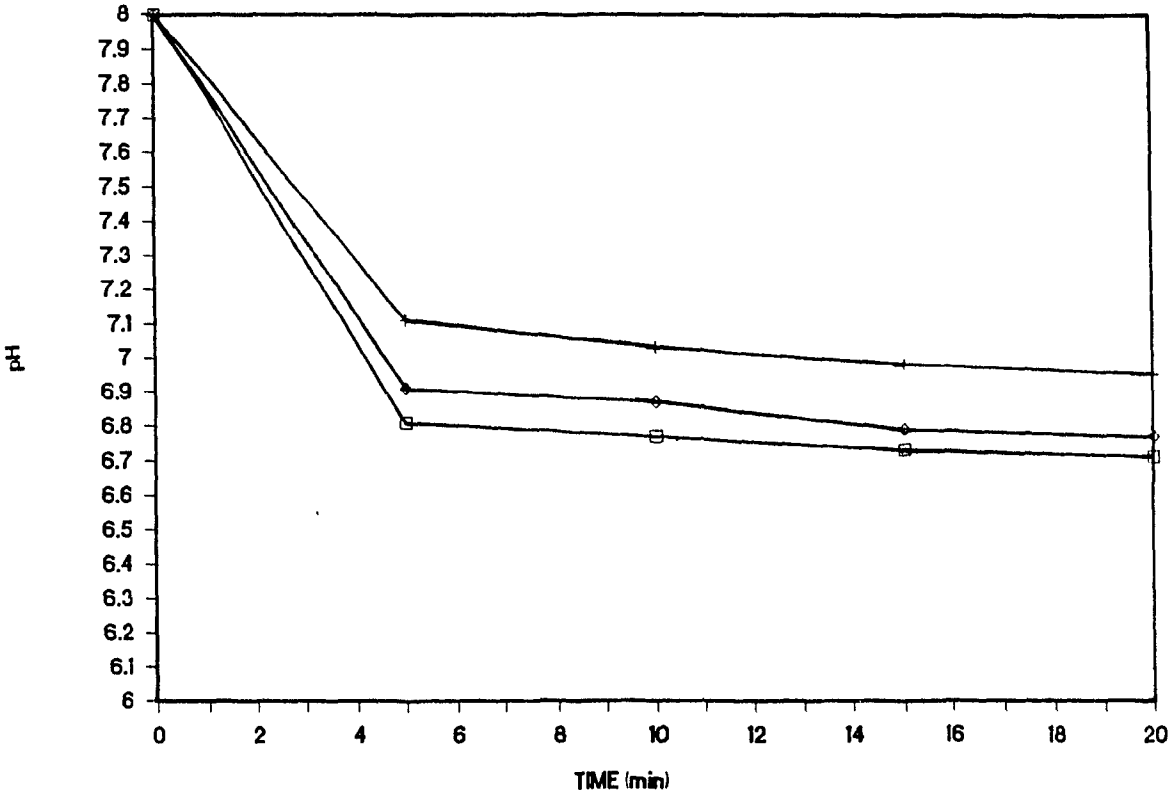


Figure 15. **pH vs time curves for determination of *in vitro* protein digestibility of casein and soymilk obtained from unprocessed and micronized soybeans.**

Casein
Unprocessed
Micronized

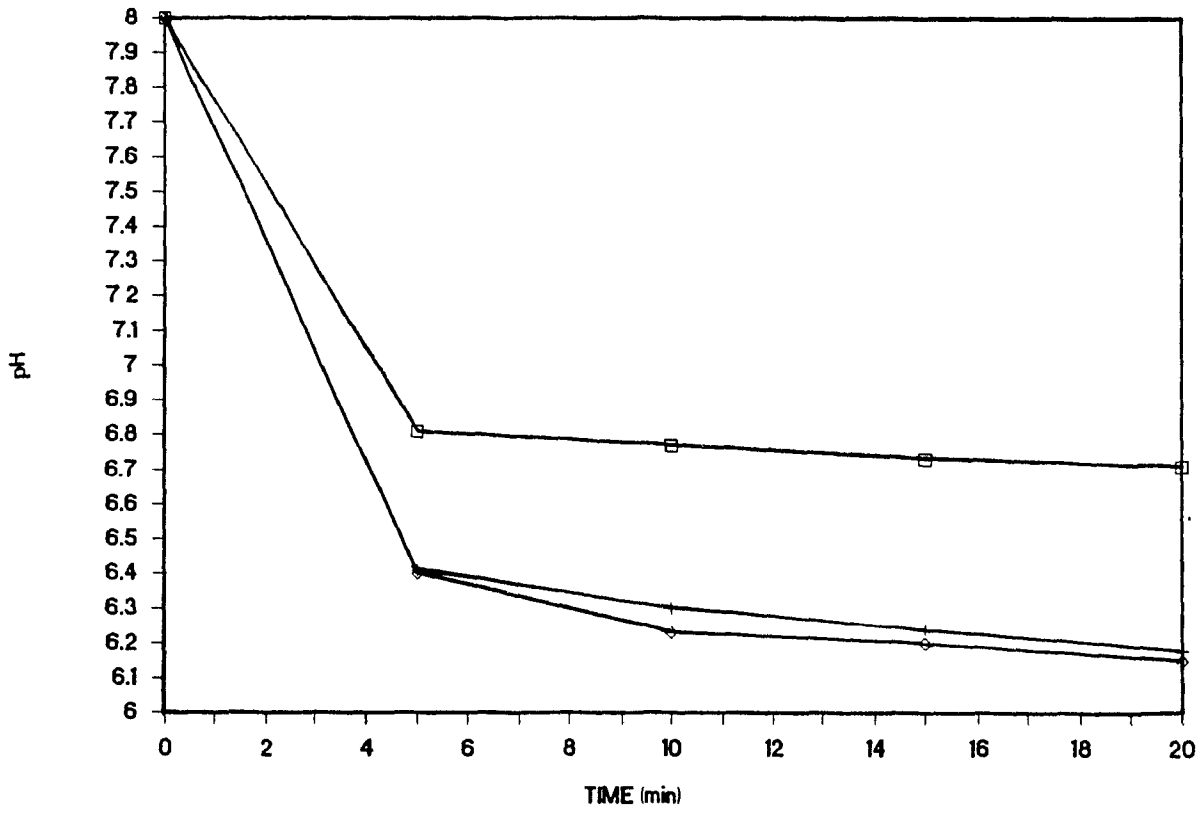


Figure 16. pH vs time curves for determination of *in vitro* protein digestibility of casein and tofu prepared from unprocessed and micronized soybeans.

Casein
Unprocessed
Micronized

4.2: Available lysine

The effect of micronization on the available lysine contents of full-fat soybean, soy isolate, soymilk and tofu is shown in Table 27. The results indicate that heat treatment of the micronization process has little effect on the available lysine content of the soybeans. The quantity of available lysine is generally regarded as indicator of overheating since it is reduced by excessive heat (Kouzeh-Kanani *et al.*, 1981). Shiau *et al.* (1976) reported a destruction of 15% to 23% lysine in sorghum at elevated temperatures. Lawrence (1978) reported 1.76% available lysine (on dry matter basis) in raw soybeans compared with 2.18% of micronized soybeans. In the present work, the available lysine content found in unprocessed and micronized soybeans were 3.74% and 3.71%, respectively. These results indicated that the heat treatment of micronization did not result in the reduction of available lysine. The available lysine contents of soy isolate (5.50%). soymilk (6.14%) and tofu (6.60%) from micronized beans were higher than those of these corresponding products derived from unprocessed beans (soy isolate, 3.14%; soymilk, 4.64%; tofu, 5.94% available lysine).

The factors which influence the loss of available lysine include the type of carbohydrate present in the foodstuffs, the temperature at which the foodstuff is heated and the time of heating (Smith and Friedman, 1984); these workers reported that heating casein at 121^{O}C for 1 h in the presence of glucose, sucrose and starch resulted in 54%, 66% and 20% decrease in lysine availability, respectively. In this study, the temperature of the micronization process was 110^{O}-115^{O}C, and the time of heating was 90 seconds. These conditions of heating are relatively mild and could explain the relatively little loss in available lysine which occurred during the infrared heating of the full-fat soybeans.

Table 27. Available lysine content of unprocessed and micronized soybeans and soybean derived products

Sample	Available lysine (g/16g N)[1]	
	Unprocessed	Micronized
Soybean	3.74(0.34)	3.71(0.08)
Soy isolate	3.14(0.30)	5.50(0.12)
Soymilk	4.64(0.11)	6.14(0.30)
Tofu	5.94(0.13)	6.60(0.32)

[1]Results are means (standard deviations) of four determinations (DM basis)

5.0: Effect of micronization on functional properties of soybean and derived products

5.1: Foam capacity

Foam capacities obtained for protein slurries (2% weight/volume) of unprocessed and micronized soybean flour, soy protein isolate and tofu and commercial soy protein isolate (Ardex D) are shown in Table 28. The results indicate that micronization resulted in a reduction in the foam capacity of the soybean flour and the protein isolated from the beans. The unprocessed soybean flour showed foam capacity in the range 116.7-176.7 with maximum capacity at pH 9.0. The micronized soybean flour showed no foam capacity at pH 3.0 and 5.0 and similar foam capacities at pH 7.0 and 9.0. The results obtained for soy protein isolated from the micronized beans were similar to those of the micronized soy flour. In addition, soy protein isolate from unprocessed beans showed negligible foam capacity at pH 5.0. The results suggest that the foaming properties of the unprocessed and micronized soybean flour and soy protein isolates are pH dependent. Since pH affects the overall charge on the protein molecule, it is likely that the unprocessed and micronized soybeans and soy isolates may have different

isoelectric points. Towsend and Nakai (1983) reported that numerous factors including pH, temperature, the presence of salts, sugars and lipids and the protein source affect the foaming behaviour of proteins.

The tofu prepared from unprocessed and micronized soybeans showed no foam capacity over the pH range studied. Hudson (1987) reported that not all proteins exhibit the same foaming properties and suggested that foamability depends on the molecular weight, surface hydrophobicity and internal bonding of the protein molecule. As heating is involved in preparation of tofu, the temperature ($100^{o}C$) used in this study may play an important role in determining the nature of the proteins solubilised. The commercial soy isolate showed foam capacity ranging from 121.7 to 146.7 over the range of pH values studied (3 to 9). The foam capacity of the commercial soy isolate was higher than that of the soy isolate of unprocessed and micronized beans over the range of the pH values.

