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THE PHYTIC ACID AND TRACE MINERAL CONTENT OF SOYBEANS AND THEIR EFFECTS ON TOFU TEXTURE

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The phytic acid and trace mineral content of soybeans and their effects on tofu texture

by

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INTRODUCTION

Soybeans were introduced to the United States as a cash crop in the early 1920s. The harvest in 1924 was 5 million bushels (Wolf, 1976). The initial interest in soybeans was as an oil source for human consumption with the soybean meal being used for animal feed. Since that time soybeans have become a major crop with the United States producing the vast majority of the world supply of soybeans. In 1982, over ninety percent of the world crop was produced in the United States (World Food Institute, 1984). Despite the large soybean production the direct food use of soybean protein is extremely small. Less than two percent of the crop is utilized as a human protein source (Brown, 1981).

In contrast to the United States, soybeans have been a major protein source in the Orient for centuries. Japan alone imports substantial quantities of soybeans, at one time importing approximately one fourth of the total world soybeans (Schmidt, 1975). Much of this usage may be accounted for in the consumption of tofu or soybean curd.

Preparation of tofu was first recorded around 150 BC in China where its preparation and use was associated with Buddhist temples (Shurtleff and Aoyagi, 1975). Buddhist monks carried the technique of tofu preparation to Japan, where its use flourished. Tofu is now described as the most popular food item in the Japanese diet (Saio, 1979). Consumption of tofu in Japan has been compared to U.S. bread consumption with the per capita tofu consumption being seventy twelve ounce cakes per year (Brown, 1981).

Although tofu consumption has not reached these proportions in the United States, the use of soyfoods, including tofu, has increased substantially (Leviton, 1982; Fukushima, 1981). Tofu has grown from a nearly unknown food to a common item in supermarkets across the U.S. In addition, California and Georgia have begun use of tofu in their school lunch programs. The USDA Food and Nutrition service has begun to develop specifications for use of tofu in the National School Lunch Program (Brown, 1981).

Despite the increasing popularity of tofu, there are a number of factors which limit its acceptability. Of these, one major factor is texture. The bland flavor of tofu tends to emphasize the importance of texture and makes the factors affecting tofu texture important.

The purpose of this study was to investigate some of the factors involved in determination of tofu texture. Three specific objectives were outlined. The first was to determine the effect of soybean composition on composition and textural characteristics of tofu with special reference to phytic acid, copper, and iron. The second was to determine the distribution of soybean components among soymilk, tofu, and whey. The final objective was to investigate the effects of variation in temperature of soymilk coagulation and coagulant concentration on tofu composition and texture.

REVIEW OF LITERATURE

Soybeans

Soybean physiology

Soybean seeds are composed of protein bodies covered by a cytoplasmic network in which lipid bodies or spherosomes are embedded (Bair and Snyder, 1980). The oil-containing spherosomes range in size from 0.2 to 0.3 μ (Tombs, 1967). They are surrounded by a single membrane and contain from 60% to 70% of the total soybean protein. The protein bodies are approximately 97% protein with the bulk of the remainder being carbohydrate (Tombs, 1967). They may be separated into light and heavy fractions based on density, with the light and heavy fractions containing 1.4% and 5.2% phytic acid, respectively (Tombs, 1967). Upon germination, the protein bodies develop a granular appearance with a decrease in membrane integrity (Tombs, 1967).

Soybean composition

Soybeans are cultivated as a source of protein and of oil and in soybeans, percentages of these two components are high. A typical proximate analysis of soybeans on a dry basis is follows: protein 40%, lipid 21%, carbohydrate 34%, and ash 4.9% (Wolf and Cowan, 1975). A number of factors have been shown to affect soybean composition. One major source of variation in composition is soybean variety. DeMan et al. (1975) determined proximate composition of 55 soybean varieties grown in Ontario. Protein contents in the samples tested ranged from 30.30% to 46.13%, with a mean of 40.84%. Fat contents ranged from

14.46% to 21.26%, with a mean of 17.18%. The mean percentage of moisture was 5.0% and the moisture content ranged from 4.21% to 7.8%. Ten soybean varieties grown at experiment stations in the Midwest, including Iowa, were found to have considerable variation in protein content. In these samples, protein content ranged from 36.62% to 53.19% (Cartter and Hopper, 1942). Variation due to variety was also noted in oil, ash, and calcium content. Similar results were noted in protein and oil contents of soybeans grown in Greece (Papadopetros and Katsikas, 1981). Smith et al. (1960) compared Japanese and U.S. soybean varieties and found Japanese beans to have a mean protein content which was higher than that found in the American soybean varieties.

Cartter and Hopper (1942) reported that, in addition to varietal differences, growing location had an effect on protein, lipid, and mineral content. 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 methods, crop year, and planting date (Beatty et al., 1982; Krivoruchco et al. 1979; Taira et al. 1977).

Effect of soybean storage

The effect of storage conditions and length of storage on soybeans has been studied by several groups. Yoshino et al. (1982) stored soybeans at temperatures from 30° to 50°C for up to eight months. No marked change in either color or water absorption was found after

storage at 30°C for eight months. When the beans were stored at 40°C, however, darkening and an impaired water absorbing capacity were noted after storage periods of four months or longer. Further, soymilk prepared from beans stored at 40°C contained less nitrogen and formed a softer tofu curd than that prepared from soybeans stored at lower temperatures.

Similar results were found by Saio and Arisaka (1978). These workers stored soybeans at 40°C with a relative humidity of 75.2% for one month. Nitrogen solubility index (NSI) decreased with storage. The pH of a water extract prepared from the beans dropped to an acidic range after one month, while the ratio of 7S/11S proteins in the water extract increased with storage time. Tofu prepared from the soybeans which had been stored was softer than that prepared from unstored beans.

Protein extractability from whole soybeans and soymeals after long term storage was examined by Saio et al. (1982). Again, reduction in nitrogen solubility with improper storage was found; the effect was significant in whole beans but was more pronounced in soy meal.

Saio et al. (1982) found not only a quantitative change in the amount of protein extracted after storage, but a qualitative change in the protein as well. The 11S protein extractability decreased more rapidly than the 7S and 15S components while the extractability of the 2S component remained constant. The decrease in 11S extractability was thought to be the result of formation of an insoluble aggregate which involved disulfide bond formation.

Soy Protein

Soy protein structure

Soybean protein may be separated into four major fractions by ultracentrifugation on the basis of molecular size. The 2S, 7S, 11S, and 15S components comprise approximately 22, 37, 31, and 10% of the total soybean protein, respectively (Wolf, 1969).

2S and 15S soy protein fractions

The 2S fraction of soybean protein includes cytochrome c and the group of trypsin inhibitors (Wolf, 1969). This fraction, sometimes refered to as α conglycinin, seems to be relatively stable in that there is no change in its extractability with prolonged storage (Saio et al., 1982). There is little information available regarding the 15S component of soybean protein.

7S soy protein fraction

The 7S soy protein fraction includes a group of proteins the most important of which are hemagglutinin, lipoxygenase, and 7S globulin (the major protein), referred to as β conglycinin. This protein has a molecular weight of 180,000 to 210,000 (Koshiyama, 1968). It may be fractionated into electrophoretic forms designated B1 through B6. These six forms are themselves composed of groupings of 4 subunits: α', α, β, and a minor γ fraction (Thanh and Shibasaki, 1976a; Thanh and Shibasaki, 1978). The α and β subunits contain carbohydrate in the form of mannose and glucosamine, making the 7S globulin a glycoprotein (Thanh and Shibasaki, 1977).

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 by Thanh and Shibasaki (1977). Hoshi et al. (1982) found some sulfhydryl groups in 7S globulin but noted that they were located on the interior of the molecule with limited potential for disulfide interchange reactions. Loss of solubility of 7S globulin with storage has been related to polymerization reactions involving disulfide bond formation (Hoshi et al., 1982).

Thanh and Shibasaki (1979) studied the dissociation behavior of conglycinin with pH and ionic strength variation. At pH values of less than 3, dissociation of the protein into subunits was time dependent but was still incomplete after 12 days. Dissociation induced by alkali occurred at a pH of 11.5. At ionic strengths of greater than 0.1, the quaternary structure was stable over a pH range of 2 to 11. At ionic strengths of over 0.5 the pH range for stability was 1 to 11.5, but conglycinin was only stable at a pH range of 2 to 4 at an ionic strength of less than 0.2. When the pH was raised from 5 to 11 at this ionic strength a dimeric form predominated. At ionic strengths of less than 0.01 irreversible dissociation occurred even under mildly acid or alkaline conditions.

11S soy protein fraction

The 11S fraction of soy protein consists almost entirely of the 11S globulin called glycinin which has a molecular weight of approximately 362,000 (Kitamura et al., 1976). Peng et al. (1984) have recently published an excellent review of literature relative to the 11S soy protein.

Catsimpoolas (1969) published results showing that glycinin can dissociate into three acidic and three basic subunits. More recently, other workers have reported larger but varying numbers of subunits. Kitamura et al. (1976) found four acidic and four basic subunits which were grouped into three intermediary subunits (IS1 - IS3). Nielsen et al. (1981) found six acidic and five basic subunits and indicated that nonrandom pairing among acidic and basic subunits occurs to provide the intermediary structural units. Mori et al. (1981) reported that the numbers of acidic and basic subunits differed among soybean cultivars, but they found all glycinins to contain three major subunit proteins having molecular weights of 38,000, 34,800, and 17,900 to 19,000. Variation in glycinin subunit composition with soybean cultivar was also shown by Utsumi et al. (1981).

In 1969, Catsimpoolas proposed an arrangement of subunits to form two 'doughnut' shapes. Badley et al. (1975) proposed the combination of three acidic and three basic subunits alternating to form a hexagonal ring shaped monomer. Two monomers are 'stacked' to form the dimeric glycinin molecule. The acidic/basic subunits seem to be joined via hydrophobic and disulfide bonds while the two monomer units are

held together by electrostatic or hydrogen bonds.

Glycinin contains over twice the methionine of 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 two times the number 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.

Varietal differences in soy protein

The presence of varietal differences in soy protein has been examined by several groups. Larsen (1967) investigated electrophoretic patterns of soy protein extracts from 64 soybean varieties from the U.S. and Canada. Types of molecular bonding were found to be highly reproducible within variety and were not affected by differences in growing season or location. The main difference among varieties was seen to be the variable presence of two proteins designated A and B. Of 61 varieties tested, 13 contained A but not B while 48 contained B but not A. The A and B proteins never appeared together in any variety.

Wolf et al. (1961) used ultracentrifugation to compare the proteins in U.S. and Japanese soybean varieties. The percentage of the 2S fraction was similar in the two groups; however, the U.S. beans contained a lower proportion of the 7S and a larger amount of the 11S fraction relative to those grown in Japan. The percentage of 7S was

40% in the U.S. beans compared to 52% in the Japanese. Percentages of 11S fraction were 36% and 26% for U.S. and Japanese soybeans, respectively. The soybeans grown in the U.S. contained 6% 15S fraction compared to only 3% in the Japanese beans. These authors stated that the similarity among the Japanese varieties suggests that the differences seen between U.S. and Japanese beans were the result of environmental factors.

Hughes and Murphy (1983) studied the amount of glycinin in 10 U.S. and 5 Japanese soybean varieties. A significant difference in glycinin content with variety was observed although no overall difference between U.S. and Japanese beans was seen. The following year Murphy and Resurreccion (1984) concluded that the environmental effect was greater than that of variety on glycinin and conglycinin contents of soy protein.

Soy protein gel formation

Gelation of soy protein has been extensively studied by a number of investigators. Gels of significance in food systems are of two types: thermally induced gels and gels induced by a combination of heat and increased ionic strength. Hermansson (1977; 1978; 1979) has published a number of articles which discuss the gelation phenomenon in general and gelation of soy protein in particular. Gel network formation involves a dissociation and denaturation step before to protein/protein interaction (or aggregation). Gelation implies formation of a certain degree of order in the process of gel network formation. Higher molecular weight complexes develop through the

formation of disulfide bonds. The character of the gel will be determined (in part) by the kinetics of the denaturation/dissociation and aggregation reactions.

Heat induced gelation of soy protein isolate has been studied by a number of researchers. Circle et al. (1964) studied the gels formed when soy protein solutions in cans were heated at 100°C for 30 minutes. Protein concentrations of from 8% to 20% were tested. Gel viscosity (measured by Brookfield Viscometer with helipath stand) increased with protein concentration as did gel stability with heat stress. The addition of reducing agents such as sodium sulfite and cysteine resulted in decreased viscosity which was attributed to disulfide bond cleavage. Circle et al. (1964) concluded that gel formation involved an initial unfolding of the protein molecule which exposed reactive groups and increased hydrophobicity, followed by association to form a three dimensional network. This association involves covalent as well as hydrogen bonding. Aoki and Kakurai (1968) also found lowered viscosity in soy protein gels with the addition of reducing agents. They further noted that protein denaturants, including urea and guanidine HCl, prevented gelation.

Furukawa et al. (1979) have studied the effect of heating temperature on texture of soy protein gels measured by texturometer. Gel hardness was seen to increase with heating temperature from 25° to 80°C. A weakening of the structure occurred at temperatures over 90°C and was especially pronounced after heating to 120°C and higher. Cohesiveness was not affected by heating until temperatures over 105°C

were reached. Based on the solubility of the protein gel in mercaptoethanol and urea, these authors concluded that as gel hardness increased, hydrogen and hydrophobic bonds increased in number. At higher temperatures where gel strength decreased, a degradation of cysteine was hypothesized. This hypothesis is supported by work of Hazshizume and Watanabe (1979), who reported increasing numbers of half cysteine residues with heating of soy protein from 60° to 80°C. No further increase in number of residues was noted at temperatures over 85°C. Nakamura et al. (1984) observed greater gel hardness with increased heating time until a plateau was attained after 20 minutes.