Table 28. The effect of pH on the foam capacity (%) of unprocessed and micronized soybean and soybean derived products[1]

Sample	Foam capacity			
	pH 3.0	pH 5.0	pH 7.0	pH 9.0
Soy bean flour				
Unprocessed	116.7(5.77)[2]	136.7(2.89)	146.7(2.89)	176.7(12.58)
Micronized	NF	NF	106.7(2.89)	110.0(5.0)
Soy isolate				
Unprocessed	118.3(2.89)	NF	116.7(2.89)	120.0(5.0)
Micronized	NF	NF	116.7(2.89)	116.7(2.89)
Tofu				
Unprocessed	NF	NF	NF	NF
Micronized	NF	NF	NF	NF
Soy isolate[3] (Ardex D)	121.7(7.64)	141.7(10.41)	138.3(5.77)	146.7(5.77)

[1]2% protein dispersion
[2]Results are means (standard deviations) of triplicate measurements
[3]Commercial soy protein isolate
NF: no foaming

5.2: Foam stability

Table 29 shows the foam stability of the protein slurries (2% weight/volume) of the unprocessed and micronized soybeans, soy isolate and tofu and commercial soybean protein isolate (Ardex D). The results show that micronization resulted in reduction of foam stability of the soybean flour and the protein isolated from the beans. Tofu of both unprocessed and micronized beans exhibit no foaming properties over the pH range studied (3.0 to 9.0). Foam stability values ranged from 90 to 99 for the unprocessed and micronized soybean flour, and 94 to 100 for the commercial soy protein isolate. The micronized soybean flour showed no foaming properties at pH 3.0 and 5.0. Maximum foam stabilities values were 99 and 100 obtained at pH 3.0 for unprocessed soybean flour and commercial soy isolate,

respectively and 96 at pH 7.0 for micronized soybean isolate. Hudson (1987) reported that proteins stabilise foams by forming a flexible, cohesive film around air bubbles, as the pH moves away from the isoelectric point (pI), net charges increase and film strength and foam stability decrease. At pH 5.0, the foam stability of the commercial soy protein isolate was different from that of the soy isolates prepared from unprocessed and micronized soybeans. The soy isolate investigated in this study was obtained from full-fat soybeans, while the commercial soy isolate is obtained from defatted meal. The presence of lipid materials in soy preparations can destabilize the protein foams; hexane and aqueous alcohol treatment of soy proteins which remove neutral and bound polar lipids, respectively, markedly enhance foaming properties (Glabe *et al.*, 1956). Treatment of commercial soy preparations with aqueous alcohol significantly improved their foaming properties (Eldridge *et al.*, 1963).

Table 29. The effect of pH on foam stability (%) of unprocessed and micronized soybean and soybean derived products[1]

Sample	Foam stability			
	pH 3.0	pH 5.0	pH 7.0	pH 9.0
Soybean				
Unprocessed	99.2(1.31)[2]	92.7(0.16)	90.9(4.05)	96.2(1.67)
Micronized	NF	NF	96.9(1.51)	92.5(2.40)
Soy isolate				
Unprocessed	98.6(2.41)	NF	94.3(2.57)	93.2(4.47)
Micronized	NF	NF	92.9(2.41)	95.5(0.12)
Tofu				
Unprocessed	NF	NF	NF	NF
Micronized	NF	NF	NF	NF
Soy isolate[3] (Ardex D)	100.0(0.0)	94.0(2.32)	97.6(2.07)	100.0(0.0)

[1]2% protein dispersion
[2]Results are means (standard deviations) of triplicate measurements
[3]Commercial soy isolate
NF: No foaming

5.3: Water absorption

In general, there was little effect of micronization on water absorption capacity of soybean flour, soy isolate and tofu (Table 30). The water absorption capacities of the unprocessed soybean flour, soy isolate and tofu were 264.1%, 163.2% and 360.6%, respectively and of the micronized soybean flour, soy isolate and tofu were 284.6%, 166.1% and 358.4%, respectively. The results show that the infrared heat of micronization did not lower the imbibing capacities of soybeans and the derived products.

A marked difference in water absorption capacity was observed for the unprocessed and micronized derived soy products and the commercial soy protein isolate. The commercial soy isolate had a water absorption capacity (596.4%)

approximately twice that of the unprocessed and micronized soybean flour and tofu, about 3 times that of unprocessed and micronized soy protein isolate. This higher water absorption capacity of the commercial soy protein isolate suggests that it is more hydrophillic in nature than the unprocessed and micronized derived soy products. Difference in soy preparations between commercial and laboratory prepared soy isolate may also attribute to the marked difference in the water absorption capacity between the two samples; commercial soy isolate is prepared from defatted meal while the laboratory prepared soy isolate was obtained from full-fat unprocessed and micronized soybeans.

Table 30. Water absorption (%) of unprocessed and micronized soybeans and soybean derived products

Sample	Water absorption (%)	
	Unprocessed	Micronized
Soybean flour	264.1(2.97)[1]	284.6(2.42)
Soy isolate	163.2(3.95)	166.1(4.05)
Tofu	360.6(1.57)	358.4(7.27)
Soy isolate[2] (Ardex D)	596.4(6.62)	

[1]Results are means (standard deviations) of triplicate analyses
[2]Commercial soy protein isolate

5.4: Fat absorption

The fat absorption capacities of the unprocessed and micronized soybean flour, soy isolate and tofu and commercial soy protein isolate (Ardex D) are shown in Table 31. The results indicated that the infrared heat of micronization had no effect on the fat absorption capacity of the soybeans and the derived products. The

fat absorption of the unprocessed soybean flour (199.3%) was similar to that of micronized soybean flour (192.7%). Hutton and Campbell (1977) and Lin and Humbert (1974) reported that fat absorption by soy preparations is closely related to protein content and is little affected by pH or temperature.

The unprocessed and micronized soybeans and derived products had oil absorption values ranging from 192.7% for the flour to 430.4% for the isolate (Table 31). Both the unprocessed and micronized soy isolate and tofu bound more oil than the commercial soy protein isolate (223.3%). This suggests that unprocessed and micronized soy isolate and tofu are more lipophilic than the commercial soy isolate and the unprocessed and micronized soybean flour. Lin and Humbert (1974) reported that soy products had oil absorption values ranging from 84.4% to 154.5%, the author also reported that all sunflower products bound more oil than soy products and suggested that it could be likely that sunflower proteins contain numerous nonpolar side chains that have been believed to bind the paraffin chains (hydrocarbon chains) of fats (Przylecki *et al.*, 1935), thereby, contributing to higher absorption of oil. The same mechanism could probably explain the higher absorption of oil of the unprocessed and micronized soy isolate and tofu when compared to unprocessed and micronized soybean flours and commercial soy isolate.

Table 31. Fat absorption (%) of unprocessed and micronized soybeans and soybean derived products

Sample	Fat absorption (%)	
	Unprocessed	Micronized
Soybean flour	199.3(0.76)[1]	192.8(11.32)
Soy isolate	399.2(0.06)	430.4(10.15)
Tofu	419.7(0.05)	421.8(5.84)
Soy isolate[2] (Ardex D)	223.36(3.26)	

[1]Results are means (standard deviations) of triplicate analyses
[2]Commercial soy protein isolate

6.0: Effects of micronization on the biochemical properties of soybean and derived products

The interaction of protein with lipids or with carbohydrates in food and food products are of fundamental importance in food processing. Both interactions could be naturally occurring or chemically or physically induced (Karel, 1973). As these interactions also have significant effect on functional properties such as gelation, water binding capacity, foaming and emulsification (Kinsella, 1979), a better understanding of these interactions could enhance the utilization of various food proteins.

The effects of micronization on the interactions between protein-lipid and protein-carbohydrate in soybean, soy isolate, tofu and soymilk prepared from the unprocessed and micronized beans were investigated using polyacrylamide-disc gel electrophoresis (PAGE) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

6.1: Polyacrylamide-disc gel electrophoresis

Soybean flour:

Electropherograms of proteins obtained from unprocessed (A_1, A_2 and A_3) and micronized soybean flour (B_1, B_2 and B_3) are shown in Figure 17. The results show slight differences in the electrophoretic behaviour of proteins in unprocessed soybean flour (A_1) and micronized soybean flour (B_1). The protein bands (3,4 and 13) obtained for the micronized soybean flour (B_1) are less intense and some of the minor protein bands (7-12) are not present when compared to the electropherogram of the unprocessed soybean flour (A_1). This indicates that heat treatment by micronization has some slight effect on the protein constitution of the soybean. The infrared heat of micronization could cause insolubilization of some soy protein fraction and these insoluble soy proteins would not undergo electrophoresis. This might explain the slight difference in the electrophoretic behaviour between the unprocessed and micronized samples.

The electrophoretic patterns also show that at least two of the protein components of the soybean proteins are bound to carbohydrate as shown by the two pink bands in electropherograms A_2 and B_2. The similarity in electrophoretic behaviour of glycoproteins from the unprocessed (A_2) and micronized (B_2) soybeans suggests that micronization has little effect on the protein-carbohydrate complex present in the soybean flour.

When stained for lipoprotein (electropherograms A_3 and B_3), the results showed that at least three of the soy protein fractions of the micronized bean (B_3) were bound to lipid while for the unprocessed soybean flour (A_3) only a single lipoprotein band was observed. This suggests that heating by micronization induce some interactions of protein with lipid component in the soybeans.

Soy protein isolate:

Figure 18 shows the electropherograms of proteins obtained from the soy protein isolate of unprocessed bean (C_1, C_2 and C_3) and micronized bean (D_1, D_2 and D_3). The results showed that there was no difference in behaviour of the protein isolates from unprocessed and micronized beans (C_1 and D_1). The electrophoretic behaviour of protein fractions in soy isolate from unprocessed bean (C_1) and from micronized beans (D_1) were similar both in the number (10) and intensity of the bands and in relative mobilities. The importance of this is that it indicates that micronization has no effect on the behaviour of the proteins that are extractable during the protein isolation. As the soy isolate is the primary soy products used as protein ingredients in food systems (Kinsella, 1979), this result suggests that soy isolate from micronized beans could be just as useful as protein ingredients as soy isolate from the unprocessed beans.

The results also show that at least two protein fractions of the soy isolate of the unprocessed (C_2) and micronized (D_2) beans are bound to carbohydrate. Both electropherograms (C_2 and D_2) showed similar electrophoretic patterns, indicating that micronization had little effect on the protein-carbohydrate interactions in the soy protein isolates.

Electropherograms C_3 and D_3 showed that at least one protein fraction in the unprocessed soy isolate (C_3) and two protein fractions in the micronized soy isolate (D_3) were bound to lipid. This indicates that micronization has an effect on the interactions between the protein and lipid present in the soy protein isolate.

Soymilk:

Electropherograms of proteins obtained from the soymilk prepared from unprocessed (E_1 and E_2) and micronized (F_1 and F_2) are shown in Figure 19. The results showed that there was no difference in the behaviour between proteins of soymilk prepared from unprocessed (E_1) and micronized (F_1) soybeans. This

indicates that micronization has no effect on the protein components of soymilk. Most of the protein materials remaining in the soymilk of both unprocessed and micronized beans appeared as unresolved diffused bands (E_2 and F_2), and these unresolved protein components are bound to carbohydrate as shown by the diffused glycoprotein bands (pink) in electropherograms of soymilk from unprocessed (E_2) and micronized (F_2) beans. No lipoprotein band was observed in soymilk of both unprocessed and micronized beans. This suggests that the process for preparation of soymilk from the unprocessed and micronized soybeans affected the protein-lipid interactions in the soymilk.

Tofu:

Figure 20 shows the electropherograms of proteins obtained from tofu prepared from unprocessed (G_1 and G_2) and micronized (H_1 and H_2) beans. The results show that the electrophoretic behaviour of protein fractions of the tofu from unprocessed (G_1) and micronized (H_1) beans were similar. One of the major protein fractions of tofu from both of unprocessed and micronized beans was bound to the carbohydrate as shown by the single glycoprotein (pink) band in electropherograms G_2 and H_2. No lipoprotein bands were observed in proteins of tofu from unprocessed and micronized beans. This indicated that either the protein-lipid complexes component was not precipitated by the coagulant ($CaSO_4.2H_2O$) or the process for preparation of the tofu affected the interactions between the protein and lipid components.

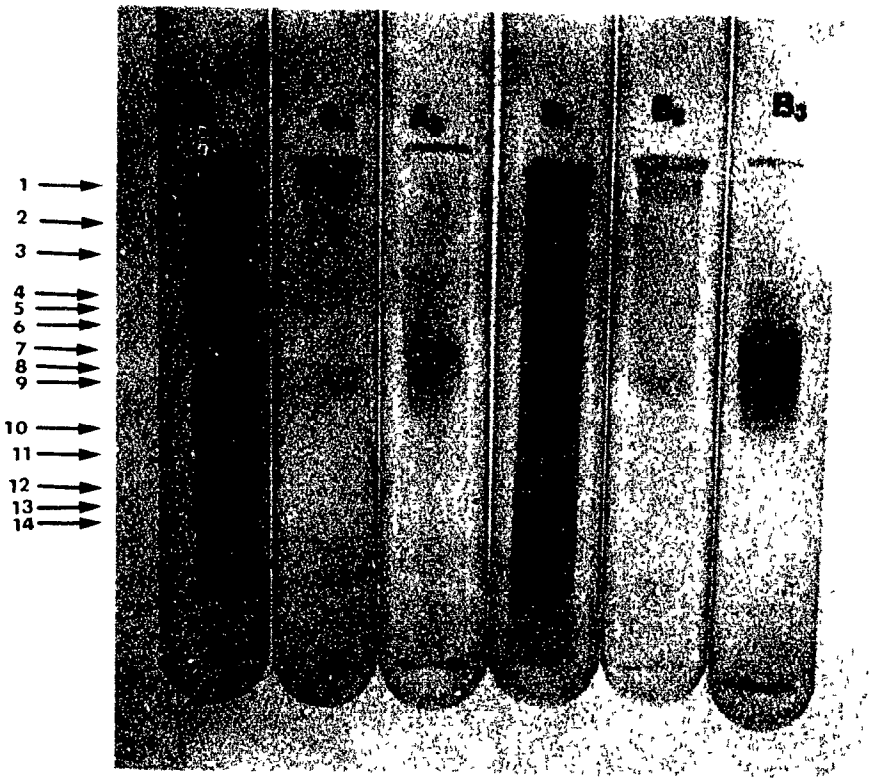


Figure 17. Electropherograms for polyacrylamide disc gel electrophoresis with tris aminoethane-glycine buffer (pH 8.9).

A_1, A_2, A_3 : Soybean flour from unprocessed beans
B_1, B_2, B_3 : Soybean flour from micronized beans

Gels of each protein sample were stained for protein (A_1, B_1), glycoprotein (A_2, B_2) and for lipoprotein (A_3, B_3).

Figure 18. Electropherograms for polyacrylamide disc gel electrophoresis with tris aminoethane-glycine buffer (pH 8.9).

C_1, C_2, C_3 : Soy protein isolate from unprocessed beans
D_1, D_2, D_3 : Soy protein isolate from micronized beans

Gels of each protein sample were stained for protein (C_1, D_1), glycoprotein (C_2, D_2) and for lipoprotein (C_3, D_3).

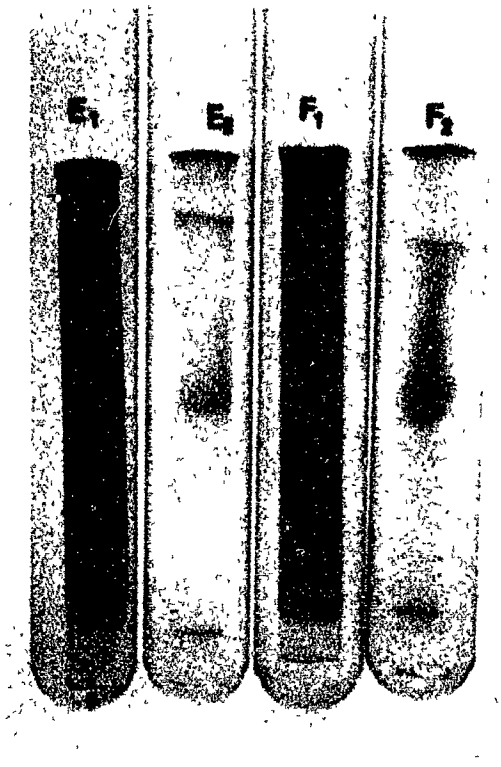


Figure 19. Electropherograms for polyacrylamide disc gel electrophoresis with tris aminoethane-glycine buffer (pH 8.9).

E_1, E_2 : Soymilk from unprocessed beans
F_1, F_2 : Soymilk from micronized beans

Gels of each protein sample were stained for protein (E_1, F_1) and for glycoprotein (E_2, F_2).

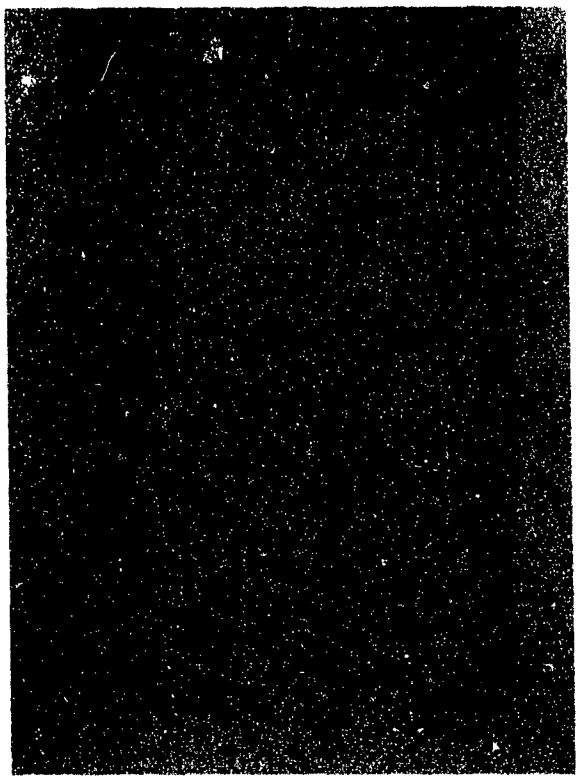


Figure 20. Electropherograms for polyacrylamide disc gel electrophoresis with tris aminoethane-glycine buffer (pH 8.9).

G_1, G_2 : Tofu from unprocessed beans
H_1, H_2 : Tofu from micronized beans

Gels of each protein sample were stained for protein (G_1, H_1) and for glycoprotein (G_2, H_2).

6.2: SDS-Electrophoresis

Phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme with molecular weights of 97000, 66200, 42699, 31000, 21500 and 14400 daltons, respectively were used as molecular weight markers. A linear regression equation (r = -0.9954) was generated from the plot (Figure 21) of the log of the molecular weight of the standard proteins versus their migration distances (Weber and Osborne, 1972).

$$Y = 5.2022 - 0.0163X$$

where Y represents the molecular weight

and X the migration distance

In the present work, the various protein preparations of unprocessed and micronized soybeans and the derived products were subjected to electrophoresis in the presence of SDS; and gels were stained for proteins, glycoproteins and lipoproteins. However, when stained for lipoprotein, no lipoprotein band was observed in any of the unprocessed and micronized soybeans and the derived products. This suggests that interaction between protein and lipid present in the unprocessed and micronized soybeans and derived products might be affected by the presence of the SDS.

Figure 22 shows the electrophoretic patterns obtained by electrophoresis of the protein standards (electropherogram s), the unprocessed (electropherograms a_1, c_1, e_1 and g_1) and micronized (electropherograms b_1, d_1, f_1 and h_1) soybeans, and the derived products in the presence of SDS. The molecular weights of the unprocessed and micronized soybeans and the derived products ranged from 11954 to 113632 daltons.

Soybean flour:

SDS-PAGE electrophoresis revealed the presence of at least eleven subunits for both the unprocessed (electrophoragram a_1) and micronized (electropherogram

b_1) soybean flours (Figure 23). The molecular weights ranged from 12885 to 117978 daltons (Table 33). Three of the bands represented major protein components; these major components and one minor component appeared to be glycoproteins (electropherograms a_2 and b_2). The results indicate that the electrophoretic patterns of the proteins obtained from the unprocessed and micronized soybean flours were somewhat similar. This suggests that micronization does not have an effect on the cleavage of peptide bonds of protein.

Soy protein isolate:

Figure 24 shows the electropherograms obtained from electrophoresis of soy protein isolate of unprocessed (electropherograms c_1 and c_2) and micronized (electropherograms d_1 and d_2) soybeans in the presence of SDS. The electrophoretic pattern (electropherogram c_1) of the unprocessed soy protein isolate showed at least eleven bands. The molecular weights ranged from 13378 to 97791 daltons (Table 34). Three of the bands represented major protein components; two of the major proteins appeared to be glycoproteins (electropherograms c_2). The electrophoretic pattern (electropherogram d_1) of the micronized soy protein isolate shows at least twelve bands with molecular weights ranging from 13890 to 117978 daltons (Table 34). Three of the bands represented major protein components; one of the major protein appeared to be glycoprotein (electropherograms d_2). The results indicate that the electrophoretic patterns of the proteins obtained from the soy protein isolate of unprocessed and micronized beans were somewhat different. This suggests that micronization might have a minor effect on the constitution of protein of the soy isolate.

Soymilk:

Figure 25 shows the electropherograms obtained from electrophoresis of soymilk of unprocessed (electropherograms e_1 and e_2) and micronized (electropherograms f_1 and f_2) soybeans in the presence of SDS. The electrophoretic

pattern (electropherogram e_1) of the soymilk of unprocessed beans shows at least eleven bands. The molecular weights ranged from 13890 to 97791 daltons (Table 35). Three of the bands represented major protein components; two of the major proteins appeared to be glycoproteins (electropherogram e_2). The electrophoretic pattern (electropherogram f_1) of the soymilk of micronized beans shows at least twelve bands with molecular weights ranged from 12885 to 113632 daltons (Table 35). Three of the bands represented major protein components; one of the major protein and a minor component appeared to be glycoproteins (electropherogram f_2). The results indicate that electrophoretic patterns obtained from the soymilk of unprocessed and micronized soybeans were somewhat different; this suggests that micronization might have an effect on the nature of proteins in the soymilk.