It has become clear that thermal gelation of soy protein is largely a function of the glycinin component. Heating seems to cause dissociation of the 11S fraction into subunits separable by electrophoresis while no dissociation is apparent in the 7S fraction (Hashizume and Watanabe, 1979). These workers suggest that, on heating, amino acid residues capable of hydrophobic bonding (that are normally in the 11S molecule interior) are exposed, which results in protein aggregation. The nature of this aggregation seems to be different with and without a reducing agent. In the presence of a reducing agent a precipitate is formed composed primarily of a basic monomer (Yamagishi et al., 1982). Sulfhydryl compounds able to cause monomer formation were, in decreasing order of effectiveness: mercaptoethanol, glutathione, and cysteine. Thermal aggregates induced without the addition of sulfhydryl compounds have a network structure similar to that found in the tofu gel, in which acidic and basic

subunits are both important. In either case, disulfide interchange accounts at least partially for the aggregate formation (Yamagishi et al., 1982, Yamagishi and Yamagishi, 1983). Utsumi et al. (1983) found hardness of heat-induced gels prepared from soybean glycinin to be dependent on both the acidic and basic subunits. Nakamura et al. (1984) reported that glycinin gel hardness was directly proportional to the percentage of one acidic subunit. These authors stated that it was unclear whether the effect of the acidic subunit on gel hardness was a result of the acidic component itself, or was due to some paired basic counterpart.

Damodaran and Kinsella (1982) recently investigated the effect of conglycinin on the thermal aggregation of glycinin. The heat gelation of glycinin was found to be the result of aggregation of basic subunits following the thermal dissociation from their acidic counterparts. The presence of a reducing agent facilitated the dissociation step. These workers found that glycinin failed to form a gel in the presence of conglycinin due to the interaction of the acidic conglycinin with the basic subunits, with the subsequent formation of a soluble complex. The conglycinin/basic subunit interaction was apparently not specific in nature and it was observed that any electrostatic or hydrophobic interactions would have a similar effect. Bresnahan et al. (1981) also noted that the purified 11S soy protein fraction produced a firmer gel than the soy protein isolate from which it was derived.

The effect of sodium chloride on gelation

The effect of monovalent salts on the gelation of soy protein has been studied. It seems that the effect is dependent on salt concentration and on whether the salt is added before or after protein denaturation and dissociation. It has been found that the addition of sodium chloride at levels of 0.2M and above reduces gel viscosity (Catsimpoolas and Meyer, 1970; Ehninger and Pratt, 1974; Babajimopoulos et al., 1983). This effect is related to the ability of NaCl to suppress aggregation when it is added following protein dissociation. When salt is added before the protein denaturation and dissociation phase, the gel viscosity is also decreased, due to suppression of dissociation by stabilization of the quaternary structure. At low sodium chloride levels the protein tends to become unstable (Wolf and Tamura, 1969; Hashizume and Watanabe, 1979). In contrast, at higher ionic strengths the temperature necessary to denature proteins is substantially increased (Hermansson, 1978; Hashizume and Watanabe, 1979; Iwabuchi and Shibasaki, 1981).

The effect of sodium chloride on the gelation of 7S and 11S soy protein fractions has also been examined. Thanh and Shibasaki (1976b) found that a decrease in salt concentration from 0.5M to 0.1M resulted in a lowered level of 7S protein and suggested that dissociation occurs at this concentration. Later work by this group (Thanh and Shibasaki, 1979) revealed the presence of a protomer at 0.5 ionic strength. This protomer changed to a dimeric 10S form at an ionic strength of 0.1. At ionic strengths of 0.5 the 11S fraction was converted to a fast

sedimenting aggregate and a 4S fraction (Wu and Ingle, 1974). Shimada and Matoushita (1980) compared the gelation behavior of 7S and 11S protein fractions. Sodium chloride at a concentration of 0.3M decreased the viscosity of the 11S gel while the viscosity of the 7S gel was increased.

The effect of other salts on soy protein gelation

Sodium sulfate affected gelation of soy protein in the same manner as sodium chloride; that is, by acting as a structure stabilizer and reducing gel viscosity (Babajimopoulos et al., 1983). In contrast, sodium thiocyanate destabilized protein structure and produced a stronger gel. The effect of salts on gel viscosities followed the lyotropic series $SO_4^{-2} < Cl^- < Br^- < SCN^-$ (Babajimopoulos et al., 1983).

The effect of sugar on gelation of soy protein

The addition of sucrose at a level of 5% increased the gel viscosity of a soy protein dispersion, but 10% added sucrose produced a drop in viscosity. When dextrose was used an increase in viscosity was seen at both the 5% and 10% levels (Ehninger and Pratt, 1974).

Bonding involved in gel formation

There are conflicting opinions regarding the bonding forces of primary importance in gelation of soy protein. In 1974, Saio et al. concluded that disulfide bonds were the major contributors to gelation of 11S soy protein, but bonds other than disulfides were of greater significance in 7S gel formation. More recently, Babajimopoulos et al.

(1983) concluded that the major forces involved in gelation of soy protein are hydrogen bonding and van der Waals attractive forces. There is minimal contribution by hydrophobic and electrostatic interactions. Involvement of hydrophobic bonds was seen as important in 7S interactions while hydrogen and ionic bonds were found to be major contributors to 11S gelation by Shimada and Matoushita (1980).

The involvement of disulfide bonds in 11S gelation seems unquestionable. Further, it seems clear that the relative importance of bonding types in the 7S fraction and in whole soy protein is dependent upon the conditions existing at the time of gel formation.

Mineral/soy protein interactions

Interactions of a number of minerals with soy protein have been studied. In 1958, Wolf and Briggs noted a more rapid degradation of the 11S fraction of soy protein upon the addition of EDTA. They suggested the possibility that divalent cations were involved in stabilizing the glycinin molecule. More recently, Appu Rao and Narasinga Rao (1975a) have also observed 11S dissociation into subunits when EDTA was added to soy protein.

In 1976, Appu Rao and Narasinga Rao (1976a) investigated zinc binding by the 11S fraction of soy protein. These workers found two sets of zinc binding sites, the total number of which corresponded to the number of histidine residues in the 11S fraction; binding through the imidazole was hypothesized. The binding of zinc by the 7S fraction was also investigated (Appu Rao and Narasinga Rao, 1976b). Zinc binding by 7S protein was found to be at a single set of sites; no

location for these sites was proposed. The addition of sodium chloride resulted in a decrease of zinc binding by 11S protein (Appu Rao and Narasinga Rao, 1976b). The ability of soy protein to bind zinc exhibits a pH dependence with a greater degree of binding with increasing pH (Allred et al., 1964). Little binding occurred at pH values of from 3.7 to 4.3, but binding increased with increasing pH to a value of 5.3 (which was the upper limit tested).

Like zinc, binding of magnesium is thought to occur at two sites in the 11S protein and at a single site in the 7S protein (Appu Rao and Narasinga Rao, 1975b; Appu Rao and Narasinga Rao, 1975b). The addition of sodium chloride almost completely suppresses magnesium binding by the 11S protein (Appu Rao and Narasinga Rao, 1975b). No evidence indicates that the protein changes conformation as the result of magnesium binding in either the 7S or 11S fractions.

It has been suggested that calcium shares an 11S binding site with magnesium (Appu Rao and Narasinga Rao, 1975b). Only a single binding site was found for calcium in the 11S protein fraction by Appu Rao and Narasinga Rao (1976b). More recently, however, Kroll (1984) located two types of binding sites for calcium when soy protein isolate was tested. Binding was thought to involve imidazolic groups and carboxylic groups of aspartic and glutamic acids. The addition of sodium chloride decreased the binding of calcium to the 11S fraction (Appu Rao and Narasinga Rao, 1975a).

Calcium precipitation of soy protein

The precipitation of soy protein by calcium has been studied by a large number of workers. The extent of precipitation of soy protein increases with increasing calcium concentration to a point, after which increasing protein solubilization occurs (Wolf and Briggs, 1956; Appu Rao and Narasinga Rao, 1976b). Calcium sensitivity of the 7S and 11S fractions differs. Saio and Watanabe (1973) found the 11S fraction to precipitate at a lower calcium concentration than the 7S fraction. Minimum calcium concentrations required for precipitation were 0.4mM and 1.1mM for the 11S and 7S protein fractions, respectively. Precipitation of the 11S protein by calcium was seen to be unrelated to an ability of the protein to bind calcium; instead, precipitation was related to the induction of a protein conformational change (Appu Rao and Narasinga Rao, 1975a; Sakakibara and Noguchi, 1977).

The effect of sodium chloride on precipitation of soy protein by calcium is to reduce the amount of the precipitate. This effect was seen in the 7S fraction (Appu Rao and Narasinga Rao, 1976b) and in the 11S fraction (Appu Rao and Narasinga Rao, 1975a). Saio and Watanabe (1973) noted this effect and hypothesized that the decrease in protein precipitation was related to competition of sodium for calcium binding sites.

The effect of phytic acid on the ability of calcium to precipitate soy protein has been investigated. Appu Rao and Narasinga Rao (1975a) found only 50% precipitation of soy protein containing phytic acid at a calcium level of 0.1M. On removal of the phytic acid, the precipitate

increased to 80% at 0.01M calcium.

Precipitation of soy protein by other minerals

Minerals other than calcium have been shown to be effective in precipitation of soy protein. Magnesium quantitatively precipitated 11S protein at a concentration of 8mM and produced a more homogeneous 11S fraction than that produced by calcium precipitation (Appu Rao and Narasinga Rao, 1975b). Precipitation by magnesium was found to be suppressed by sodium chloride in a manner similar to that seen with calcium precipitation (Appu Rao and Narasinga Rao, 1976b). Despite the similarities between calcium and magnesium precipitation, Appu Rao and Narasinga Rao (1975b) concluded that the mechanism of soy protein precipitation differed for calcium and magnesium.

Zinc was shown to produce more complete precipitation of 7S protein than either calcium or magnesium. The precipitation of the 7S fraction by zinc was less complete than that of of the 11S fraction by this mineral (Appu Rao and Narasinga Rao, 1976b).

Phytic Acid

A number of comprehensive reviews of literature relative to phytic acid have been published (Erdman, 1979; Cosgrove, 1980; Erdman, 1981; Reddy et al., 1982; Oberleas, 1983). Phytic acid is found in many plant products, especially cereals and legumes. The most common function proposed for phytate is a phosphorus reserve, although Cosgrove (1980) has suggested a number of other roles. Phytic acid may account for over 80% of the total plant phosphorus in some products

(O'Dell et al., 1972). It is often localized in a specific plant part such as wheat bran or corn germ, which contain 87% and 88% of the total phytate of wheat and corn, respectively (O'Dell et al., 1972). In soybeans, phytic acid seems to be associated with the protein fraction. Prattley and Stanley (1982) found that the cotyledon of soybean contained 90% of the phytate of the seed. In the cotyledon, phytate is concentrated in the protein bodies, probably in the form of a protein/phytic acid complex. Significant amounts (from 10% to 15%) were specifically located in globoid inclusions in an insoluble form. These authors suggested that the 7S fraction was the soy protein most likely to be involved in soy phytic acid binding.

Content of soy products

The concentration of phytic acid varies slightly in soybeans. Lolas et al. (1976) determined the phytic acid content of 15 varieties of soybeans. Values ranged from 1.00% to 1.47% on a dry weight basis, with a mean level of 1.14%. Harland and Prosky (1979) reported a slightly higher level of 2.22% phytic acid in soybeans.

Phytic acid levels have also been determined in a number of soy products. deBoland et al. (1975) reported an approximate phytic acid percentage in soybean products of 1.5%. Schuster and Bodwell (1980) found a mean phytic acid percentage in soy isolate of 1.27%, with a range of 0.97% to 1.69%. Soy concentrate contained 1.38% phytic acid and, in this product, phytic acid ranged from 1.21% to 1.72%. Phytate in soy flour ranged from 1.30% to 1.63%, with a mean of 1.47%. Samples of textured soy protein contained a mean level of 1.46% phytic acid,

with a range of from 1.19% to 1.63%. A range of from 0.37% to 1.66% phytate was found in a number of textured products by Harland and Prosky (1979).

Recently Brooks and Morr (1984) investigated the variation of phytic acid and phytate phosphorus concentrations in soy protein fractions. Soy flakes contained 1.41% phytic acid. 7S protein prepared from these flakes contained 1.40% phytate; the 11S fraction contained only 0.07% phytic acid. Insufficient sample was available to determine phytic acid in fractions prepared from whole soybeans. Based on analysis of total phosphorus, however, the results seem to parallel those found in fractions prepared from soy flakes.

Structure and chemistry

Phytic acid is the hexaphosphoric acid of myoinositol. Lower esters containing from 1 to 5 phosphate groups exist in immature plants but are not considered to be phytic acid.

Two models for the structure of phytic acid have been proposed. Neuberg proposed a hydrated pyrophosphate form, but an orthophosphate form was suggested by Anderson (1914). Although evidence has been presented supporting both structures, the Anderson form is favored based on nuclear magnetic resonance and x-ray crystallographic data (Oberleas, 1983).

The phytic acid molecule has twelve hydrogen atoms. Six of these are strongly dissociated at a pK of 1.8. Two are weakly dissociated with a pK of 6.3, and the remaining four are very weakly dissociated with a pK of 9.7 (Crean and Haisman, 1963).