Tofu:

Figure 26 shows the electropherograms obtained by electrophoresis of tofu of unprocessed (electropherograms g_1 and g_2) and micronized (electropherograms h_1 and h_2) soybeans in the presence of SDS. The electrophoretic pattern (electropherogram g_1) of the tofu of unprocessed beans shows at least ten bands with molecular weights ranging from 11954 to 87378 daltons (Table 36). Three of the bands represented major protein components, one of which appeared to be glycoprotein (electropherogram g_2). The electrophoretic pattern (electropherogram h_1) of the tofu of micronized beans shows at least eleven bands with molecular weights ranging from 11954 to 81058 daltons (Table 36). Three of the bands represented major protein components, one of which appeared to be glycoprotein. The results indicate that electrophoretic patterns of the proteins obtained from unprocessed and micronized tofu were somewhat different; suggesting that micronization might have an effect on the nature of proteins in the tofu.

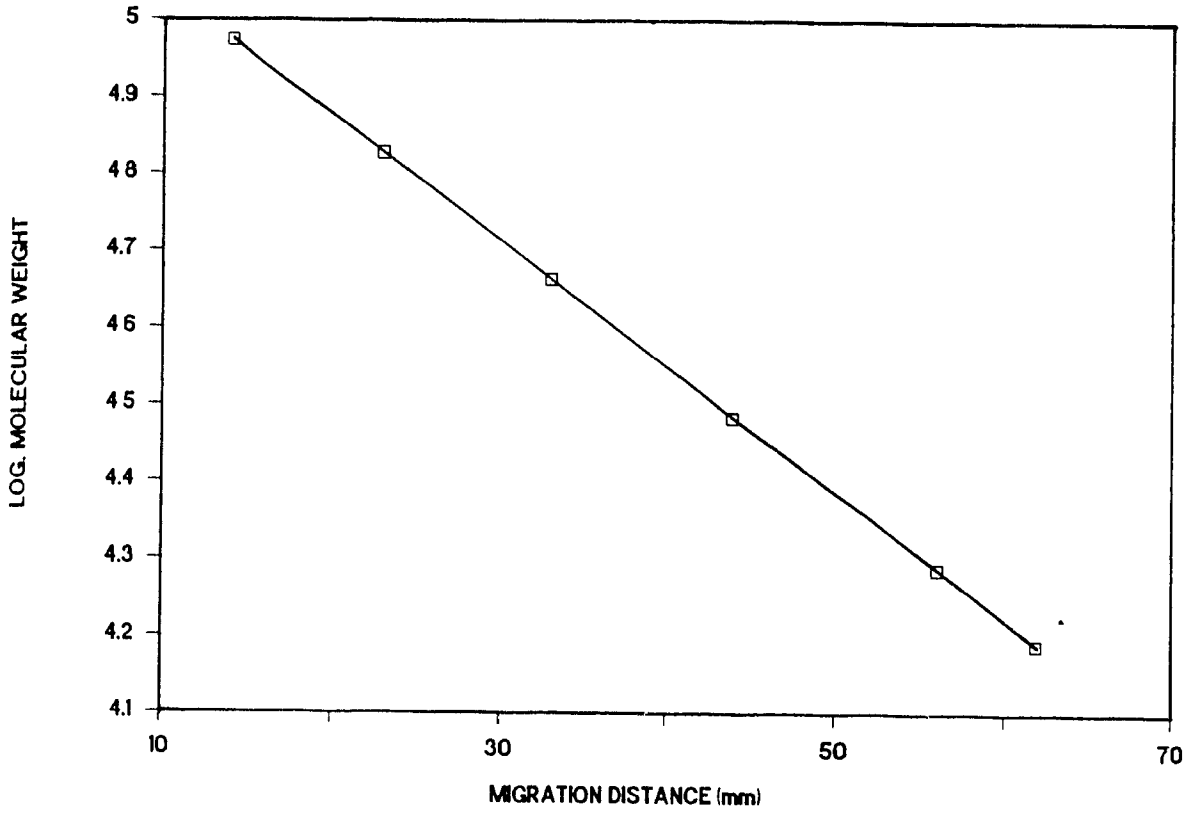


Figure 21. **Plot of log of molecular weight versus migration distance of standard proteins.**

Table 32. Migration distance of six standard proteins

Protein	Molecular weight (Daltons)	Migration Distance (mm)
Lysozyme	14400	62
Soybean trypsin inhibitor	21500	56
Carbonic anhydrase	31000	44
Ovalbumin	42699	33
Bovine serum albumin	66200	23
Phosphorylase b	97400	14

Table 33. Migration distance and molecular weights of the proteins obtained from unprocessed and micronized soybeans

Molecular weight (Daltons)		Migration distance (mm)	
U[1]	M[2]	U[1]	M[2]
12885	12885	67	67
20989	21792	54	53
30549	31718	44	43
36855	35498	39	40
42825	42825	35	35
49762	49762	31	31
67190	67190	23	23
75197	72427	20	21
78073	78073	19	19
90719	90719	15	15
117978	117978	8	8

[1]Unprocessed beans
[2]Micronized beans

Table 34. Migration distance and molecular weights of the proteins obtained from unprocessed and micronized soy protein isolates

Molecular weight (Daltons)		Migration distance (mm)	
U[1]	M[2]	U[1]	M[2]
13378	13890	66	65
20989	20989	54	54
30549	30549	44	44
34190	36855	41	39
44463	44463	34	34
53641	51665	29	30
62330	64714	25	24
67190	69760	23	22
72427	75197	21	20
81059	78073	18	19
97791	01531	13	12
	117978		8

[1]Unprocessed beans
[2]Micronized beans

Table 35. Migration distance and molecular weights of proteins obtained from unprocessed and micronized soymilk

Molecular weight (Daltons)		Migration distance (mm)	
U[1]	M[2]	U[1]	M[2]
13890	12885	65	67
21792	20216	53	55
31718	29424	43	45
36855	34190	39	41
44463	41248	34	36
53641	51665	29	30
67190	62330	23	25
69760	67190	22	23
75197	72427	20	21
78073	75197	19	20
97791	94189	13	14
	113632		9

[1]Unprocessed beans
[2]Micronized beans

Table 36. Migration distance and molecular weights of the proteins obtained from unprocessed and micronized tofu

Molecular weight (Daltons)		Migration distance (mm)	
U[1]	M[2]	U[1]	M[2]
11954	11954	69	69
18754	19472	57	56
28340	28347	46	46
31718	32931	42	43
38265	38265	38	38
46164	47929	33	32
62330	57823	25	27
67190	62330	23	25
72427	69760	22	21
87378	72427	16	21
	81058		18

[1]Unprocessed soybeans
[2]Micronized soybeans

FIgure 22. Electropherograms for sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis of soybeans and derived products.

s : Protein standards (1- phosphorylase b, 2- bovine serum albumin, 3- ovalbumin, 4- carbonic anhydrase, 5- soybean trypsin inhibitor, 6- lysozyme)
a_1: Soy flour (unprocessed)
b_1: Soy flour (micronized)
c_1: Soy isolate (unprocessed)
d_1: Soy isolate (micronized)
e_1: Soymilk (unprocessed)
f_1: Soymilk (micronized)
g_1: Tofu (unprocessed)
h_1: Tofu (micronized)

Gels of each protein sample were stained for protein.

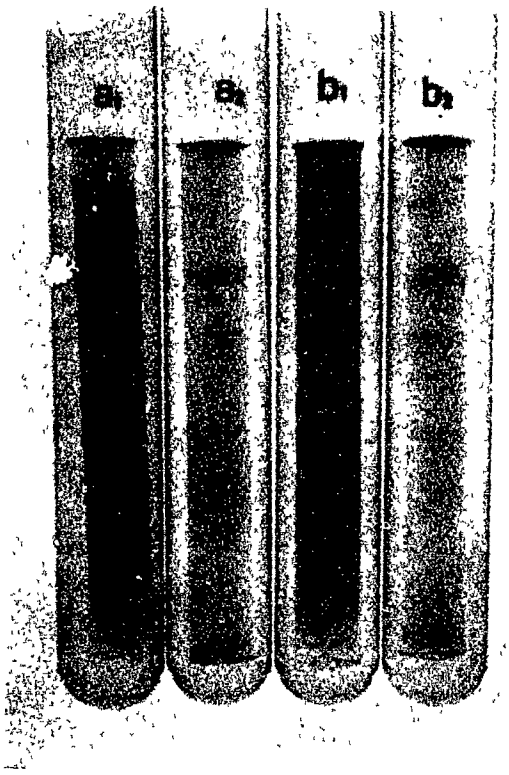


Figure 23. **Electropherograms for sodium dodecyl sulfate- polyacrylamide disc gel electrophoresis of soybean flours.**

a_1, a_2 : Soybean flour (unprocessed)
b_1, b_2 : Soybean flour (micronized)

Gels of each protein sample were stained for protein (a_1, b_1) and for glycoprotein (a_2, b_2).

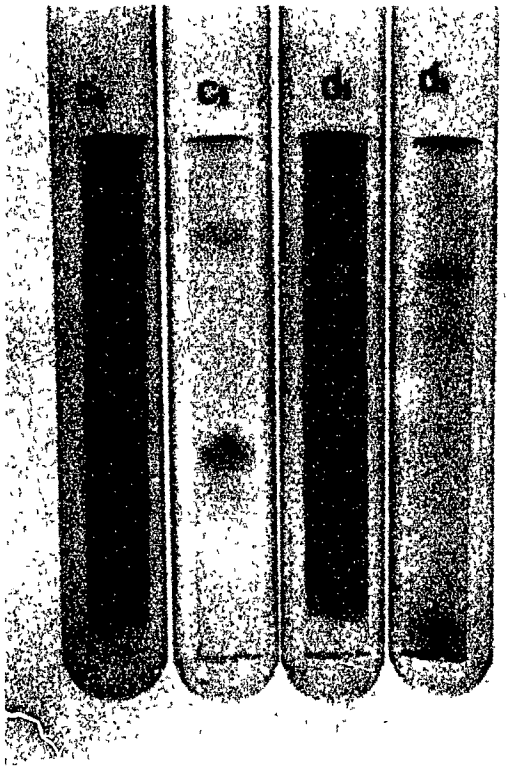


Figure 24. **Electropherograms for sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis of soy protein isolate.**

c_1, c_2 : Soy protein isolate (unprocessed)
d_1, d_2 : Soy protein isolate (micronized)

Gels of each protein sample were stained for protein (c_1, d_1) and for glycoprotein (c_2, d_2).

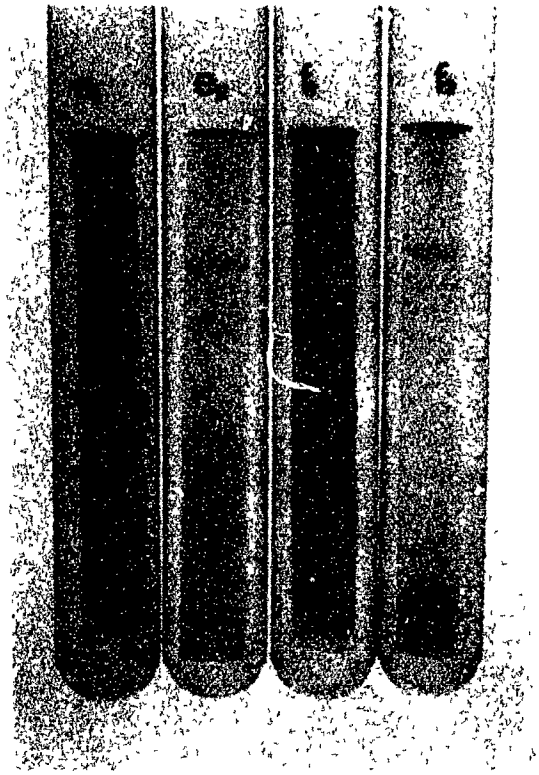


Figure 25. **Electropherograms for sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis of soymilk.**

e_1, e_2 : Soymilk (unprocessed)
f_1, f_2 : Soymilk (micronized)

Gels of each sample were stained for protein (e_1, f_1) and for glycoprotein (e_2, f_2).