Mineral binding

The importance of mineral binding by phytic acid became apparent when a decrease in the bioavailability of zinc from products containing phytic acid was noted (O'Dell, 1969). The lowering of zinc availability by phytic acid has been related to the formation of insoluble salts at the pH of the small intestine (Halsted et al., 1974). Forbes et al. (1983) studied the effect of incorporation of tofu into the diets of rats on the bioavailability of zinc from that diet. A decrease in zinc availability from the tofu-based diets relative to a control diet of albumen was demonstrated. No difference in zinc availability was seen in the tofu diet compared to other soy protein diets, indicating that there was no effect of the tofu process per se on zinc availability from soy products.

Phytic acid has also been implicated in decreased availability of other minerals such as iron, magnesium, calcium, and copper. Reddy et al. (1982) have summarized this work.

The relative affinity of phytic acid for various minerals is studied using a potentiometric titration. Maddaiah et al. (1964) ranked phytate/mineral complex stability in the following decreasing order: zinc, copper, cobalt, manganese, and calcium. Using a similar procedure, Vohra et al. (1965) found the relative stabilities of copper and zinc reversed. These authors ranked a greater number of minerals with the decreasing order of affinity as follows: Cu^{+2}, Zn^{+2}, Ni^{+2}, Co^{+2}, Mn^{+2}, Fe^{+2}, Ca^{+2}.

Soy protein binding

The interaction between phytic acid and plant proteins in general has long been recognized. In 1946, Fontaine et al. observed that phosphorus solubility from soybeans, peanuts and cottonseed was dependent on nitrogen solubility. The pH dependence of this association was also noted. More recently, a similarity in the percentages of nitrogen and phosphorus dialyzed from soybean flakes was seen by O'Dell and deBoland (1976).

The phytic acid/protein interaction is dependent upon the charge on the protein and thus is pH dependent. At pH values lower than the protein isoelectric point of 4.2, the phytic acid/protein interaction is electrostatic in nature. It reaches its maximum strength at a pH of 2.5. At this pH the protein positive charge is high and disruptive effects of higher orders of protein structure are decreased (Okubo et al., 1976). As the pH increases above the protein isoelectric point, the strength of the interaction decreases, and reaches a minimum at a pH of 5.0. The maximal amount of free phytic acid has been reported at pH 5.0 (Prattley and Stanley, 1982; Prattley et al., 1982). When the pH increases further, the interaction of phytic acid and protein seems to be mediated by a divalent cation which forms a salt bridge between the anionic phytic acid and the negatively charged protein groups. Prattley and Stanley (1982) demonstrated a strong calcium dependence of protein/phytic acid binding under alkaline conditions. A doubling of bound (non-dialyzable) phytic acid (from 40% to 80%) was found by deRham and Jost (1979) when calcium was added to a soy protein

preparation.

Possible protein binding sites for phytic acid have been discussed. Histidine is considered to be a likely site of interaction as are the carboxyl groups of aspartate and glutamate and the α and ϵ amino groups of lysine and the guanidino group of arginine (Cosgrove, 1980; Erdman, 1981).

Methods of analysis

The initial step in any determination of phytic acid is its extraction, which usually involves the use of a dilute acid. McCance and Widdowson (1935) found that a two hour HCl treatment gave complete extraction of phytic acid. More recently, Latta and Eskin (1980) found that a one hour extraction with 2.4% HCl was as effective in phytic acid removal as a two hour procedure with 1.2% HCl. Other investigators have reported more complete extraction when 3% trichloroacetic acid (TCA) was used instead of HCl. Further, the TCA extract was clearer and lacked a white flocculance found in the HCl extract (Wheeler and Ferrel, 1971).

Following extraction, a method of isolating phytic acid is required. Methods are generally based on either precipitation by ferric ion or separation using an ion exchange procedure. Precipitation methods are described as either direct or indirect, based on what analytical step follows isolation. If phosphorus is determined in the ferric phytate precipitate, the method is direct. Alternatively, iron may be determined either in the precipitate, or as unprecipitated iron in the supernatant; these methods are described as

indirect procedures.

Both direct and indirect methods have been criticized. It is a basic assumption of both methods that phytic acid, the hexaphosphoric acid of inositol, is the only compound precipitating with the ferric ion. It has been stated that phytic acid is the only phosphate which precipitates with ferric ion (Oberleas, 1971; deBoland et al., 1975). Other workers have reported precipitation of other phosphates, particularly the higher phosphates, by this method (Anderson, 1963; Posternak, 1965; deBoland et al., 1975). Ellis et al. (1977) reported co-precipitation of inorganic phosphorus with phytate phosphorus which resulted in an apparent increase in phytic acid concentration as inorganic phosphorus levels increased.

The indirect procedure involving iron determination is complicated by additional problems. This method assumes an iron to phosphorus ratio of 4:6. This ratio is, however, dependent on a number of factors which may be difficult to control. Thompson and Erdman (1982b) have shown the iron to phosphorus ratio to be related to the presence of sulfate anion, with a higher sulfate level increasing the ratio. The amount of iron also had a marked effect on this ratio. As the amount of excess iron decreased from 3.6 fold to 1.2 fold, the iron to phosphorus ratio decreased from 4.22:6 to 3.69:6. Variability in the ratio of these minerals has also been noted by Wheeler and Ferrel (1971). Excess iron has also been reported to increase the solubility of ferric phytate (Anderson, 1963; Wheeler and Ferrel, 1971). Samotus and Schwimmer (1962) have also found the indirect method to give high

results in the presence of reducing agents such as ascorbic or chlorogenic acids. The addition of an oxidizing agent such as H_2O_2 eliminated this problem. Despite these problems, Thompson and Erdman (1982b) found similar results of 1.4% and 1.38% phytic acid in soybean meal when indirect and direct methods were used, respectively. But variability was substantially lower in the phosphorus determination than in the iron determination.

As an alternative to precipitation methods, ion exchange procedures have been used. In a typical method, the sample extract containing phytic acid is placed on an anion exchange column and water is used to remove inorganic phosphorus. A dilute NaCl solution of 0.05M elutes more inorganic phosphorus. Phytic acid is removed from the column with 0.7M NaCl and is analyzed for phosphorus following a digestion procedure. Ellis and Morris (1983) have modified the procedure by the addition of EDTA and NaOH (to a pH of 6.0) to reduce interference by minerals and protein, respectively.

Precipitation methods and ion exchange methods of phytic acid analysis have been compared by several groups. In general, ion exchange procedures result in phytic acid levels that are lower than those obtained by precipitation (Marrese et al., 1961; Harland and Oberleas, 1977; Ellis and Morris, 1982). Marrese et al. (1961) attributed this to inclusion of lower esters by the precipitation method, producing an incorrectly high result. Ellis and Morris (1982), however, found from 97% to 100% recovery of phytic acid by iron precipitation, compared to approximately 63% recovery using an ion

exchange method. This effect was related to an interferant of proteinaceous nature in the ion exchange extract. In subsequent work, this group (Ellis and Morris, 1983) used a modified procedure and found similar results using ion exchange and precipitation techniques. It was also noted that the iron precipitation method lacked the sensitivity of the ion exchange technique.

Recently, methods of phytic acid analysis by high performance liquid chromatography (HPLC) have been developed. The first procedure using HPLC was published by Tangendja et al. (1980). The sample was extracted with 3% TCA, injected on a reverse phase C-18 column, eluted with sodium acetate, and phytic acid was detected using both ultraviolet (UV) and refractive index (RI) detectors. Knuckles et al. (1982) have criticized this procedure for resulting in column deterioration through injection of a low pH sample. A number of other methods based on HPLC have been published (Camire and Clydesdale, 1982; Graf and Dintzis, 1982b; Graf and Dintzis, 1982b; Knuckles et al., 1982), most of which involve a filtration and neutralization step before injection, and may include an ion exchange clean-up step. Lee and Abendroth (1983) have criticized these methods since phytic acid elutes quite rapidly with the solvent front. They have developed a procedure for phytic acid analysis using a tetrabutyl formate ion pair on an octadecyl column. Although both UV absorption and RI have been used to detect phytic acid, Lee and Abendroth (1983) found UV detection to lack sensitivity and give a nonlinear response.

Uppstrom and Svensson (1980) developed a procedure for phytic acid analysis based on complete hydrolysis of phytic acid by the addition of wheat phytase. Phosphorous was then determined on the sample.

Food Texture

Definitions of food texture

It is difficult to arrive at an exact definition for the term "food texture". A number of authors, including Szczesniak (1963) and Bourne (1982) have considered this problem. Although Bourne does not give an exact definition for texture, he does provide a list of characteristics of food texture as follows:

1. It is a group of physical properties that derive from the structure of the food.
2. It belongs under the mechanical or rheological subheading of physical properties. Optical properties, electrical and magnetic properties, and temperature and thermal properties are physical properties that are excluded from the texture definition.
3. It consists of a group of properties, not a single property.
4. Texture is sensed by the feeling of touch, usually in the mouth, but other parts of the body may be involved (frequently the hands).
5. It is not related to the chemical senses of taste or odor.
6. Objective measurement is by means of functions of mass, distance, and time only.

Bourne (1982) states that it is preferable to discuss "textural properties" of a food rather than "texture".

Szczesniak (1963) divided textural characteristics into three classes . These classes are mechanical characteristics, geometrical characteristics, and other characteristics, primarily those relating to fat and moisture content. Mechanical characteristics describe the reaction of a food to stress and include the primary characteristics of hardness, viscosity, cohesiveness, elasticity, and adhesiveness. Secondary mechanical properties are derived from the primary properties and include brittleness, chewiness, and gumminess. Geometrical properties refer to the arrangements of food constituents and are primarily apparent visually. Such properties include cellularity, as in porous fruits or flakiness, as in pastry. The final category of textural properties includes properties relating to moisture or fat content. The description of fat content includes factors for oiliness and greasiness.

Methods of texture measurement

Textural properties of food may be measured either by sensory tests or by objective measures of mass, time, and distance. Szczesniak (1972) cautions that no objective texture measurement device measures texture but rather measures 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 only well-defined rheological parameters. They are classical material testing techniques and may be

difficult to relate to data obtained using sensory evaluation.

In contrast, empirical tests measure parameters which have been shown to be related to textural properties of foods but which themselves are not well understood. By far the bulk of texture measurement devices fall into this class. An example of a test in this category would be the Warner Bratzler Shear which measures the force required to shear through a meat sample. This force has been shown to relate to the perception of toughness of meat, even though the sensation of toughness of meat is far more complex than simply the force required to shear through it.

Imitative tests are so named because they more closely imitate the conditions to which the food is subjected during mastication or processing. The farinograph is an example of a test of this type in which the force required to mix a dough is measured over a period of time as the dough is being mixed. The test simulates the agitation and stretching that occurs when dough is mixed in food preparation.

Szczesniak (1972) states that all devices used to measure textural characteristics have four common elements: 1) a probe which contacts the food sample, 2) a mechanism to drive the probe, 3) an element to sense force, distance, or time, and 4) a system which reads or records the parameter measured by the sensory element. The most commonly used instruments that measure textural attributes are those involving a measurement of force. Devices falling into this category include compressimeters, viscometers, and penetrometers. Szczesniak (1972) and Bourne (1982) have discussed many of these instruments.

Texture profile analysis

The General Foods Texturometer and the General Foods Texture Profile analysis were developed to describe the range of textural characteristics of foods. The texture profile consists of parameters of hardness, cohesivness, 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 by Bourne (1968). A sample tracing from an Instron is shown in Figure 1. The parameters measured are described in Table 1.

Texture measurement in tofu

A number of objective techniques have been utilized in the study of tofu texture. The most commonly used tool for textural evaluation of tofu is the Instron Universal Testing Machine. Lu et al. (1980) measured tofu hardness with an Instron using 75% compression. Similar procedures have been used by other researchers (Kamel and DeMan, 1982; Wang and Hesseltine, 1982; Wang et al., 1983). 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.

The General Foods Texturometer has also been extensively used in studies of tofu texture. Saio et al. (1969a) have measured hardness, springiness, adhesiveness, chewiness and gumminess using the texture

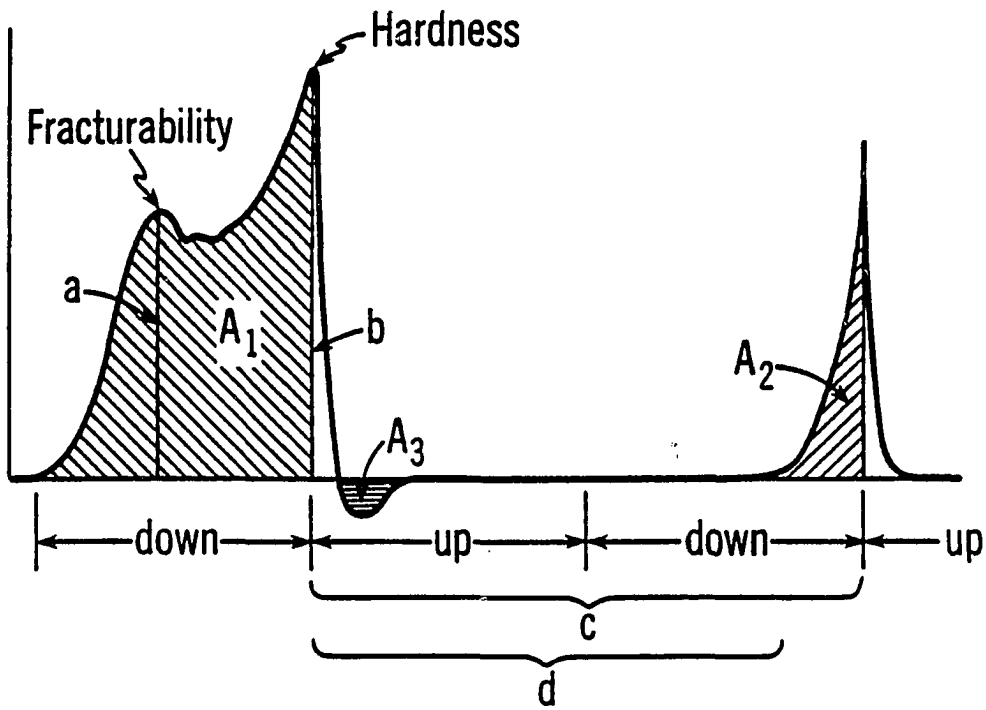

Figure 1. Tracing of force/distance curve obtained using an Instron Universal Testing Machine. Modified from Bourne (1968)

Table 1. Texture profile parameters[a]

Term	Definition	Texture Profile Definition	Represented in Figure 1
Hardness	Force necessary to attain a given deformation	Height of first compression	b
Cohesiveness	Strength of internal bonds	Ratio of 2nd peak area to 1st peak area	A_2/A_1
Elasticity	Rate at which deformed material returns to undeformed condition after removal of deforming force	Distance food recovers between 1st and 2nd compressions	c-d
Adhesiveness	Work needed to overcome attractive forces between food and the surface of other materials	Area under curve on upward stroke	A_3
Brittleness	Force with which a material fractures	Height of 1st significant break in the 1st compression	a
Chewiness	Energy required to masticate a solid food to a state ready for swallowing	Product of hardness, cohesiveness and elasticity	$a * \frac{A_2}{A_1} * (c-d)$
Gumminess	Denseness which persists through chewing	Product of hardness and cohesiveness	$a * (A_2/A_1)$

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

profile analysis. Other work by this group has also utilized the texturometer in evaluation of tofu texture (Saio et al., 1971; Saio, 1979). Furukawa et al. (1979) and Furukawa and Ohta (1983) have studied the texture of thermally induced soy protein gels using the General Foods Texturometer.

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. Bresnahan et al. (1981) used the Warner-Bratzler shear and the Ottowa Texture Test to evaluate integrity of a tofu-like product. Tsai et al. (1981) measured tofu gel strength, softness, and chewiness using a rheometer.

Tofu

Preparation of soymilk

A traditional method of tofu preparation is described by Shurtleff and Aoyagi (1975). Traditional tofu preparation begins with hydration of the dried soybeans. This step has two functions: 1) the grinding step is facilitated, and 2) a better dispersion of soybean solids is attained in the extraction (Lo et al., 1968b). The length of the hydration step has been examined by a number of workers. Smith and Nash (1961) compared water absorption by U.S. versus Japanese soybean varieties. These investigators found the principal controlling factor in time required for water absorption to be seed coat integrity. Hard beans and those with a lower water content exhibited a decrease in water uptake. Although a tendency for lower water contents was found

in U.S. beans, as compared to Japanese beans, no fundamental difference in water absorption in soybeans from the two sources was found. Wang et al. (1979) observed the most rapid water absorption during the first two hours of soaking, with a declining rate of absorption with longer times.

Wang et al. (1979) also found that the rate of water absorption increased with increasing hydration temperature. As the temperature increased from 20° to 37°C, the time required for maximum hydration decreased from 5.5 hours to 2.5 hours. A similar trend was found by Johnson and Snyder (1978), who reported 14 hours for peak water absorption at 4°C compared to 9 and 2 hours for absorption at 21° and 100°C, respectively.

A greater loss of solids in the soak water seems to accompany long soak times. A 5% loss of solids was seen after 24 hours at 1°C while the loss increased to 10% at 72 hours (Lo et al., 1968a). The solids lost were predominantly (76.4%) carbohydrate with the remaining 23.6% being protein. The loss of protein apparently occurred during the initial 24 hour period, since as the nitrogen found in the soak water was constant after 24 hours. Wang et al. (1979) found larger losses with higher hydration temperatures and also found that the ratio of protein to total solids lost increased with increasing temperature.

In the grinding step in soymilk preparation, the protein is extracted from the beans and is dispersed, with the lipid, in the aqueous medium. In the traditional tofu preparation process the beans are ground with unheated water and the resulting slurry is heated.

Water is usually added to hydrated beans to supply a water to dry bean ratio of 10:1 (Lo et al., 1968b; Smith and Nash, 1961; Bourne et al., 1976; Wang, 1981).

In addition to the decrease in protein extractability seen by Johnson and Snyder (1978) with higher temperature of grinding, Yoshino et al. (1982) found a decrease of water soluble nitrogen with longer bean storage times. Saio and Arisaka (1978) reported similar results and also found a higher ratio of 7S to 11S soy protein fractions with longer storage times.

A number of alternative methods of soymilk preparation have been suggested, the most common of which is the 'hot grind' technique. In this method the beans are ground with boiling water to inactivate the lipoxygenase enzyme. Wilkins et al. (1967) found that this technique produced soymilk having a blander flavor than that produced by some other methods. Lo et al. (1968b) also reported improved flavor with higher grinding temperatures but they noted substantial reductions in yield at temperatures greater than 85°C. Johnson and Snyder (1978) compared the effectiveness of a 30 minute blanch at 100°C before a 20 to 25°C grind with that of a 95 to 100°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 (as in the boiling water grind) allowed greater release of protein.

The grinding step is followed by heating to a boiling temperature. The slurry is held at or near boiling usually for 10 to 20 minutes, and

is then filtered to remove the insoluble material or okara.

Preparation of tofu

Following preparation of soymilk, the hot milk is gelled by the addition of a coagulant. A number of coagulants have been used and may be classified generally into three types: chloride, sulfate, and acid types. Chloride type coagulants include mainly magnesium and calcium chloride. Nigari, the most traditional coagulant, is prepared from dehydrated sea water with the sodium chloride removed. It is composed primarily of magnesium chloride. Chloride types seem to be faster reacting than sulfate coagulants (Saio, 1979). The coagulant in greatest use commercially is calcium sulfate (Lu et al., 1980). Magnesium sulfate is the only other coagulant in the sulfate group. The primary acid type of coagulant is glucono delta lactone (GDL), which is used in the manufacture of silken tofu, a very soft tofu product which is not pressed. Acetic and citric acids may also be used in tofu preparation on a home scale.

A number of other coagulants have been used with varying success. Calcium acetate and calcium gluconate failed to produce a curd in work by Tsai et al. (1981). Kamel and DeMan (1982) were successful in producing curd with calcium acetate, but they found no coagulation using calcium citrate. Failure to coagulate was also seen when calcium hydroxide, calcium carbonate and calcium hydrogen phosphate were used, although calcium acetate and calcium gluconate were successfully employed (Lu et al., 1980).

Coagulant concentration

The concentration of coagulant required for curd formation varies. Wang (1981) found curd formation in the 0.008M to 0.080M range using calcium sulfate, calcium chloride, magnesium sulfate, and magnesium chloride. No curd formed at concentrations below 0.006M and above 0.1M. Saio (1979) reported that the optimum concentration of calcium salt for coagulation was 0.02N.

The type of coagulant used affects the amount of coagulant required (Kamel and DeMan, 1982; Wang and Hesseltine, 1982). Minimum coagulant levels necessary for coagulation were 0.1% using a chloride coagulant, 0.25% for sulfate coagulants, and 0.20% for an acetate coagulant (Kamel and DeMan, 1982).

Other factors may also influence the amount of coagulant needed. Skurray et al. (1980) found that the amount of calcium sulfate required was inversely related to the amount of protein in the soybeans from which the tofu was prepared. Using a calcium coagulant, Nakashima and Murakami (1980) found that when a sodium salt was present in the soymilk, a larger amount of calcium was needed for coagulation. The effect became more pronounced as the anion size of the sodium salt increased.

Lu et al. (1980) have 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. For these coagulants, precipitation of protein began when a sufficient amount of coagulant was added for the pH to fall 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. Similar results were reported by Kamel and DeMan (1982). Addition of sufficient chloride ion to coagulate soymilk lowered the pH of the soymilk from 6.58 to 5.91. Reductions to pH values of 5.74 and 6.08 were seen when sulfate and acetate ions were used as coagulants, respectively.

Soymilk may be coagulated over a range of temperatures. Wang (1981) has reported that the optimum temperature for tofu curd formation is between 60° and 70°C. Alteration in coagulation temperature affects tofu moisture content and texture, with higher temperatures resulting in a decrease in percentage moisture and a firmer product (Wang and Hesseltine, 1982).

Following the addition of coagulant to form the tofu curds, a short settling period is necessary for completion of curd formation. The curds are then transferred to a cloth-lined perforated pressing box. They are weighted and whey is allowed to drain, usually from 20 minutes to one hour.

Tofu gel formation

Gel formation in tofu has received a considerable amount of attention, with much of it centering on the types of bonding involved. Many workers have stressed the importance of disulfide bonding in this product (Saio et al., 1971; Hashizume et al., 1978; Hashizume and Ka, 1978; Saio and Watanabe, 1978; Furukawa and Ohta, 1982). When a sulfhydryl blocking agent was added to soymilk, a weaker tofu gel

resulted (Saio et al., 1971). Saio (1979) stated that the formation of disulfide bonds is important in coagulation with calcium or GDL but not in acid coagulated tofu. The addition of cysteine to soymilk was shown to result in an increased protein solubility caused by disulfide disruption (Furukawa and Ohta, 1982). Other types of bonding which have been suggested as being of importance in tofu gel formation are hydrogen bonding, hydrophobic interactions, and, in calcium coagulated tofu, calcium bridges (Saio et al., 1971; Hashizume and Ka, 1978; Saio and Watanabe, 1978).

The importance of disulfide bond formation in gel formation has also been demonstrated by studying heated soymilk. More sulfhydyl groups seem to be exposed in soymilk after heating than before heating, resulting in a more extensive three dimensional network formation. A number of investigators have reported a greater number of sulfhydryl groups with heating (Saio et al., 1971; Hashizume et al., 1978; Saio, 1979). Saio (1979) reported that heating soymilk initially increased the number of sulfhydryl groups and that continued heating resulted in a decrease in number of sulfhydryls. Long heating of soymilk was also shown by Hashizume et al. (1978) to result in a reduction of sulfhydryls through oxidation. The loss of sulfhydryls was related to a weakening of the tofu gel structure.

As in the case of heat-induced soy protein gels, there seem to be differences between gelation of 7S and 11S protein in tofu. This difference in gelation has been related to difference in sulfhydryl group content of the two fractions and is affected by soymilk heating

time. Maximum exposure of sulfhydryls at 100°C occurred after 20 to 30 seconds of heating the 11S protein fraction. A longer heating time of from 1 to 5 minutes was required for maximum exposure of sulfhydryls in the 7S protein (Saio et al., 1971). In 1978, Saio and Watanabe found that the optimum temperature for gelation was higher in the 11S than in the 7S fraction implying a greater dependence on disulfide bond formation in gelation in the 11S fraction. Firmer tofu gels are seen with coagulation of the 11S protein than with the 7S fraction (Saio, 1979), and some authors have concluded that the properties of tofu prepared from 7S protein cannot be related to sulfhydryl group content (Saio et al., 1971). Hashizume et al. (1975) suggested that in unheated milk or at lower temperatures, gelation results from 7S aggregation, while at higher temperatures the 11S fraction is primarily involved.

Tofu texture

Tofu texture is affected by many factors, a number 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 produced using other coagulants, while that prepared with GDL was the softest. When

cohesiveness and elasticity were measured no difference was seen among the coagulants tested. The coagulants included calcium and magnesium sulfate and calcium and magnesium chloride. The chloride type coagulants did result in a more brittle product than did the sulfate types (Wang and Hesseltine, 1982).

Coagulant concentration also influences tofu texture. Increasing coagulant concentration increases the firmness of the product. Due to 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.

The temperature at which the soymilk was coagulated has been shown to influence the texture of the final product. Saio (1979) found greater hardness of tofu with higher coagulation temperatures. In 1982, Wang and Hesseltine confirmed this phenomenon, and also noted greater elasticity with higher coagulation temperatures. No effect on tofu cohesiveness was seen. Agitation of the coagulated product also will increase tofu firmness by disrupting the gel network. The subsequent release of whey produces a less moist, firmer curd (Saio, 1979; Wang and Hesseltine, 1982).

It seems logical that the weight used in pressing and length of pressing would affect the texture of tofu although there has been a limited amount of research in this area. Schroeder and Jackson (1972) found that a pressure of 1 psi resulted in a smooth rubbery textured

tofu, with lower pressures yielding tofu with a pastelike consistency.

As mentioned previously, 7S and 11S proteins have different gelation characteristics and thus have different effects on the texture of tofu. 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 fraction 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. When tofu was prepared from 7S and 11S fractions mixed in varying proportions, hardness was seen to decrease 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. Kantha et al. (1983) prepared a tofu-like product by the coagulation of winged bean protein. A very soft curd was produced which was attributed to lack of the 11S component. Murphy and Resurreccion (1984) found high correlations between glycinin content and hardness, brittleness, elasticity, and gumminess of tofu. No relationship between conglycinin content and textural characteristics of tofu was seen. In contrast to these studies, Skurray et al. (1980) found no relationship between the 7S to 11S ratio and tofu quality.

Variation in tofu texture with soybean variety has been attributed to variation of the 7S:11S ratio with variety (Saio et al., 1969b). Other work has not demonstrated this effect. Smith et al. (1960) prepared tofu from 15 U.S. and 15 Japanese soybean varieties. Average hardness values of tofu from beans from the two sources were not

significantly different. More recently, Wang et al. (1983) also compared U.S. and Japanese soybean varieties with regard to preparation of tofu. These authors also found no overall difference in tofu produced from beans of the two origins. They did find significant variation in firmness of tofu prepared from beans of different varieties. This variation could not be accounted for on the basis of 7S/11S ratio alone; these authors suggested that other factors outweighed differences in 7S/11S ratio in determination of tofu texture.