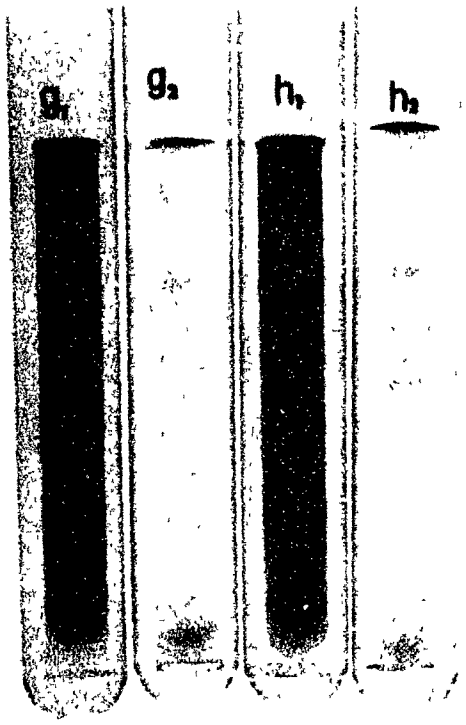


Figure 26. Electropherograms for sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis of tofu.

g_1, g_2 : Tofu (unprocessed)
h_1, h_2 : Tofu (micronized)

Gels of each sample were stained for protein (g_1, h_1) and for glycoprotein (g_2, h_2).

SUMMARY

1. There was little difference in the overall proximate composition between the micronized and unprocessed soybeans. The protein content of soymilk from the micronized beans was higher than that of soymilk prepared from the unprocessed beans. The protein content of tofu from the micronized beans was lower than that from the unprocessed beans which suggested that micronization affected the coagulation properties of the proteins.

2. Tofu made from micronized beans using standard procedures ($70^{o}C$ and calcium sulphate as coagulating agent) showed lower yield, protein content and textural properties than those of untreated beans. Tofu prepared from micronized beans using a mixture of citric acid (0.01M) and calcium sulphate (0.03M) as coagulating agent and a temperature of $90^{o}C$ gave a product of comparable firmness and cohesiveness to tofu from unprocessed soybeans.

3. Scanning electron microscopy demonstrated differences in the microstructure of tofu prepared from the unprocessed and the micronized beans. The microstructure of tofu prepared from micronized beans lacked the regularity of honeycomb-like structure as shown by tofu from unprocessed beans.

4. The digestibility of micronized soybeans (84.3%) was higher than that of the unprocessed soybeans (76.5%). Soy protein isolate, soymilk and tofu prepared from the unprocessed and the micronized beans showed similar digestibilities and the digestibilities of tofu obtained from the unprocessed (97.2%) and micronized beans (96.7%) were found to be significantly high when compared to casein (88.2%).

5. The available lysine contents of the unprocessed and micronized soybeans were 3.74% and 3.71%, respectively, which indicated that heat treatment of the micronization process had little effect on the available lysine contents of the soybeans. The available lysine contents of the soy isolate (5.50%), soymilk (6.14%) and tofu (6.60%) from the micronized beans were higher than the corresponding products derived from the unprocessed beans (soy isolate, 3.14%; soymilk, 4.64%; tofu, 5.94% available lysine).

6. The foaming properties of the unprocessed and the micronized soybean flour and soy protein isolates were pH dependent. The unprocessed soybean flour displayed maximum foam capacity at pH 9.0, the micronized soybean flour showed no foaming at pH 3.0 and 5.0 and similar foam capacities at pH 7.0 and 9.0. The tofu prepared from the unprocessed and micronized beans showed no foam capacity over the pH range studied (3 to 9).

7. The unprocessed soybean flour, soy isolate and tofu showed water absorption capacities of 264.1%, 163.2%, and 360.6%, respectively which were similar to the water absorption capacities of the micronized soybean flour (284.6%), soy isolate (166.1%), and tofu (358.4%). The results showed that the infrared heat of micronization did not lower the water imbibing capacities of soybeans and the derived products. The unprocessed and the micronized soybean flour, soy isolate and tofu showed similar fat absorption capacities which indicated that the infrared heat of micronization had little effect on the fat absorption capacity of the soybeans and the derived products.

8. Polyacrylamide-disc gel electrophoresis of soybean flour revealed that heat treatment by micronization had little effect to the protein constitution of the soybean and on the protein-carbohydrate interaction but induced some

interactions of protein with lipid component in the soybeans. Electrophoresis of soy protein isolates showed that micronization had no effect on the behaviour of the proteins that were extractable during the protein isolation and had little effect on the protein-carbohydrate interactions in the soy protein isolate but had an effect on the interactions between the protein and lipid present in the soy protein isolate. Electrophoresis of soymilk and tofu prepared from the unprocessed and micronized beans revealed that micronization had no effect on the protein components of soymilk and tofu and on the protein-carbohydrate interactions in the soymilk and tofu. No lipoprotein band was observed in the electrophoregrams of both soymilk and tofu from the unprocessed and micronized soybeans indicating that the process for preparation of soymilk and tofu from the unprocessed and the micronized beans affected the protein-lipid interactions in the soymilk and tofu.

9. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the unprocessed and micronized soybean flour revealed the presence of at least eleven protein bands with four of the bands (three major and one minor protein components) were bound to carbohydrate. The molecular weights ranged from 12885 to 117978 daltons. SDS-PAGE of the unprocessed and micronized soy protein isolates revealed the presence of at least eleven and twelve protein bands, respectively, with two of the major protein components of the unprocessed soy isolate and one major protein component of the micronized soy isolate bound to carbohydrate. The molecular weights of the protein subunits ranged from 13378 to 97791 daltons and from 13890 to 117978 daltons, for the isolates from unprocessed and micronized beans, respectively. SDS-PAGE of the soymilk from the unprocessed and

micronized beans revealed the presence of at least eleven and twelve protein bands, respectively, with two of the major protein components of the soymilk from unprocessed and one major and one minor protein components of the soymilk from micronized beans bound to carbohydrate. The molecular weights of the protein subunits ranged from 13890 to 97791 daltons and from 12885 to 113632 daltons, for the soymilk from the unprocessed and the micronized beans, respectively. SDS-PAGE of the tofu from the unprocessed and micronized beans revealed the presence of at least ten and eleven protein bands, respectively, with one major protein component of the tofu from the unprocessed and the micronized beans bound to carbohydrate. The molecular weights of the protein subunits ranged from 11954 to 87378 daltons and from 11954 to 81058 daltons, for tofu from the unprocessed and the micronized beans, respectively.

REFERENCES

Adams, C.F. 1975. Nutritive value of American Foods. Agriculture Handbook 456. U.S. Government Printing Office, Washington, D.C.

Aguilera, J.M., and Lusas, E.W. 1981. Review of earlier soya-protein-fortified foods to relieve malnutrition in less developed countries. J. Am. Oil Chem. Soc. 58:514.

Alberecht, W.J., Mustakas, G.C., McGhee, J.E., and Griffin Jr., E.L. 1967. A simple method for making full-fat soy flour. Cereal Sci. Today 12:81.

Anderson, R. 1974. Ultracentrifugation and binding studies of acid-sensitive soybean proteins. Cereal Chem. 51:707.

A.O.A.C. Handbook 1984. Official Methods of Analysis of the Association of Official Analytical Chemists. 14th edition. Williams, S. (Ed.).

Aoki, H., and Sakurai, M. 1969. Studies on the gelation of soybean proteins V. On the effects of heat denaturation. J. Agric. Chem. Soc. (Japan) 43:448.

Arnold, J.B., Summers, J.D., and Bilansky, W.K. 1971. Nutritional value of heat treated whole soybeans. Can. J. Anim. Sci. 51:57.

Babajimopoulos, M., Damodaran, S., Rizvi, S.S.H., and Kinsella, J.E. 1983. Effect of various anions on the rheological and gelling behavior of soy proteins: thermodynamic observations. J.Agric. Food Chem. 31:1270.

Badley, R.A., Atkinson, D., Houser, H., Oldani, D., Green, J.P., and Stubs, J.P. 1975. The structure, physical and chemical properties of the soy protein glycinin. Biochem. Biophys. Acta 42:214.

Barraquio, V.L., and van de Voort, F.R. 1988. Milk and soy proteins: Their status in review. Can. Inst. Food Sci. Technol. J. 21:477.

Beatty, K.D., Eldridge, I.L., and Simpson Jr., A.M. 1982. Soybean response to different planting patterns and dates. J. Agron. 74:859.

Beddows, C.G., and Wong, J. 1987a. Optimization of yield and properties of silken tofu from soybeans. I. The water:bean ratio. International Journal of Food Science and Technology 22:15.

Beddows, G.C., and Wong, J. 1987b. Optimization of yield and properties of silken tofu from soybeans. II. Heat processing. International Journal of Food Science and Technology 22:23.

Beddows, C.G., and Wong, J. 1987c. Optimization of yield and properties of silken tofu from soybeans. III. Coagulant concentration, mixing and filtration pressure. International Journal of Food Science and Technology 22:29.

Bookwalter, G.N., Mustakas, G.C., Kwolek, W.F., Mcghee, J.E., and Alberecht, W.J. 1971. Full-fat soy flour extrusion cooked: properties and food uses. J. Food Sci. 36:5.

Booth, V.H. 1971. Problems in the determination of FDNB - in available lysine. J. Sci. Food Agr. 22:658.

Borchers, R., Manage, L.D., Nelson, S.O., and Stetson, L.E. 1972. A research note. Rapid improvement in nutritional quality of soybeans by dielectric heating. J. Food Sci. 37:333.

Bourne, M.C. 1968. Texture profile of ripening pears. J. Food Sci. 33:223.

Bourne, M.C. 1982. In "*Food texture and viscosity: concept and measurement*", Academic Press, New York.

Bourne, M.C., Clements, M.G., and Banzon, J. 1976. Survey of suitability of thirty cultivars of soybeans for soymilk manufacture. J. Food Sci. 41:1204.

Bradford, M.M., and Orthoefer, R.T. 1983. Nutritional properties of soy protein concentrate. Cereal Foods World. 28:457.

Bresnahan, D.P., Wolf, J.C., and Thompson, D.R. 1981. Potential for utilizing 11s soy globular protein to study texture formation. J. Food Process Eng. 5:113.

Bressani, R. 1977. In "*Evaluation of Proteins for Humans*", Bodwell, C.E. (Ed.), p.81, AVI Publishing Co., Westport, Connecticut.

Bressani, R. 1981. The role of soybeans in food systems. J. Am. Oil Chem. Soc. 58:392.