Length of heating of soymilk in tofu preparation also affects tofu texture and, as previously discussed, is related to oxidation of sulfhydryl groups. 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 minutes of heating. Escueta (1979) found increases in hardness and gumminess of tofu which were not significant when soymilk was boiled for up to 12 minutes. After 30 minutes a significant decrease in these characteristics was seen. Cohesiveness of tofu was not affected after boiling 30 minutes, but it decreased after 60 minutes. Tofu elasticity was not affected by boiling for over 60 minutes.

Because phytic acid is able to combine with divalent cations such as calcium and with soy protein, it is not surprising that the effect of phytic acid on tofu texture has been investigated. Saio et al. (1969b) found that hardness of tofu decreased with an increase in the total phosphorus to nitrogen ratio. Whether the phosphorus was in the

form of native or added phytic acid was not clear. When four varieties of soybeans were coagulated using a calcium salt, phytic acid decreased the rate of coagulation with the ultimate effect of producing softer tofu. This effect was noted with either added or native phytic acid. The softest tofu was produced when the calcium:phosphorus ratio was 1:3. As this ratio decreased to 1:1.5 a firmer tofu of smaller volume resulted. Other work by this same group has shown a greater effect of phytic acid on texture of tofu prepared from 11S protein than on that prepared using the 7S fraction (Saio et al., 1969a; Saio and Watanabe, 1978). But Skurray et al. (1980) were unable to find a relationship between phosphorus content and tofu quality.

Tofu yield

Tofu yield is closely related to tofu texture since higher yields are normally the result of greater water retention. Greater water retention leads to a softer tofu. Comparison of yield data are somewhat difficult since percent yield may be expressed based on a number of different standards. Weight of tofu per gram of dry or hydrated bean may be used, as may tofu weight per liter of soymilk. In some cases protein yield is reported.

The effect of type of coagulant on tofu yield has been investigated by several workers. Lu et al. (1980) found the highest tofu yield when calcium chloride was the coagulant while GDL gave the lowest yield. Tsai et al. (1981) found calcium sulfate to give a higher yield than the other calcium coagulants tested (calcium acetate, gluconate, and chloride), but lower than GDL.

In general, after a sufficient amount of coagulant is added to completely precipitate the protein, higher coagulant levels result in lowered yields. Tsai et al. (1981) found this to be true with calcium coagulants, but noted a continued increase in yield when GDL was used. Wang and Hesseltine (1982) reported increasing yields at coagulant concentrations of 0.01M to 0.02M with calcium chloride, magnesium sulfate, and magnesium chloride. At coagulant conditions of between 0.02M and 0.04M yields remained high, but fell at higher coagulant levels. When calcium sulfate was used as the coagulant, no change in yield was seen between 0.01M and 0.04M, and between 0.04M and 0.08M calcium sulfate yields decreased slightly.

A number of other factors influence yield of tofu. Heating soymilk for up to 30 minutes resulted in a slightly higher yield, compared to unheated soymilk, while continued heating for 60 minutes resulted in a significant yield decrease (Escueta, 1979). The temperature at which the soymilk was coagulated also affected yield, with lower yields seen as coagulation temperature increased from 60°C to 80°C. Stirring the coagulated curds before pressing also decreased yield (Wang and Hesseltine, 1982). Saio et al. (1969b) found increased yield of tofu as the calcium to phosphorus ratio increased from 1:1.5 to 1:3 with added sodium phytate.

Soymilk composition

The composition of soymilk has been determined by several groups. Chang and Murray (1949) found a moisture level of 91.0% in soymilk. Protein, fat, and ash percentages were 4.4%, 1.7%, and 0.5%,

respectively. DeMan et al. (1975) determined composition of soymilk prepared from 55 soybean varieties. Mean percent moisture was 94.02. Mean protein and lipid percentages were 2.71 and 1.24, respectively. Soymilk composition reported in The Book of Tofu (Shurtleff and Aoyagi, 1975) gave the following percentages of moisture, protein, fat, and ash, respectively: 90.8%, 3.6%, 2.0%, and 0.5%.

Preparation of soymilk apparently concentrates lysine and the aromatic amino acids in the milk. But a decrease in threonine, tryptophan, leucine, and valine relative to the bean has been found (Del Valle, 1981). Hackler and Stillings (1967) found no significant effect on amino acid composition by heating soymilk to 93°C for up to four hours. Miller et al. (1952) investigated partitioning of B vitamins with tofu preparation. From 50% to 90% of the thiamin, 90% of the riboflavin, and 60% to 80% of the niacin found in soybeans was retained in soymilk.

The effect of various lengths of soybean hydration on soymilk composition was investigated by Lo et al. (1968a). These authors concluded that soaking beans for as long as 72 hours did not have a significant effect on the gross composition of the soymilk.

Data on the mineral content of soymilk are more limited. When analyzed on a dry basis, calcium, iron, and phosphorus percentages in soymilk were 0.195, 0.0072, and 0.65, respectively (Chang and Murray, 1949). On a moist basis (mean 5.98% moisture), mean calcium, phosphorus, and magnesium concentrations in soymilk were 0.10, 0.53 and 0.20 mg/g, respectively. Recovery of bean calcium in the milk was

roughly 62%. Recoveries of phosphorus and magnesium were respectively, 82% and 72% (DeMan et al., 1975).

Bourne et al. (1976) examined the suitability of 30 soybean cultivars for use in soymilk preparation. Some varieties were found to produce dark brown or off-flavored milks. Variability in milks from beans of the same variety but different sources led the authors to conclude that factors other than variety influence the composition of soymilk. It was also noted that protein content of the soybean was not necessarily a good indicator of protein content of soymilk prepared from that bean.

Tofu composition

Although the composition of tofu may vary with a number of factors such as coagulant used and processing conditions, data on tofu composition have been published. Proximate analysis values of 88% moisture, 6% protein, and 3 to 3.5% lipid have been given (Kinsella, 1978; Fukushima, 1981). The Book of Tofu (Shurtleff and Aoyagi, 1975) gives values of 84.9%, 7.8%, 4.3%, and 0.7% for moisture, protein, lipid, and ash, respectively. Earlier work reported the following composition of tofu: 76.5% moisture, 15.0% protein, 7.1% lipid, and 0.9% ash (Chang and Murray, 1949).

The mineral content of tofu has also been investigated. The concentration of calcium in tofu varies considerably and depends on whether a calcium salt was used as a coagulant. Tseng et al. (1977) reported calcium levels on a wet basis in tofu of approximately 0.21% when measured by atomic absorption, and 0.17% when determined by EDTA

titration. In contrast, tofu coagulated with GDL had a calcium content of only 0.04%. Chang and Murray (1949) found calcium concentrations on a dry basis of 0.241% and 0.690% when magnesium chloride and calcium chloride, respectively, were the coagulants. More recently, Kantha et al. (1983) found 1.638% calcium in tofu on a dry basis. Phosphorous levels in tofu have been reported as 0.80% on a dry basis (Chang and Murray, 1949) and approximately 0.08% on a wet basis (Tseng et al., 1977).

Little data are available on trace mineral content of tofu. Chang and Murray (1949) found 105 ppm iron in tofu on a dry basis, while Kantha et al. (1983) found only 70 ppm iron. Miller et al. (1952) did not report mineral concentrations in tofu, but they noted that tofu retains from 50% to 60% of the iron found in the soybean. This ratio is low when compared to calculations made from data supplied by Chang and Murray (1949) and Kantha et al. (1983) of 140% and 100% of the soybean iron, respectively. The zinc content of tofu was reported to be 60 ppm by Kantha et al. (1983).

Data on the phytic acid content of tofu are extremely limited, although there seems to be agreement that a substantial amount of soybean phytic acid is retained in tofu. Saio (1979) states that most of the soybean phytic acid is extracted in soymilk and is co-precipitated with protein in tofu preparation, although no data are supplied supporting this conclusion. Thompson and Erdman (1982a) found that the tofu they prepared contained 1.96% phytic acid. These authors suggested that the calcium precipitation of tofu tended to concentrate

phytic acid. Zinc deficiency was induced in rats by feeding a tofu-based diet and was attributed to the phytic acid content of the tofu. No difference in tofu composition was seen, however, between calcium- and magnesium-precipitated tofu on a dry basis, nor was a difference in zinc status apparent between rats receiving the two types of tofu.

It is well established that the coagulation conditions used in tofu preparation affect tofu texture. A range of coagulant concentrations and temperatures of coagulation used in tofu preparation has been reported in the literature (Saio, 1979; Wang and Hesseltine, 1982; Wang, 1981; Tsai et al., 1981). Some investigators utilize "test tube" preparation techniques in which tofu is coagulated in small batches using less than 50 ml of soymilk (Saio, 1979; Wang and Hesseltine, 1982; Tsai et al., 1981). These procedures may or may not accurately reflect results obtainable using a practical scale method. Thus, studies are needed to determine the effects of various coagulation temperatures and coagulant concentrations on the composition of tofu and whey, and on the textural characteristics of tofu using a home scale procedure. Because of its extensive use for tofu preparation in the food industry (Fukushima, 1981; Shurtleff and Aoyagi, 1975), calcium sulfate should be used as the coagulant in these studies.

The effects of soybean composition on tofu texture are less clear than those of coagulation conditions. In light of the soy protein/phytic acid interaction, some effect of phytic acid on tofu texture might be expected. Identification of some soybean component

which is predictive of the texture of tofu prepared from those soybeans would be valuable to tofu processors; studies in this area are needed.

Finally, there is little data available on the partitioning of soybean components among soymilk, okara, tofu, and whey. These data could provide information on solubility and associations among soybean components. More importantly, such information is valuable from a nutritional perspective.

MATERIALS AND METHODS

Materials

Soybeans were obtained from seed companies in the Ames, Iowa area and were stored in plastic bags at 5°C. Amsoy 71 soybeans were used for experiments investigating the effects of coagulation conditions on tofu composition and texture. For other work, eight soybean samples were chosen according to soybean variety, growing location, and crop year to provide a range in concentration of components (Table 2). Reagent grade chemicals were used throughout the study except that food grade $CaSO_4$ was used in tofu preparation.

Methods

Tofu preparation

Equipment used in tofu preparation was either stainless steel, glass, or plastic. Deionized water was used in experiments where iron and copper were determined. Tap water was used in other experiments.

Soybeans were rinsed and soaked in water at 5°C to hydrate. Times were as needed for complete hydration or longer, usually 12 to 24 hours. Hydrated beans were allowed to warm to room temperature. They were drained and homogenized with water. The homogenization step was carried out in a Waring blender for 2 minutes on high speed. In experiments in which the effects of coagulation conditions were determined, 454 g of soybeans were homogenized with 3600 ml of water. Other experiments used 120 g soybeans slurried with 950 ml of water.

Table 2. Soybean samples used for tofu preparation

Sample	Soybean Variety	Growing Location	Crop Year
1	Amsoy 71	Beaman	1982
2	Amsoy 71	Hudson	1981
3	Amsoy 71	Hudson	1982
4	Amsoy 71	Pilot Mound	1982
5	Vinton	Hudson	1980
6	Vinton	Hudson	1982
7	Vinton 81	Ames	1982
8	Vinton 81	Hudson	1982

The soybean slurry was heated to boiling, and was simmered for 15 minutes. The slurry was stirred occasionally during heating. The hot slurry was filtered through 4 layers of cheesecloth to separate the soymilk from the insoluble okara. The milk was refrigerated overnight.

Percentage solids were determined in the soymilk using a modification of a method described by Johnson and Wilson (1984). One gram of soymilk was weighed into a 250-ml volumetric flask and diluted to volume. Absorbance of diluted soymilk was determined at 400nm using a Bausch and Lomb Spectronic 20 spectrophotometer. Two determinations were made per sample. Absorbance was related to percentage soymilk solids by a standard curve prepared by serial dilution of a

concentrated soymilk sample. Solids were determined for the standard curve by vacuum-drying the soymilk at 100°C until constant weight was attained.

Based on percentage solids in soymilk, the amount of water to be added to an aliquot of soymilk to provide 650 ml of 6% solids milk was calculated. The bulk of this water was added to the soymilk, with 20 to 25 ml being retained for dispersion of the coagulant.

In determination of the effects of coagulation conditions on tofu composition and texture, the diluted soymilk was rapidly heated to 90°C on an electric range and was either coagulated immediately or allowed to cool to 80°C or 70°C. The hot soymilk was added to a dispersion of $CaSO_4 2H_2O$ supplying a coagulant concentration of 0.020M, 0.025M or 0.030M in the soymilk. The soymilk was stirred until small curds began to appear (less than 5 seconds), and was then allowed to settle for 5 minutes. The coagulated soymilk was poured gently into a perforated stainless steel pressing box (4x5x3.5 inches) which had been lined with 3 layers of cheesecloth. The cheesecloth was folded over the curds, weighted with 4 kg, and the curds were pressed for 20 minutes. The box was supported by Lucite bars over a Pyrex dish in which the whey was collected. The same procedure was used for tofu preparation in experiments other than those investigating coagulation condition effects, except that soymilk was heated to 80°C and was coagulated immediately with 2.24 g $CaSo_4 2H_2O$ making a coagulant concentration of 0.025M.

After pressing, the tofu cake was carefully unwrapped and weighed. The cake was quartered and two diagonally opposite quarters were placed in a container, covered with water, and refrigerated for texture analysis on the following day. The remaining tofu was homogenized in a Waring blender, and was refrigerated until moisture and Kjeldahl nitrogen determinations were complete. A portion was freeze-dried and stored at 0°C for subsequent analysis. Soymilk not used in tofu preparation was also freeze-dried and stored at 0°C for subsequent analysis.

Whey transparency

Whey transparency was determined by measuring whey absorbance at 400nm in a Bausch and Lomb Spectronic 20 spectrophotometer immediately after collection.

Tofu yield

Tofu yield was calculated based on weight of tofu/weight of dry soybeans. A constant volume of 6% solids soymilk was used for each tofu cake; however, the volume of soymilk and soymilk percentage solids obtained from each portion of soybeans varied. Therefore, the percentage of dry beans represented by the tofu cake was calculated based on the proportion of the volume of soymilk coagulated relative to the volume of soymilk produced.

Percentage solids determination

Moisture was determined in samples of tofu obtained immediately after pressing. Okara was stored frozen at 0°C prior to moisture determination. Portions of homogenized samples were weighed into aluminum weighing boats and freeze-dried to remove most of the water. Samples were transferred to a vacuum-oven and dried at 100°C until constant weight was attained.

Protein determination

A macro-Kjeldahl procedure was used for determination of nitrogen content. Tofu was homogenized and weighed into Kjeldahl digestion tubes. Whey samples were pipetted directly into tubes. Soymilk and okara were dried before nitrogen determination. Fifteen ml concentrated H_2SO_4 and 2 Pro-Pac tablets (MT-37, Alfie Packers Inc,. Omaha, NE) were added and the tubes were allowed to stand overnight. The samples were digested and distilled using the Tecator digestion block and distillation apparatus as outlined in the Tecator Digestion System 20 manual. Following distillation, samples were immediately titrated with approximately 0.1N HCl using a methyl red - bromcresol green mixed indicator. Percentage nitrogen was calculated and converted to percentage protein using the 6.25 conversion factor.

Crude lipid determination

Crude lipid was determined in dried samples with a Goldfisch fat extractor using hexane (60-70°C boiling range) as the solvent. Samples of approximately 1 g were extracted 5 to 6 hours. Extracted lipid was

heated briefly at 100°C to volatilize any residual solvent and was weighed to give percent crude lipid.

Digestion for mineral analysis

Freeze-dried and vacuum-dried tofu, okara, and soymilk, vacuum-dried soybeans and whey obtained immediately after pressing were wet ashed using the following procedure. Acid soaked micro-Kjeldahl flasks were used for the H_2SO_4/H_2O_2 digestion; additional H_2O_2 was added as necessary to complete the digestion. Samples were heated until dense H_2SO_4 fumes were evolved and no further sample charring was observed. Cooled samples were diluted slightly with deionized water and were heated to dissolve silicates before dilution.

Calcium determination

Digested samples were diluted to give calcium concentrations of 0.05 mg calcium/ml or higher and calcium was determined using a procedure described in the Food and Feeds Analysis Handbook (Hach Inc., 1982). The method, developed by Hach Corporation, is based on an EDTA titration. Potassium cyanide was added to samples to prevent interference by other minerals and the end point was determined visually.

Trace mineral determination

Copper and iron were determined in digested soybean, tofu, soymilk, okara, and whey samples. Glassware used in trace mineral analysis was acid-soaked and deionized water was used throughout. Diluted samples were immediately transferred to acid-washed

polyethylene bottles. Trace minerals were determined as soon as possibile after dilution, usually the following day, by atomic absorption spectroscopy. A single beam Unicam (Model SP .90) atomic absorption spectrophotometer (AAS) was used for the analysis of whey samples. A Perkin Elmer model 460 AAS was used for analysis of the remainder of the samples. Absorption was related to trace mineral concentration through the use of a regression equation or graph prepared from a series of standard copper or iron solutions. Standards were prepared fresh for each sample run, and were acidified with 1 ml of 6N HNO_3 per 100 ml standard solution.

Phytic acid determination

Phytic acid was determined on dried tofu, soymilk, okara, and soybeans. Samples of from 0.2 to 0.5 g were extracted with 15 to 25 ml 2.4% HCl containing 10% Na_2SO_4. Following filtration a 5 ml aliquot was precipitated with $FeCl_3$ in 0.6% HCl (supplying approximately 4 mg iron). Samples were placed in a boiling water bath for 45 minutes to complete precipitation of ferric phytate. After cooling, samples were centrifuged and decanted and the pellet was washed with 0.6% HCl containing 10% Na_2SO_4. The tubes were heated in a boiling water bath for 5 minutes, then cooled and centrifuged. The supernatant was discarded and the pellet was washed with deionized water. Following centrifugation and decantation, the pellet was dispersed in 1 ml concentrated H_2SO_4 and transferred quantitatively to a 100 ml Kjeldahl flask. samples were digested, with 30% H_2O_2 being added as necessary to prevent charring and complete the digestion. Following dilution to

give concentrations in an appropriate range, phosphorus was determined on the digestate using the method of Chen et al. (1956). Phytic acid content was calculated from phosphorus content based on 28.16% phosphorus in phytic acid.

Texture measurement

Textural characteristics of tofu samples were measured using the Instron Universal Testing Machine (model 1122) equipped with a compression anvil and a 500 kg tension-compression load cell. Compression force was recorded on a strip chart recorder at a chart speed of 200 mm/minute. Cubes of tofu measuring 2 cm per side were cut from the interior of the tofu cake. Side exterior portions of the cake were not tested, nor was the top surface included, however, the bottom surface was not trimmed before texture measurement. A texture profile analysis (Bourne, 1968) was conducted on as many cubes as could be cut (usually 10 to 12 cubes). Samples were compressed to 25% of original height (5mm) at a crosshead speed of 200 mm/minute. Full scale load varied with tofu firmness but was either 2 or 5 kg.

Statistical Analysis

Experiments investigating the effects of coagulation conditions on tofu composition and texture were replicated two times and duplicate analyses were performed on each sample. Other experiments were replicated four times, however, all types of analyses were not completed for all replications. Analyses of soybean samples were repeated two or three times. Analyses of soymilk, tofu, okara, and

whey samples were repeated three or four times. A minimum of duplicate determinations was completed for each analysis.

Statistical analysis of data was carried out on the Iowa State University computer system using the Statistical Analysis System (SAS) package (Statistical Analysis System, 1982).

Means obtained for each parameter measured were analyzed by the analysis of variance. Associations among variables were tested using linear correlations.

RESULTS AND DISCUSSION

Effects of Coagulation Conditions on Composition and Texture of Tofu

A study was conducted to determine suitable coagulation conditions for tofu preparation in our laboratory and to determine whether an interaction between coagulation temperature and coagulant concentration existed.

Effects of coagulation temperature

The yield and composition of tofu and whey and the textural characteristics of tofu that was coagulated at 70°, 80°, and 90°C are shown in Table 3. Differences between samples coagulated at 70° and 80°C were significant only in the case of tofu protein. Differences were more pronounced for samples coagulated at 80° and 90°C. These tofu samples differed in percentage solids and protein, and the clarity of the whey produced at 80° differed from that produced at 90°. Tofu coagulated at 90° was harder and more fracturable than tofu coagulated at 70°C. Saio (1979) reported increased hardness of tofu when soymilk was heated to temperatures greater than 80°C, while little difference was found for tofu hardness when soymilk was heated from 70° to 80°C. Although the results in Table 3 seem to confirm the observations of Saio (1979), the experiments conducted by Saio (1979) are not identical to the experiments reported here. Soymilk in the study conducted by Saio (1979) had not been heated before the coagulation step, while the soymilk used in our studies had been simmered for 15 minutes. Heat treatment of soymilk has been shown to alter the levels of 7S and 11S

components which could be separated using electrophoresis (Hashizume et al., 1975). Increased ionic strength results in a decrease in the ability of soy protein to be aggregated when soy protein has been heated (Hashizume et al., 1975; Hashizume and Watanabe, 1979). Thus, the quantity and characteristics of the protein in the soymilk used by Saio (1979) were probably different than in the soymilk used in our studies, even though coagulation temperatures were identical.

Based on visual observations of the samples prepared in our laboratory, soymilk coagulated at higher temperatures tends to form smaller, discrete curds. In contrast, the curd formed at lower temperatures consists of a continuous network, which traps liquid efficiently. These differences in the nature of the curd formed at different coagulation temperatures presumably account for the differences in percentage solids and in volume of whey collected. Significantly more whey was separated from the curd coagulated at 90° than at 70°C, and tofu produced from soymilk coagulated at 90°C had a significantly higher solids content than tofu coagulated at 70°C (Table 3).

The percentage protein in whey was low at all temperatures, indicating that precipitation of protein was essentially complete regardless of coagulation temperature. Therefore, differences in tofu yield primarily reflected differences in retention of whey in the tofu cake rather than differences in protein recovery. Similarly, the increase in tofu protein percentage with increasing coagulation temperature is due to retention of lesser amounts of whey in the

Table 3. Composition and texture of tofu coagulated at three temperatures

Coagulation Temperature °C	Yield %	Solids %	Tofu Protein %	Tofu Calcium mg/g	Whey Volume ml	Whey Protein %
70	$294^{a}\pm 7$	$12.68^{a}\pm 0.30$	$6.72^{a}\pm 0.17$	$1.66^{a}\pm 0.05$	$322^{a}\pm 16$	$0.45^{a}\pm 0.01$
80	$272^{ab}\pm 9$	$13.68^{a}\pm 0.35$	$7.36^{b}\pm 0.20$	$1.66^{a}\pm 0.06$	$394^{ab}\pm 19$	$0.44^{a}\pm 0.01$
90	$250^{b}\pm 7$	$14.82^{b}\pm 0.30$	$8.05^{c}\pm 0.17$	$1.82^{a}\pm 0.05$	$409^{b}\pm 16$	$0.45^{a}\pm 0.01$

[a]LS Means±SEM based on two replications.

[b]Superscripts in common within any column indicate no significant difference ($P < 0.05$).

Whey Calcium mg/ml	Whey Clarity %T	Hardness Kg	Fractur-ability Kg	Cohesiveness	Elasticity mm
$0.52^{a}\pm$ 0.02	$50.40^{a}\pm$ 1.44	$0.40^{a}\pm$ 0.05	$0.14^{a}\pm$ 0.02	$0.16^{a}\pm$ 0.01	$3.28^{a}\pm$ 0.19
$0.56^{a}\pm$ 0.02	$47.60^{a}\pm$ 1.69	$0.53^{ab}\pm$ 0.06	$0.17^{ab}\pm$ 0.02	$0.18^{a}\pm$ 0.01	$3.58^{a}\pm$ 0.23
$0.54^{a}\pm$ 0.02	$40.70^{b}\pm$ 1.44	$0.65^{b}\pm$ 0.05	$0.20^{b}\pm$ 0.02	$0.18^{a}\pm$ 0.01	$3.66^{a}\pm$ 0.19

products coagulated at higher temperatures. The data in Table 3 also indicate that greater values for hardness and fracturability were found in tofu coagulated at the higher temperature. Again, increased loss of moisture on pressing the tofu coagulated at the higher temperature resulted in a higher percentage solids curd that was harder and more fracturable.

Wang and Hesseltine (1982) also have reported decreased yield and increased percentage solids as the coagulation temperature for tofu increased from 60° to 80°C. These authors also found increased hardness and elasticity of tofu with increased coagulation temperatures. Hashizume et al. (1975) found that maximum hardness of tofu coagulated with glucono delta lactone resulted from coagulation at 80°C, with a drop in hardness at higher temperatures.

Saio (1979) found both hardness and yield of tofu to increase as coagulation temperature increased from 60° to 100°C. These authors used a low calcium sulfate concentration of 0.008M. Protein coagulation was probably not complete at the lower temperatues used in their study. As coagulation temperature increased, more protein may have been precipitated, resulting in both increased yields and firmer texture.

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

precipitated. Based on these studies, whey clarity would be expected to increase with increasing coagulation temperature since it is likely that protein precipitation would be most complete under those conditions. It was found, however, that whey clarity was lowest in the sample coagulated at the highest temperature (90°C). The finer curds formed at 90°C are difficult to retain in the tofu block during the pressing step. It is possible that very fine tofu curds were separated with the whey at higher temperatures, leading to lowered whey clarity at these temperatures. This hypothesis (that fine tofu curds were separated in whey at higher temperatures) is not supported by values for whey protein (Table 3). The levels of protein in whey were quite low, however, and small differences in whey protein may not have been detectable. Thus, 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 if protein precipitations and separations are similar among samples.

Effects of coagulant concentration

The effects of coagulant concentration on composition of tofu and whey, and on the textural characteristics of tofu are shown in Table 4. Differences were significant among all three sample means only in the case of calcium concentrations in tofu and whey. In general, differences among means were significant only when low and high coagulant concentrations were compared.

Table 4. Composition and texture of tofu coagulated at three coagulant concentrations[a,b]

Coagulant Concentration Molarity	Yield %	Solids %	Tofu Protein %	Tofu Calcium mg/g	Whey Volume ml	Whey Protein %
0.020	284[a] ± 7	13.04[a] ± 0.30	7.03[a] ± 0.17	1.33[a] ± 0.05	348[a] ± 0.16	0.44[a] ± 0.01
0.025	274[a] ± 7	13.55[ab] ± 0.30	7.31[ab] ± 0.17	1.66[b] ± 0.05	377[a] ± 0.16	0.45[a] ± 0.01
0.030	259[a] ± 9	14.60[b] ± 0.35	7.80[b] ± 0.20	2.15[c] ± 0.06	379[a] ± 0.19	0.44[a] ± 0.01

[a] LS Means ± SEM based on two replications.

[b] Superscripts in common within any column indicate no significant difference ($P < 0.05$).