Bressani, R., Murillo, M., and Ellias, L.G. 1974. Whole soybeans as a means of increasing protein and calories in maize based diets. J. Food Sci. 39:577.

Bressani, R., Navarrete, D.A., Ellias, L.G., and Braham, J.E. 1979. In "*Soy Protein and Human Nutrition*", Wilcke, H.L., Hopkins, D.T., and Waggle, D.H. (Eds.), p.313, Academic Press, New York.

Briggs, D.R., and Mann, R.L. 1950. An electrophoretic analysis of soybean protein. Cereal Chem. 27:243.

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

Brooks, J.R., and Morr, C.V. 1984. Phosphorus and phytate content of soybean protein components. J.Agric. Food Chem. 32:672.

Carpenter, K.J. 1960. The estimation of available lysine in animal protein foods. Biochem. J. 77:604.

Carter, J.L., and Hopper, T.H. 1942. Influence of variety, environment, and fertility level on the chemical composition of soybean seed. p.737. U.S. Dept. Agric. Technical Bulletin.

Catsimpoolas, N., and Eckenstain, C. 1969. Isolation of α-, B- and γ- conglycinin in soybean seeds. Phyto-chemistry 15:161.

Catsimpoolas, N., and Meyer, E.W. 1970. Gelation phenomena of soybean globulins. I. Protein-protein interactions. Cereal Chem. 47:150.

Catsimpoolas, N., and Meyer, E.W. 1971a. Gelation phenomena of soybean globulins. II. Protein-water miscible solvent interactions. Cereal Chem. 48:150.

Catsimpoolas, N., and Meyer, E.W. 1971b. Gelation phenomena of soybean globulins. III. Protein-lipid interactions. Cereal Chem. 48:159.

Catsimpoolas, N., Campbell, T.G., and Meyer, E.W. 1969. Association dissociation phenomena in glycinin. Arch. Biochem. Biophys. 131:557.

Catsimpoolas, N., Kenny, J.A., Meyer, E.W., and Szuhaj, B.F. 1971. Molecular weight and amino acid composition of glycinin subunits. J. Sci. Food Agric. 22:448.

Chang, I.C.L., and Murray, H.C. 1949. Biological value of the protein and the mineral, vitamin and amino acid content of soymilk and curd. Cereal Chem. 26:297.

Chang, K.C., Kendrick, J.G., Marshall, H.F., and Saterlee, L.D. 1985. Effect of partial methionine oxidation on the nutritional quality of soy isolate and casein. J. Food Sci. 50:849.

Chavez, J.F. 1981. Consideration of regulations of baby foods containing soybeans in Venezuela. J. Am. Oil Chem. Soc. 58:448.

Chiu, W.L. 1960. The calcium content and palatibility of soybean curd from field and vegetable varieties. M.Sc. Thesis, University of Illinois, Urbana, IL.

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

Coppock, J.B.M. 1974. Soy proteins in foods: retrospect and prospect. J. Am. Oil. Chem. Soc. 51:59A.

Cowan, J.C. 1979. Dry roasting of beans. J. Am. Oil. Chem. Soc. 56:168.

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

Damodaran, S., and Kinsella, J.E. 1982. Effect of conglycinin on the thermal aggregation of glycinin. J. Agric. Food Chem. 30:812.

Del Valle, J.R. 1981. Nutritional qualities of soya protein as affected by processing. J. Am. Oil Chem. Soc. 58:419.

DeMan, J.M., Stanley, K.W., and Rasper, V. 1975. Composition of Ontario soybeans and milk. Can. Inst. Food Sci. Technol. J. 8:1.

DeMan, J.M., DeMan, L., and Gupta, S. 1986. Texture and microstructure of soybean curd (tofu) as affected by different coagulants. Food microstructure 5:83.

Derbyshire, E., Wright, D.J., and Boulter, D. 1976. Legumin and vicillin-storage protein of legume seeds. Phyto-chemistry 15:3.

Dubois, D.K., and Hoover, W.J. 1981. Soya protein products in cereal grain foods. J. Am. Oil Chem. Soc. 58:343.

Eldridge, A.D., Hull, P.K., and Wolf, W.J. 1963. Stable foams from unhydrolyzed soybean protein. Food Technol. (Chicago). 17:1592.

Escueta, E.E. 1979. Effect of boiling treatment and gata (coconut cream) addition to soymilk on the chemical, rheological and sensory properties of tofu (soybean curd). Diss. Abstr. Int. B39:5304

Fan, T.Y., and Sosulski, F.W. 1974. Dispersibility and isolation of proteins from legume flour. Can. Inst. Food Sci. Technol. J. 7:256,

Featherston, W.R., and Rogler, J.C. 1966. A comparison of processing conditions of unextracted soybeans for utilization by the chicks. Poult. Sci. 45:330.

Finnigan, J.J.A., and Lewis, M.J. 1985. Nitrogen extraction from defatted rapeseed with particular reference to United Kingdom commercial rapeseed meal. J. Sci, Food Agric. 36:520.

Fleming, S.E., Sosulski, F., Kilara, A., and Humbert, E.S. 1974. Viscosity and water absorption characteristics of slurries of sunflower and soybean flours, concentrates and isolates. J. Food Sci. 39:188.

Freeland, J.H., Ebangit, M.I., and Bodzy, P.W. 1977. Trace mineral content of vegetarian foods: zinc and copper. Fed. Proc. 36:1124.

Friedman, H.H., Whitney, J.E., and Szczesniak, A.S. 1963. The texturometer - a new instrument for objective texture measurement. J. Food Sci. 28:390.

Friedman, M., Gumbmann, M.R., and Grosjean, O.K. 1984. Nutritional improvement of soy flour. J. Nutr. 114:2241.

Fukushima, D. 1968. Internal structure of 7s and 11s globulin molecules in soybean proteins. Cereal Chem. 45:203.

Fukushima, D. 1981. Soy proteins for foods centering around soy sauce and tofu. J. Am. Oil Chem. Soc. 58:346.

Furukawa, T., and Ohta, S. 1982. Mechanical and water holding properties of heat-induced soy protein gels as related to their structural aspects. J. Texture Stud. 13:59.

Furukawa, T., Ohta, S., and Yamamoto, A. 1979. Texture structure relationships in heat-induced soy protein gels. J. Texture Stud. 10:333.

Gayler, K.R., and Sykes, G.E. 1981. β-conglycinins in developing soybean seeds. Plant Physiol. 67:958.

German, B., Damodaran, S., and Kinsella, J.E. 1982. Thermal dissociation and association behaviour of soy proteins. J. Agric. Food Chem. 30:807.

Gierhart, D.L., and Potter, N.N. 1978. Effects of ribonucleic acid removal methods on composition and functional properties of Candida Utilis. J. Food Sci. 43:1705.

Glabe, E.F., Goldman, P.F., Anderson, P.W., Finn, L.A., and Smith, A.K. 1956. Uses of gelsoy in prepared foods. Food Technol. 10:51.

Graham, G.G., and Baertl, J.M. 1974. Nutritional effectiveness of soy cereal foods in undernourished infants. J. Am. Oil Chem. Soc. 51:152A.

Hartman, R.J., and Cheng, L.T. 1936. Iso-electric point of glycinin. J. Phys. Chem. 40:453.

Hasegawa, K., Kusano, T.T., and Nitsuda, H. 1963. Fractionation of soybean protein by gel filtration. Agr. Biol. Chem. (Tokyo). 27:878.

Hashizume, K., Nakamura, N., and Watanabe, T. 1975. Influence of ionic strength on comformation changes of soybean proteins caused by heating and relationship of its conformation changes to gel formation. Agric. Biol. Chem. 39:1139.

Hashizume, K., Maeda, M., and Watanabe, T. 1978. Relationships of heating and cooling to hardness of tofu (in Japanese, English summary). Nippon-Shokuhin Kogyo Gakkaishi 25:387.

Hermansson, A.M. 1978. Physicochemical aspect of soy protein structure formation. J. Texture Stud. 9:33.

Honig, D.H., Warner, K., and Rackis, J.J. 1976. Toasting and hexane: ethanol extraction of defatted soy flakes. Flavor of flours, concentrates and isolates. J. Food Sci. 41:642.

Hopkins, D.T. 1980. "Protein Quality in Humans and *In Vitro* Estimation", Proceedings Conference, Airlie House, Warrenton, VA.

Hopkins, D.T., and Steinke, F.H. 1981. Use of soy protein in mixed protein systems to meet nutritional needs. J. Am. Oil Chem. Soc. 58:452.

Horiuchi, T., Fukushima, D., Sugimoto, H., and Hattori, T. 1978. Studies on enzyme modified-proteins as foaming agents: Effect of structure on foam stability. Food Chem. 3:35.

Hoshi, Y., Yamauchi, F., and Shibasaki, K. 1982. On the role of disulfide bonds in polymerization of soybean 7s globulin during storage. Agric. Biol. Chem. 46:2803.

Hsu, H.W., Vavak, D.L. Satterlee, L.D., and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42:1269.

Hudson, B.J.F. 1987. In "*Developments in Food Proteins*", Vol.2, Applied Science Publishers Ltd., England.

Hull, S.J., Waldroup, P.H., and Stephenson, E.L. 1968. Utilization of unextracted soybeans by broiler chicks. 2. Influence of pelleting and re-grinding on diets with infrared cooking and extracted soybeans. Poult. Sci. 47:1115.

Hutton, C., and Campbell, A.M. 1977. Functional properties of soy concentrate and soy isolate in simple systems. Nitrogen solubility index and water absorption. J. Food Sci. 42:454.

Ibuchi, C., and Imahori, K. 1978a. Heterogeneity and its relation to the subunit structure of soybean 7s globulin. Agric. Biol. Chem. 42:31.

Ibuchi, C., and Imahori, K. 1978b. Interconversion between monomer and dimer of the 7s globulin of soybean seed. Agric. Biol. Chem. 42:25.

Jaffe, G. 1981. Phytic acid in soybeans. J. Am. Oil Chem. Soc. 58:493.

Johnson, L.D. 1984. Influence of soybean variety and method of processing on tofu manufacturing quality and consumer acceptability. M.Sc. Thesis, Iowa State University.

Johnson, K.W., and Snyder, H.E. 1978. Soymilk: A comparison of processing methods on yield and composition. J. Food Sci. 43:349.

Johnson, L.D., Wilson, L.A., and Love, J. 1983. Influence of soybean variety and method of processing in tofu manufacture. In "Research in Food Science and Nutrition", Vol. 1, McLoughin, J.V., and McKenna, B.M. (Eds.), p.102, Proc. 6th International Congress Food Sci. and Technol., Dublin.

Jones, D.B., and Csonka, F.A. 1932. Precipitation of soybean proteins and various concentrations of ammonium sulfate. Proc. Am. Soc. Biol. Chemists 26:29.

Kakade, M.L. 1974. Biochemical basis for the differences in plant protein utilization. J. Agric. Food Chem. 22:550.

Kakade, M.L., Simmons, N.R., Liener, I.E., and Lambert, J.W. 1972. Biochemical and nutritional assessment of different varieties of soybeans. J. Agric. Food Chem. 20:87.

Kamat, V., Graham, G., and Davis, M. 1978. Vegetable protein:lipid interactions. Cereal Chem. 55:295.

Kamel, B.S., and DeMan, J.M. 1982. Composition and properties of bean curd made from Ontario soybeans. Can. Inst. Food Sci. Technol. J. 15:273.

Kantha, S.S., Hettiarachchy, N.S., and Erdman, Jr. J.W. 1983. Laboratory scale production of winged bean curd. J. Food Sci. 48:441.

Karel, M. 1973. Protein-lipid interaction. J. Food Sci. 38:756.

Kawaguchi, Y. 1979. Effects of heat treatment of soymilk, coagulants and yeast inoculation on the texture of soy curds. J. Japan Soc. Food Sci. Technol. 26:434.

Kelley, J.J., and Pressey, R. 1966. Studies with soybean protein and fiber formation. Cereal Chem. 43:195.

Kellor, R.L. 1974. Defatted soy flour and grits. J. Am. Oil Chem. Soc. 51:77A.

Kilara, A., and Sharkasi, T.Y. 1986. Effects of temperature of food proteins and its implications on functional properties. CRC Crit. Rev. Food Sci. Nutr. 23:323.

Kinsella, J.E. 1978. Textured proteins: Fabrication, flavouring and nutrition. Crit. Rev. Food Sci. Nutr. 10:147.

Kinsella, J.E. 1979. Functional properties of soy proteins. J. Am. Oil Chem. Soc. 56:242.

Kinsella, J.E., and Damodaran, S. 1980. In "*Analysis and Control of Less Desirable Flavours in Foods and Beverages*", Charalambous, G. (Ed.), p.165, Academic Press, New York.

Kitamura, K., Takagi, T., and Shibasaki, K. 1976. Subunit structure of soybean 11s globulin. Agric. Biol. Chem. 40:1837.

Knox, J.N., Morrison, J.L., Probst, A.H., and Mertz, E.T. 1965. Unusual chromatographic pattern of Adams soybean whey proteins. Soybean Digest 25:20.

Kollar, C.W., Cho, I.C., and Waltrous, W.L. 1979. Vegetable protein application in yoghurt, coffee creamers and whip toppings. J. Am. Oil Chem. Soc. 56:389.

Koshiyama, I. 1969. Isolation of a glycopeptide from a 7s protein in soybean globulins. Arch. Biochem. Biophys. 130:370.

Koshiyama, I., and Iguchi, N. 1965. Studies on soybean proteins. Part I. A ribonucleoprotein and ribonucleic acids in soybean casein fraction. Agr. Biol. Chem. (Tokyo) 29:144.

Koshiyama, I., and Fukushima, D. 1976. Purification and some properties of α-conglycinin in soybean seeds. Phyto-chemistry 15:161.

Kouzeh-Kanani, M., van Zuilichem, D.J., Roozen, J.P., and Pilnik, W. 1981. A modified procedure for low temperature infrared radiation of soybeans. Food Science and Technology 14:225.

Krivoruchco, D., Kaba, H., Sambucetti, M.E., and Sanahaja, J.C. 1979. Maturation time and some seed composition characters affecting nutritive value in soybean varieties. Cereal Chem. 56:217.

Larsen, A.L. 1967. Electrophoretic differences in seed proteins among varieties of soybean, Glycine max (L) Merrill. Crop Science 7:311.

Lawrence, T.L.J. 1978. Effects of micronization on the digestibility of whole soya beans and rapeseeds for the growing pig. Animal Feed Science and Technology 3:179.

Lee, Y.H., and Marshall, R.T. 1979. Rennet curd from milk-soy protein mixtures. J. Dairy Sci. 62:1051.

Liener, I.E. 1978. In "*Soybeans: Chemistry and Technology*", Smith, A.K. and Circle, S.J. (Eds.), Vol 1, p.203, AVI Publishing Co. Inc., Westport, Connecticut.

Liener, I.E. 1980. Anti-nutritional factors as determinants of soybean quality. In "*Proc. World Soybean Res. Conf. II* ", Corbin, F.T. (Ed.), p.703, Westview Press, Boulder, CO.

Liener, I.E. 1981. Factors affecting the nutritional quality of soya products. J. Am. Oil Chem. Soc. 58:406.

Lin, M.J.Y., and Humbert, E.S. 1974. Certain functional properties of sunflower meal products. J. Food Sci. 39:368.

Lin, S.H.C., Bax, B.J., Yang, A., and Morr, C.V. 1976. Note on a light microscope technique to preview specimen mounting for electron microscopy. Cereal Chem. 53:153.

Livingston, H.G. 1977. Heat processing of cereals and oilseeds, Micronizing Co. (UK) Framlingham, Suffolk.

Lo, W.Y., Steinkraus, K.H., Hand, D.B., Hackler, L.R., and Wilkins, W.F. 1968a. Soaking soybeans before extraction as it affects chemical composition and yield of soymilk. Food Technol. 22:1188.

Lo, W.Y., Steinkraus, K.H., Hand, D.B., Wilkins, W.F., and Hackler, L.R. 1968b. Yields of extracted solids in soymilk as affected by temperature of various pre-treatment of beans. Food Technol. 22:1322.

Lu, J.Y., Carter, E., and Chung, R.A. 1980. Use of calcium salts for soybean curd preparation. J. Food Sci. 45.32.

MacLeod, G., and Ames, J. 1988. Soy flavor and its improvement. CRC Crit. Rev. Food Sci. Nutr. 27:219.

Mauer, H.R. 1971. Disc electrophoresis and related techniques of polyacrylamide gel electrophoresis. 2nd ed. Published by Walter Gruyter (Ed.).

Meinke, D.W., Chen, J., and Beachy, R.N. 1981. Expression of storage-protein genes during soybean seed development. Planta 153:130.

Meyer, E.W. 1967. Proc. Int. Conf. on Soybean Proteins Foods, U.S. Dept. Agr. ARS-71-35, 142.

Miller, C.D., Denning, H., and Bauer, A. 1952. Retention of nutrients in commercially prepared soybean curd. Food Res. 17:261.

Mohri, M., and Matsushita, S. 1984. Improvement of water absorption of soybean protein treatment with bromelain. J. Agric. Food Chem. 32:486.

Morales, J., Bourges, H., and Camacho, J.L. 1981. Utilization of soya protein in highly nutritious low-cost products in Mexico. J. Am. Oil Chem. Soc. 58:374.

Moreira, M.A., Hermodson, M.A., Larkins, B.A., and Nielsen, N.C. 1979. Partial characterization of the acidic and basic polypeptides of glycinin. J. Biol. Chem. 254:9921.

Morrison, J.L. 1962. Column chromatography of soybean whey proteins. Ph.D. Thesis, Lafayette, Ind., Library, Purdue University.

Murphy, P.A., and Resurreccion, A.P. 1984. Varietal and environmental differences in soybean glycinin and beta conglycinin content. J. Agric. Food Chem. 32:911.

Mustakas, G.C. 1971. Full-fat and defatted soy flours for human nutrition. J. AM. Oil Chem. Soc. 48:815.

Naismith, W.E.F. 1955. Ultracentrifuge studies on soybean protein. Biochim. Biophys. Acta 16:203.

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

Neville, D.M. 1971. Glycoprotein in cell surfaces. J. Biol. Chem. 246:6338.

Norman, A.G. 1978. Background. In "*Soybean Physiology. Agronomy and Utilization*", Norman, A.G. (Ed.), Academic Press, New York. I.

Nyotu, H.G. 1986. Effect of soybean supplementation on the nutritive value of maize-based Kenyan food. M.Sc. Thesis, Mcgill University.

Nyotu, H.G., Alli, I., and Paquette, G. 1986. Soy supplementation of a maize based Kenyan food (Ugali). J. Food Sci. 51:1204.

Orthoefer, F.T. 1978. Processing and utilization. In "*Soybean Physiology, Agronomy and Utilization*", Norman, A.G. (Ed.), p.219, Academic Press, New York.

Osborne, T.B., and Campbell, G.F. 1898. Proteids of the soybeans. J. AM. Oil Chem. Soc. 20:419.

Pearson, A.M. 1983. In "*Developments in Food Proteins*", Vol 2, Hudson, B.J.F. (Ed.), p.67, Applied Science Publishers, London.

Pennington, J.T., and Calloway, D.H. 1973. Copper content of foods. J. Am. Dietet. Assn. 63:143.

Pereira, L., and De Campos, S.D.S. 1981. Soya protein products for institutional feeding systems. J. Am. Oil Chem. Soc. 58:355.

Pontecorvo, A.J., and Bourne, M.C. 1978. Simple methods for extending the shelf life of soy curd (TOFU) in tropical areas. J. Food Sci. 43:969.

Przylecki, St.J., Hofer, E., and Frajberger-Grynberg, S. 1935. Uber affinitaten zwischen proteinen und fettsauren, fetten oder lipoiden. Biochem. z. 282:362.

Rackis, J.J. 1978. Biologically active components. In "*Soybeans: Chemistry and Technology*", Vol. 1, Smith, A.K., and Circle, S.J. (Eds.), p.158, AVI. Westport, CT.

Rackis, J.J. 1981a. Flatulence caused by soys and its control through processing. J. Am. Oil Chem. Soc. 58:503.

Rackis, J.J. 1981b. Significance of soy trypsin inhibitors in nutrition. J. Am. oil Chem. Soc. 58:495.

Rackis, J.J., Smith, A.K., Babcock, G.E., and Sasame, H.A. 1957. An ultracentrifugal study on the association-dissociation of glycinin in acid solution. J. Am. Oil Chem. Soc. 79:4655.

Roberts, R.C., and Briggs, D.R. 1965. Isolation and characterization of the 7s component of soybean globulin. Cereal Chem. 42:71.

Rogler, J.C., and Carrick, C.W. 1961. Studies with unextracted soybeans for chicken and turkeys. Feedstuffs 33:26.

Saio, K. 1979. Tofu-Relationships between texture and fine structure. Cereal Food World 24:342.

Saio, K., and Arisaka, M. 1978. Deterioration of soybean during storage under high moisture and temperatue (in Japanese, English summary). Nippon Shokuhin Kogyo Gakkaishi 25:451.

Saio, K., Kamiya, M., and Watanabe, T. 1969a. Food processing characteristics of soybean 11s and 7s proteins. Part 1. Effect of difference of protein components among soybean varieties on formation of tofu-gel. Agric. Biol. Chem. 33:1301.

Saio, K., Kamiya, M., and Watanabe, T. 1969b. Protein-calcium-phytic acid relationships in soybean. Part III, Effect of pyhtic acid on coagulative reaction in tofu making. Agric. Biol. Chem. 33:36.

Saio, K., Kajikawa, M., and Watanabe, T. 1971. Food processing characteristics of soybean proteins. Part II. Effect of sulfhydryl groups on physical properties of tofu-gel. Agric. Biol. Chem. 35:890.

Saio, K., Watanabe, T., and Koji, M. 1973. Food use of soybean 7s and 11s proteins. Extraction and fuctional properties of their fractions. J. Food Sci. 38:1139.

Saio, K., Sato, I., and Watanabe, T. 1974. Food use of soybean 7s and 11s proteins. High temperature expansion characteristics of gels. J. Food Sci. 39:777.