Whey Calcium mg/g	Whey Clarity %T	Hardness Kg	Fractur-ability Kg	Cohesiveness	Elasticity mm
$0.45^{a}\pm$ 0.02	$47.50^{a}\pm$ 1.44	$0.45^{a}\pm$ 0.05	$0.15^{a}\pm$ 0.02	$0.16^{a}\pm$ 0.01	$3.25^{a}\pm$ 0.19
$0.56^{b}\pm$ 0.02	$47.40^{a}\pm$ 1.44	$0.52^{a}\pm$ 0.05	$0.17^{a}\pm$ 0.02	$0.19^{b}\pm$ 0.01	$3.68^{a}\pm$ 0.19
$0.61^{c}\pm$ 0.02	$43.80^{a}\pm$ 1.68	$0.61^{a}\pm$ 0.06	$0.19^{a}\pm$ 0.02	$0.18^{ab}\pm$ 0.01	$3.59^{a}\pm$ 0.23

Significantly greater percentages of solids and protein were found in tofu prepared using the highest coagulant concentration (0.03M). Finer curds that have a decreased ability to retain liquid were formed at higher coagulant concentrations. Similar results were found by Wang and Hesseltine (1982) who reported decreased yield and moisture content of tofu with greater coagulant concentration. These workers noted that this effect was less important when calcium sulfate was the coagulant compared to some other coagulant types such as the chlorides. They attributed this effect to the low solubility of calcium sulfate which reduces the effective coagulant concentration. Saio (1979) has reported increased hardness at higher coagulant concentrations. This observation tends to support the increased percentage solids and protein found at the high concentration in the work reported herein. No significant difference in hardness was found, however, when hardness of tofu prepared with different coagulant concentrations was compared.

Changes in coagulant concentration in the range tested had a less pronounced effect on tofu texture than did the effect of change in temperature of coagulation. Tofu yield, hardness, and fracturability and whey volume did not differ significantly when different coagulant concentrations were used. Wang and Hesseltine (1982) found no significant effect of coagulant concentration on texture at levels between 0.02M and 0.04M, supporting the similarities in texture reported in this dissertation.

No difference in whey clarity was found, indicating complete precipitation of protein at all coagulant concentrations tested. Tsai

et al. (1981) found no difference in whey clarity between coagulant concentrations of 0.02 and 0.03M. Johnson (1984) reported whey transparency to be at a maximum between 0.012 and 0.013M calcium sulfate with no further change through the maximum concentration tested (0.015M). Saio (1979) reported similar results showing no change in whey transparency between calcium sulfate concentrations of 0.01 to 0.015M.

Temperature/concentration interactions

In light of the similar effects of coagulation temperature and coagulant concentration on the composition and texture of tofu, it seems reasonable that some interaction between these two variables would exist. However, no interaction was found for any of the characteristics measured. A temperature/coagulant concentration effect might have been observed had the number of temperatures and coagulant concentrations been increased and had a greater number of replicates been included.

Conclusions

Coagulation temperature and coagulant concentration both had an effect on tofu composition and texture, however, the effect of coagulation temperature was more pronounced than that of coagulant concentration.

Coagulant concentration affected percentages of solids and protein in tofu. The calcium concentration of tofu was also higher at greater coagulant levels with use of a calcium coagulant. The effect of

coagulant concentration on textural characteristics of tofu was minimal in the range of coagulant levels tested. It may be possible, therefore, to use a higher coagulant concentration to increase dietary calcium with no adverse effect on textural characteristics. It must be noted that this assumes the use of a calcium coagulant such as calcium sulfate. A magnesium or acid type coagulant would presumably have little or no effect on concentration of tofu calcium.

Higher coagulation temperatures produced tofu containing higher percentages of solids and protein and with lower yields and greater hardness and fracturability. These effects were more pronounced at 90°C while fewer differences between tofu samples coagulated at 70° and 80°C were apparent. Use of lower coagulation temperatures is recommended if softer textures or higher yields are desired while higher temperatures are preferred if a firmer tofu texture is desired.

Partitioning of Soybean Components in Products

The distribution of soybean protein, phytic acid, copper and iron among soymilk, tofu and whey was examined. Mean values for these components in soybeans, soymilk, okara, tofu and whey are shown in Table 5. Phytic acid was not determined in whey. Also shown in Table 5 are the percentages of the components in soybeans that were found in the various products. Since quantitative collection of okara following filtration of the soymilk is very difficult, percentage recoveries of these components in okara were not calculated. It seems reasonable to conclude that most of the component not recovered in soymilk would be

found in the okara, although small losses of solids from soybeans have been found in the soak water following soybean hydration (Lo et al., 1968a).

As can be seen from Table 5, standard errors for percent recoveries are relatively high. Large variability in percent recoveries of soybean components in soymilk and tofu has also been reported by other groups (Bourne et al., 1976; DeMan et al., 1975; Kamel and DeMan, 1982). Extraction and precipitation of the components measured may be dependent on variables such as small fluctuations in coagulation temperature, coagulant concentration or soybean grinding temperature.

Protein

The recovery of protein in soymilk was 57%, with the bulk of the soymilk protein being precipitated and retained in the tofu cake. This rate of recovery is slightly lower than protein recoveries reported by some other workers. Bourne et al. (1976) found a mean of 78.5% soybean protein in soymilk , with a range of from 64% to 93%. DeMan et al. (1975) reported a similar recovery of 72.7% protein in soymilk, with a larger range of 49.2% to 94.1%. Wang et al. (1983) found recoveries of protein in soymilk to be 70.2% , with a mean tofu protein yield of 67.5%. Lee et al. (1986) did not report recovery of protein in soymilk, but found 66% of total soybean solids to be extracted during soymilk preparation. Recoveries of from 59.0% to 70.3% protein in tofu were found by Kamel and DeMan (1982), while Smith et al. (1960) found a similar recovery of 62.7%.

Table 5. Partitioning of protein, phytic acid, copper, and iron among soymilk, tofu and whey[a]

	Protein		Phytic Acid		Copper		Iron	
	Content %	Recovery %	Content %	Recovery %	Content ppm	Recovery %	Content ppm	Recovery %
Soybeans	37.78± 2.08	---	1.47± 0.20	---	10.21± 2.35	---	79.11± 7.97	---
Soymilk	2.88± 0.15	57.00± 4.00	0.13± 0.02	64.50± 5.80	0.88± 0.18	63.80± 5.30	4.24± 0.41	39.40± 4.10
Okara	4.59± 0.31	---	0.16± 0.02	---	1.23± 0.24	---	8.06± 1.12	---
Tofu	7.74± 0.93	56.40± 3.80	0.32± 0.06	60.80± 3.30	1.50± 0.30	42.00± 7.80	10.67± 1.48	38.20± 4.90
Whey	0.35± 0.03	3.60± 0.05	---	---	0.67± 0.14	23.80± 3.50	0.67± 0.19	3.10± 0.90

[a]Mean±SEM.

Protein recoveries in soymilk that were lower than those previously discussed were found by Johnson and Snyder (1978), who compared three soymilk preparation methods and various soybean:water ratios. These workers found a protein recovery of only 21.8% if whole soybeans that had been heated before grinding were used to prepare soymilk. The percentage recovery increased to 35.4% when the soybeans were ground with boiling water (the "hot grind" method) and to 47.8% when no heating step was included. Recoveries of protein also were found to increase as the water to bean ratio was increased from 1:5 to 1:10. The highest recovery obtained by these authors was 58.4% when no heating step was used at a bean:water ratio of 1:10.

The lower yields of protein in soymilk reported in Table 5 compared to the results of Kamel and DeMan (1982) and Smith et al. (1960) reflect, in part, a somewhat lower bean to water ratio than has been used by these workers. A soybean:water ratio of roughly 1:9 was used in this study to insure a soymilk percentage solids of greater than 6%, so that soymilk percentage solids could be adjusted to this level. As shown by Johnson and Snyder (1978), the ratio of beans:water would result in less than maximum recovery of protein.

Differences in protein recoveries from soymilk may be attributed to a number of causes other than bean:water ratio. Grinding temperature would presumably affect protein solubilization, although, except for lowered extraction at higher temperature (Johnson and Snyder, 1978), no temperature effect has been reported. The fineness of the grind would also have an effect on extractability of protein.

The vibroreactor used by Johnson and Snyder (1978), or the homogenizer used by Wang et al. (1983), may have produced a finer grind than the blender used in this study. The length of grinding may also have been longer in some methods yielding higher protein recoveries. Although most authors do not report the length of the grinding step, DeMan et al. (1975) used three minutes in a blender compared to the two minutes used in this study. Soybean variety and composition could also be expected to exert some influence on protein recovery in soymilk and the degree to which the soymilk was pressed from the okara would also affect the yield of protein.

Even accounting for the variability in protein recovery in soymilk discussed in the preceding paragraph, it is difficult to explain how Bourne et al. (1976) and DeMan et al. (1975) acheived protein recoveries of over 90%. These authors both reported separation of insoluble material following soybean grinding. Lower recoveries would particularly be expected in the case of the work reported by Bourne et al. (1976), since the soymilk preparation method used by this group was a "hot grind" technique, which has been shown to result in a lower recovery of protein than is acheived using the traditional method.

Recovery of soymilk protein in tofu is generally high (Wang and Hesseltine, 1982). Low protein recovery would indicate incomplete precipitation in the coagulation step. Incomplete precipitation could be caused by inadequate coagulant concentration or a coagulation temperature which was too low. Wang and Hesseltine (1982) found greater recovery of soymilk nitrogen as the concentration of the

calcium sulfate coagulant increased from 0.01M to 0.02M. These authors reported that recoveries of soymilk nitrogen in tofu were between 90% and 100%. As shown in Table 5, protein recoveries in tofu from soymilk fell within this range.

Phytic acid

The recovery of soybean phytic acid in soymilk was 64.5%, with most of the phytic acid (94%) being retained in the tofu. DeBoland et al. (1975) reported similar recoveries of 69% of the phytic acid from soybean meal following a water extraction. DeMan et al. (1975), however, reported a much higher recovery of 83.25% of soybean phosphorus in soymilk. Saio (1979) stated that most of the phytic acid of soybeans was extracted and co-precipitated with the protein as tofu is prepared, although no data were presented to support this statement.

The greater percentage recovery of phytic acid relative to protein (64.5% versus 57.0%) suggests that some of the soybean phytic acid might be present in an uncomplexed form which is more readily extractable. But the association of phytic acid with soy protein is unquestionable (Fontaine et al., 1946; deRham and Jost, 1979; Brooks and Morr, 1984; Honig et al., 1984), making a lack of soy protein/phytic acid relationship unlikely. Alternatively, the difference in extraction percentages for protein and phytic acid may indicate that the phytic acid is associated with a protein component that is more readily solubilized. Brooks and Morr (1984) have shown that the phytic acid of soy protein is separated primarily with the 7S fraction and with the soy whey precipitate, while very little phytic

acid was associated with the 11S soy protein fraction. The preferential extraction of phytic acid relative to protein and the fact that phytic acid is more closely associated with the 7S soy protein fraction may, suggest that the 7S protein fraction is preferentially extracted in soymilk preparation.

Copper

The recovery of soybean copper in soymilk was 63.8%. Yoshida et al. (1982) found a slightly lower recovery of soybean copper (55%) in a tris-HCl buffer (pH 7.4) extract from soybeans. This extract contained 47% of the soybean protein. Although the recoveries of both copper and protein reported by Yoshida et al. (1982) were lower than those shown in Table 5, the ratio of extracted percentages of copper to protein are similar in both sets of data (1:1.2 versus 1:1.1).

While over half of the copper extracted in the soymilk was retained in the tofu cake, a relatively large amount was separated in the whey. Copper may be bound to some low molecular weight component which is lost in the whey. This hypothesis is supported by work of Yoshida et al. (1982) who separated two copper containing peaks from a soybean extract. One peak was a high molecular weight (protein) fraction while the second included a group of low molecular weight compounds (sugars, peptides, and free amino acids). The low molecular weight fraction had a much higher copper binding capacity than did the protein fraction.

Iron

The recovery of soybean iron in soymilk was 39.4% (Table 5). This percentage is substantially lower than the recovery of soybean copper in soymilk (64%). Of the water-extractable iron, over 90% was retained in the tofu during soymilk coagulation. This is, again, in contrast to the results found for copper where losses of copper in whey were substantial (37%). The low extractability of iron in soymilk indicates the association of this mineral with a soybean fraction having a limited solubility. Unfortunately, there has been little research published that deals with the extractability of iron from soy products. Thiele and Schwedt (1986) tested the solubility of iron from a variety of foods including cereals, legumes, breads, fruits, and vegetables. These authors made the general statement that foods high in protein (>8%) tend to contain large amounts of total iron with small percentages of the iron being water soluble. Thiele and Schwedt (1986) further stated that "most" iron in food is not extracted with water, but list the iron in soy flour as being one exception to this rule.

Schnepf (1986) demonstrated decreased absorbability of iron from soy protein using everted rat gut. This decreased availability was attributed to iron binding by a large molecular weight peptide and by undigested soy protein isolate. These observations and the data presented in Table 5 suggest that iron in soybeans exists in a form that is not readily solubilized.

Conclusions and nutritional implications

Based on the results of this study, it may be concluded that substantial portions of soybean protein, phytic acid, copper, and iron are extracted as soymilk is prepared. The bulk of these extracted components is retained in tofu although losses of copper in whey are relatively high. The retention of protein, copper, and iron in tofu makes tofu a significant source of these nutrients, however, soymilk should be considered to be a better source of copper. The apparent concentration of phytic acid in tofu may reduce the availability of certain minerals, notably zinc, from tofu (O'Dell et al., 1964; Bodzy et al., 1977). The concentration of phytic acid in tofu is particularly significant in light of the possible replacement of meat with tofu in the diet. Meat is considered to be one of the best sources of zinc (Freeland and Cousins, 1976; Murphy et al. 1975; Haeflein and Rasmussen, 1977). Replacement of a good source of dietary zinc with a food shown to have decreased zinc availability (Forbes et al., 1983) could lead to impaired zinc status.

It should also be noted that although over 50% of soybean protein is extracted in soymilk, the quality of protein extracted may be poorer than the quality of the protein retained in the okara. Hackler et al. (1963) reported greater weight gains in rats fed okara-based diets compared to those fed diets containing tofu. The protein efficiency ratios for casein, okara, and tofu-based diets were 2.86, 2.71 and 2.20, respectively.