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

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

Satterlee, L.D., Bembers, M., and Kendrick, J.G. 1975. Functional properties of the Great Northern Bean (*Phaseolus vulgaris*) protein isolate. J. Food Sci. 40:81.

Schaefer, M.J. 1986. The phytic acid and trace mineral content of soybeans and their effects on tofu texture. Ph.D. Thesis. Iowa State University. 142pp.

Schmidt, S.C. 1976. World trade in soybeans and soybean products: past and prospects. In "*Proc. World Soybean Res. Conf.*", Lowell, L.D. (Ed.), p.639, Interstate, Danville, IL.

Schmidt, R.H, and Morris, H.A. 1984. Gelation properties of milk proteins, soy proteins and blended protein systems. Food Technol. 38:85.

Schroeder, J., and Jackson, H. 1972. Preparation and evaluation of soybean curd with reduced beany flavor. J. Food Sci. 37:450.

Scrimshaw, N.S. 1981. World need for protein. J. Am. Oil Chem. Soc. 58:389.

Seckinger, H.L., and Wolf, M.J. 1973. Sorghum protein ultrastructure as it relates to composition. Cereal Chem. 56:455.

Sgarbieri, V.C., Garruti, R., and Guzman, E.C. 1981. Soybeans as an extender of common beans. J. AM. Oil Chem. Soc. 58:522.

Shemer, M., Creinin, H.L., Macdonald, R.E., and Irwin, W.E. 1978. Functional properties of a new soy protein isolate. Cereal Chem. 55:383.

Shiau, S.Y., Yang, S.P., Tribble, L.F., and Williams, I.L. 1976. Effect of micronizing of sorghum for swine. J. Anim. Sci. 43:258 (Abstr.).

Shurtleff, W., and Aoyagi, A. 1975. The book of Tofu. Ballantine Books, New york.

Shurtleff, W., and Aoyagi, A. 1984. In "*Tofu and Soymilk Production*", Vol. 2. The Soyfood Center, Lafayette CA.

Sing-Wood, Y. 1984. Gelation characteristics of Illinois soybean beverage base. Ph.D. Thesis. University of Illinois. 131pp.

Skurray, G., Cunich, J., and Carter, O. 1980. The effect of different varieties of soybean and calcium ion concentration on the quality of tofu. Food Chem. 6:89.

Smiley, W.G., and Smith, A.K. 1946. Preparation and nitrogen content of soybean protein. Cereal Chem. 23:288.

Smith, A.K., and Nash, A.M. 1961. Water absorption of soybeans. J. Am. Oil Chem. Soc. 38:120.

Smith, A.K., and Circle, S.J. 1972. In "*Soybeans: Chemistry and Technology*", Vol.1, AVI, Connecticut.

Smith, A.K., and Circle, S.J. (Eds.). 1977. Protein products as food ingredients. In "*Soybean: Chemistry and Technology*", Vol. 1, p.339, AVI, Westport, Connecticut.

Smith, A.K., and Circle, S.J. 1978. Historical background. In "*Soybeans: Chemistry and Technology*", Vol. 1, Smith, A.K., and Circle, S.J. (Eds.), AVI, Westport, Connecticut.

Smith, G.A., and Friedman, M. 1984. Effect of carbohydrates and heat on the amino acid composition and chemically available lysine content of casein. J. Food Sci. 49:817.

Smith, A.K., Schubert, E.N., and Belter, P.A. 1955. Soybean protein fractions and their electrophoretic patterns. J. Am. Oil Chem. Soc. 32:274.

Smith, A.K., Watanabe, T., and Nash, A.M. 1960. Tofu from Japanese and United States soybeans. Food Technol. 14:332.

Smith, A.K., Rackis, J.J., McKinney, L.L., Robbins, D.J., and Booth, A.N. 1964. Tempeh-nutritive value in relation to processing. Cereal Chem. 41:173.

Sosulski, F.W. 1962. The centrifuge method for determining flour absorption in hard red spring wheats. Cereal Chem. 39:344.

Swahn, B. 1952. Microdetermination for the determination of total lipids in serum. Scand. J. Clin. and Lab. Invest. 4:247.

Szczesniak, A. 1963. Classification of textural characteristics. J. Food Sci. 28:385.

Szczesniak, A. 1972. Instrumental methods of texture measurement. Food Technol. 26:50.

Taira, H., Taira, H., and Saio, M. 1977. Effects of size of seed, variety and crop year on the chemical composition of soybean seeds. V. Potassium, phosphorus, magnesium and calcium content (In Japanese, English summary). Nihon Sakumostu Gakkai Kiji 46:483.

Thanh, V.H., and Shibasaki, K. 1976. Major proteins of soybean seeds. A straightforward fractionation and their characterization. J. Agric. Food Chem. 24:1117.

Thanh, V.H., and Shibasaki, K. 1977. Beta-conglycinin from soybean proteins. Isolation and immunological-physicochemical properties of the monomeric forms. Biochim. Biophys. Acta 490:370.

Thanh, V.H., and Shibasaki, K. 1978. Major proteins of soybean seeds. Subunit structure of B-conglycinin. J. Agric. Food Chem. 26:692.

Torun, B. 1981. Soybeans and soy products in the feeding of children. J. Am. Oil Chem. Soc. 58:460.

Townsend, A.A., and Nakai, S. 1983. Relationships between hydrophobicity and foaming characteristics of food proteins. J. Food Sci. 48:588.

Tsai, S.J., Lan, C.Y., Kao, C.S., and Chen, S.C. 1981. Studies on the yield and quality characteristics of tofu. J. Food Sci. 46:1734.

Tseng, R.Y.L., Smith-Nary, E., and Chang, Y.S. 1977. Calcium and phosphorus contents and ratios in tofu as affected by the coagulants used. Home Ec. Res. J. 6:171.

Utsumi, S., Inaba, H., and Mori, T. 1981. Heterogenicity of soybean glycinin. Phyto-chemistry 20:585.

Utsumi, S., and Kinsella, J.E. 1985. Structure-function relationships in food proteins; subunit interactions in heat induced gelation of 7s, 11s and soy isolate proteins. J. Agric. Food Chem. 33:297.

Waggle, D.H., and Kollar, C.W. 1979. In "*Soy Protein and Human Nutrition*", Wilcke, H.L., Hopkins, D,T., and Waggle, D.H. (Eds.), p.19, Academic Press, New York.

Waggle, D.H., Decker, C.D., and Kollar, C.W. 1981. Soya products in meat, poultry and seafood. J. Am. Oil Chem. Soc. 58:341.

Walker, A.F. 1983. The estimation of protein quality. In "*Developments in Food Proteins*", Vol. 2, Hudson B.J.F. (Ed.), p.293, Applied Science Publishers, London and New York.

Wang, H.L. 1981. Oriental soybean foods. Food Dev. May, p.29.

Wang, H.L., and Hesseltine, C.W. 1982. Coagulation conditions in tofu processing. Process Biochem. 17:7.

Wang, H.L., and Cavins, J.F. 1989. Yield and amino acid composition of fractions obtained during tofu production. Cereal Chem. 66:359.

Wang, H.L., Swain, E.W., Hesseltine, C.W., and Heath, H.D. 1979. Hydration of whole soybeans as affects solids losses and cooking quality. J. Food Sci. 44:1510.

Wang, H.L., Swain, E.W., Kwolek, W.F., and Fehr, W.R. 1983. Effect of soybean variety on the yield and quality of tofu. Cereal Chem. 60:245.

Watanabe, T., and Kishi, A. 1984. The book of soybeans. Japan Publications, Inc. Tokyo.

Watanabe, T., Fukumachi, C., Nakayama, O., Teramachi, Y., Abe, K., Suruga, S., and Miyanaga, S. 1964. Research into the standardisation of the tofu making

process. Part 1, regular tofu; Part 2, silken tofu; Part 3, bagged lactone silken tofu; National Food Research Institute, Tokyo, Japan.

Weber, K., Pringle, J.R., Osborne, M. 1972. Measurement of molecular weights by electrophoresis on SDS-Acrylamide gels. Methods of Enzymology 26:3.

White, C.L., Greens, D.E., Woldroup, P.W., and Stephenson, E.L. 1967. The use of unextracted soybeans for chicks. Poult. Sci. 46:1180.

Wilcke, H.L., Hopkins, D.T., and Waggle, D.H. (Eds.) 1979. In "*Soy Protein and Human Nutrition*", Academic Press, New York.

Wilkens, W.F., Mattick, L.R., and Hand, D.B. 1967a. Effect of processing on oxidative off-flavours of soybean milk. Food Technol. 21:1630.

Williams, L.D. 1970. In: Symposium on vegetable proteins (Protein Symposium 3). The British Food Manufacturing Industries Research Association, London. p.23.

Wing, R.W., and Alexander, J.C. 1975. The value of microwave radiations in the processing of full-fat soybeans. Can. Inst. Food Technol. J. 8:16.

Wolf, W.J. 1969. Chemical and physical properties of soybean protein. Bakers Dig. 43:30.

Wolf, W.J. 1970. Soybean proteins: their functional, chemical and physical properties. J. Agric. Food Chem. 18:969.

Wolf, W.J. 1976. Chemistry and technology of soybeans. In "*Advances in Cereal Science and Technology*", Pomeranz, Y. (Ed.), p.325, American Association of Cereal Chemists, St. Paul, MN.

Wolf, W.J., and Briggs, D.R. 1956. Ultracentrifugal investigation of the effect of neutral salts on the extraction of soybean protein. Arch. Biochem. Biophys. 63:40.

Wolf, W.J., and Cowan, J.C. 1971. Soybeans as a food source. CRC Crit. Rev. Food Technol. 2:81.

Wolf, W.J., and Sly, D.A. 1967. Cryoprecipitation of soybean 11s protein. Cereal Chem. 44:653.

Wolf, W.J., Rackis, J.J., Smith, A.K., Sasame, H.A., and Babcock, G.E. 1958. Behavior of the 11s protein of soybeans in acid solutions. I. Effects of pH, ionic strength and time on ultracentrifugal and optical rotatory properties. J. Am. Oil Chem. Soc. 80:5730.

Wolf, W.J., Babcock, G.E., and Smith, A.K. 1962. Purification and stability studies of the 11s component of soybean proteins. Arch. Biochem. Biophys. 99:265.

Yamauchi, F., Kwase, M., Kanbe, M., and Shibasaki, K. 1975. Separation of B-aspartamido carbohydrate fractions from soybean 7s proteins. Protein-carbohydrate linkage. Agric. Biol. Chem. 39:873.

Yoshino, U., Iwasaki, Y., Okubo, M., and Okuyama, T. 1982. Effects of storage conditions of soybean protein. Food Sci. Technol. Abstr. 14:5J619.

Young, V.R., Scrimshaw, N.S., Torun, B., and Viteri, F. 1979. Soybean proteins in human nutrition: An overview. J. Am. Oil Chem. Soc. 56:110.

Zacharius, R.M., Zell, T.E., Morrison, J.H., and Woodlock, J.J. 1969. Glycoprotein staining following electrophoresis on acrylamide gels. Anal. Biochem. 30:148.