Effects of Soybean Composition on Composition and Texture of Tofu

Composition of products

Soybeans The composition of the samples of soybeans used in the preparation of tofu is shown in Table 6. Compositional data for soybeans analyzed in this study are similar to values reported previously.

The overall mean for percentage solids in soybeans was 90.18%. This is somewhat lower than the percentage solids found in soybeans grown in Ontario (DeMan et al., 1975). Levels in the Canadian beans ranged from 95.79% to 92.20% with a mean of 95.00%.

The mean protein content in the soybeans tested in this study was 37.80%. This is within the range of protein percentages reported in soybeans grown in Ontario by DeMan et al. (1975), but lower than the mean protein percentage of 40.84 found by these authors. Cartter and Hopper (1942) found protein percentages in 10 varieties of soybeans grown in the Midwest to range from 32.62% to 53.19%.

Values for lipid in soybeans reported in the literature are similar to the mean of 17.97% found in this study. DeMan et al. (1975) reported a mean lipid percentage in soybeans of 17.18% with a range of 14.46% to 21.20%. Krivoruchco et al. (1979) found 14.8% to 22.0% lipid in soybeans with a mean percentage of 19.51. Papadopetros and Katsikas (1981) reported a slightly larger amount of lipid in soybeans grown in Greece. Lipid content in these samples ranged from 21.1% to 22.5%.

Soybean samples analyzed in this study contained an overall mean of 1.47% phytic acid. Phytic acid levels in soy products are generally

Table 6. Composition of soybeans used in tofu preparation[a]

Sample	Variety	Solids %	Protein %	Lipid %	Phytic Acid %	Calcium mg/g	Copper ppm	Iron ppm
1	Amsoy 71	89.71± 0.24	35.07± 0.18	18.69± 0.44	1.28± 0.01	1.30± 0.02	10.75± 0.17	77.27± 0.79
2	Amsoy 71	90.93± 0.14	35.57± 0.06	18.91± 0.25	1.87± 0.03	1.11± 0.03	4.55± 0.20	91.01± 2.75
3	Amsoy 71	90.87± 0.24	36.11± 0.07	18.54± 0.33	1.37± 0.01	1.31± 0.05	10.88± 0.26	67.54± 5.48
4	Amsoy 71	91.44± 0.27	37.44± 0.07	17.55± 0.61	1.31± 0.04	1.48± 0.03	12.15± 0.02	76.87± 4.34
5	Vinton	91.36± 0.32	39.79± 0.33	18.83± 0.43	1.42± 0.01	2.21± 0.22	10.64± 0.15	76.75± 0.23
6	Vinton	90.51± 0.16	39.88± 0.30	17.16± 0.24	1.34± 0.02	1.77± 0.05	11.37± 0.41	90.74± 2.70
7	Vinton 81	91.04± 0.27	39.11± 0.41	17.42± 0.15	1.60± 0.02	1.95± 0.09	10.15± 0.05	76.26± 1.10
8	Vinton 81	90.63± 0.42	39.63± 0.28	16.63± 0.40	1.60± 0.01	1.74± 0.13	11.21± 0.53	76.78± 0.43

[a]Mean±SEM based on two replications for phytic acid, copper and iron and on three replications for remaining components.

described as falling between one and two percent. Lolas et al. (1976) reported a mean level of phytic acid in soybeans of 1.14%. Harland and Prosky (1979) found a mean phytic acid content of 2.22%.

The mean level of calcium in soybeans tested was 1.61 mg/g. This value is near the lower end of the range of calcium levels in soybeans found by Cartter and Hopper (1942) of 2.63 to 4.70 mg calcium/g.

There are a limited amount of data in the literature regarding the trace mineral content of soybeans. Freeland et al. (1977) reported 24.6 ppm of copper in soybeans while a lower value of 13 ppm was found by Pennington and Calloway (1973). The mean copper content of soybeans analyzed in this study was 10.17 ppm.

Soymilk Volume and composition of soymilk prepared from different soybean samples are shown in Table 7. The mean percentage of protein in soymilk was 2.88%. Percentage protein in soymilk has been reported by a number of workers. 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) found soymilk prepared from Clark variety beans to contain 3.12% protein. Del Valle (1981) also found 3.12% protein in soymilk, while Wang et al. (1983) found the soymilk protein percentage from 10 soybean varieties to average 3.2% (percentage solids not specified).

The mean percentage lipid in the soymilk analyzed in this study was 1.28%. This is similar to values reported by Chang and Murray (1949) who found 1.14% lipid in soymilk (6% solids basis) or those

Table 7. Composition of soymilk[a]

Sample	Variety	Volume ml	Protein %	Lipid %	Phytic Acid %	Calcium mg/g	Copper ppm	Iron ppm
1	Amsoy 71	914± 42	2.68± 0.01	1.42± 0.05	0.11± 0.00	0.07± 0.00	0.92± 0.02	4.09± 0.19
2	Amsoy 71	868± 36	2.78± 0.05	1.35± 0.03	0.16± 0.00	0.06± 0.01	0.45± 0.04	4.75± 0.21
3	Amsoy 71	895± 51	2.73± 0.03	1.29± 0.10	0.12± 0.00	0.07± 0.01	0.97± 0.08	4.01± 0.25
4	Amsoy 71	900± 38	2.77± 0.02	1.36± 0.06	0.11± 0.00	0.08± 0.00	0.99± 0.03	3.87± 0.26
5	Vinton	901± 40	3.05± 0.01	1.27± 0.06	0.12± 0.00	0.14± 0.01	0.94± 0.06	4.33± 0.29
6	Vinton	942± 28	3.06± 0.01	1.25± 0.05	0.12± 0.00	0.11± 0.00	0.96± 0.02	4.45± 0.15
7	Vinton 81	870± 19	2.99± 0.02	1.15± 0.03	0.14± 0.00	0.12± 0.01	0.87± 0.03	4.14± 0.23
8	Vinton 81	921± 20	2.99± 0.01	1.15± 0.01	0.13± 0.00	0.12± 0.01	0.95± 0.01	4.23± 0.12

[a]Mean±SEM based on three replications for lipid, copper and iron and on four replications for remaining components.

reported by DeMan et al. (1975) who found a mean of 1.24% lipid in soymilk prepared from 55 soybean varieties. Wang et al. (1983) found a slightly higher lipid level of 1.53% in 10 soybean varieties (percentage solids not specified).

Phytic acid concentrations in soymilk have not been reported in the literature, however, DeMan et al. (1975) found a phosphorus content of 0.05% in soymilk on a 6% solids basis. Using the relationship of total phosphorus to phytic acid content of soybeans found by Lolas et al. (1976), this level of phosphorus would be equivalent to a phytic acid content of 0.11%. This value is similar to the mean of 0.13% phytic acid in soymilk reported herein. The applicability of the relationship reported by Lolas et al. (1976) to soymilk is not known, however.

The mean calcium level in soymilk was 0.10 mg/g. This concentration is the same as the calcium content found by DeMan et al. (1975) and quite similar to the 0.12 mg/g reported by Chang and Murray (1949).

The iron concentrations in soymilk reported by Chang and Murray (1949) were 4.32 ppm. This value is similar to the iron levels reported in this dissertation. Soymilk analyzed in this study contained an average of 4.23 ppm iron.

Okara Composition of okara produced from different soybean samples is shown in Table 8. There are a limited amount of data in the literature on the composition of okara. Bourne et al. (1976) reported okara solids, protein, and crude lipid levels of 23.2%, 5.6% and 3.7%,

respectively. Mean percentages of solids, protein and lipid reported herein were 15.0, 4.6 and 1.7, respectively.

Tofu Tofu yield and composition are shown in Table 9. The mean percentage solids in tofu was 14.25%. This level is higher than the 13.2% solids Kantha et al. (1983) found in soybean curd, but considerably below the 23.5% solids found in tofu by Chang and Murray (1949). Other sources report 15.1 to 15.2% solids in tofu (Adams, 1975; Shurtleff and Aoyagi, 1975).

The mean percentage of protein in tofu was 7.27%. Similar amounts of protein in tofu have been reported previously. Adams (1975) found 7.83% protein while Shurtleff and Aoyagi (1975) reported 7.8% protein. Del Valle (1981) found 50.79% protein in tofu on a moisture-free basis.

The percentage of lipid in tofu averaged 3.87%. This value is lower than the percentage lipid in tofu found by Adams (1975), Shurtleff and Aoyagi (1975) and Chang and Murray (1949). These authors have reported 4.2%, 4.3%, and 7.1% lipid in tofu, respectively.

The mean percentage of phytic acid in fresh tofu was 0.32%. Thompson and Erdman (1982a) found a level of 1.96% phytic acid in tofu on a moisture-free basis.

The mean level of calcium in tofu was 1.95 mg/g. Chang and Murray (1949) have reported a much lower concentration of calcium in tofu. These authors found 6.9 mg calcium/g in dry tofu when calcium chloride was used as the coagulant. Adams (1975) and Shurtleff and Aoyagi (1975) reported the calcium concentration of tofu at 1.28 mg/g and 1.46 mg/g, respectively (coagulant type not given). Kantha et al. (1983)

Table 8. Composition of okara[a]

Sample	Variety	Solids %	Protein %	Lipid %	Phytic Acid %	Calcium mg/g	Copper ppm	Iron ppm
1	Amsoy 71	15.56± 0.58	4.38± 0.18	1.94± 0.08	0.16± 0.00	0.32± 0.01	1.33± 0.01	8.70± 0.32
2	Amsoy 71	14.84± 0.58	4.22± 0.23	1.70± 0.17	0.19± 0.01	0.27± 0.00	0.58± 0.06	10.50± 0.56
3	Amsoy 71	15.04± 0.16	4.31± 0.11	1.70± 0.03	0.16± 0.00	0.27± 0.02	1.41± 0.01	7.32± 0.46
4	Amsoy 71	14.92± 0.51	4.70± 0.04	1.61± 0.02	0.14± 0.00	0.34± 0.01	1.29± 0.02	7.30± 0.89
5	Vinton	15.02± 0.02	5.05± 0.00	1.81± 0.02	0.16± 0.01	0.48± 0.02	1.33± 0.04	7.95± 0.49
6	Vinton	14.50± 0.04	4.55± 0.14	1.55± 0.33	0.15± 0.00	0.31± 0.04	1.20± 0.15	6.87± 0.20
7	Vinton 81	14.83± 0.09	4.79± 0.02	1.66± 0.02	0.18± 0.00	0.38± 0.01	1.24± 0.07	7.92± 0.10
8	Vinton 81	14.87± 0.27	4.80± 0.07	1.70± 0.15	0.18± 0.00	0.37± 0.01	1.28± 0.04	8.09± 0.19

[a]Mean±SEM based on three replications.

Table 9. Composition of tofu[a]

Sample	Variety	Yield %	Solids %	Protein %	Lipid %	Phytic Acid %	Calcium mg/g	Copper ppm	Iron ppm
1	Amsoy 71	257± 8	14.83± 0.60	7.54± 0.36	4.83± 0.62	0.30± 0.01	1.79± 0.09	1.37± 0.02	10.97± 1.17
2	Amsoy 71	248± 7	15.26± 0.34	7.78± 0.25	4.43± 0.18	0.44± 0.01	2.16± 0.06	0.88± 0.24	11.77± 0.77
3	Amsoy 71	294± 4	13.04± 0.33	6.88± 0.19	3.56± 0.14	0.28± 0.01	1.87± 0.06	1.56± 0.06	9.54± 0.34
4	Amsoy 71	286± 14	14.28± 0.64	7.42± 0.61	3.80± 0.12	0.28± 0.01	1.85± 0.04	1.70± 0.11	10.71± 0.68
5	Vinton	298± 17	13.65± 0.57	7.80± 0.38	3.64± 0.46	0.29± 0.02	1.93± 0.10	1.53± 0.24	10.28± 0.50
6	Vinton	298± 14	14.16± 0.79	8.16± 0.70	3.71± 0.48	0.29± 0.02	1.88± 0.21	1.49± 0.02	11.38± 1.31
7	Vinton 81	290± 31	14.04± 1.43	7.68± 0.72	3.40± 1.30	0.34± 0.04	1.97± 0.30	1.90± 0.30	10.12± 1.38
8	Vinton 81	276± 19	14.75± 0.84	8.46± 0.52	3.60± 0.51	0.35± 0.02	2.17± 0.21	1.54± 0.12	10.54± 0.46

[a]Mean±SEM based on three replications for lipid and minerals and four replications for remaining components.

prepared tofu using 0.5% calcium sulfate as the coagulant and found 2.16 mg calcium/g tofu.

The mean level of iron in tofu was 10.99 ppm. This iron concentration is slightly higher than the 9.24 ppm in tofu found by Kantha et al. (1983), but considerably lower than values reported by Adams (1975) and Shurtleff and Aoyagi (1975) of 19.2 and 17.0 ppm iron, respectively.

Whey Volume and composition of whey obtained during tofu preparation are shown in Table 10. Results are expressed both as concentration of component per unit volume and as the total amount of that component found in the total whey produced per batch of tofu.

Tofu texture

Table 11 shows the textural characteristics of tofu prepared from different soybean samples. The mean value for hardness of tofu was 0.39 kg. This is substantially softer than hardness values of tofu reported by Kamel and DeMan (1982). These workers found a mean hardness of 1.0 kg in tofu prepared from eleven soybean varieties. Samples were cylinders sized similarly to the cubes used in this study (20 mm diameter x 19 mm height versus 20 mm cubes). Both sets of samples were compressed to 75% of original height, although Kamel and DeMan (1982) used a substantially slower crosshead speed than was used in the work reported here (20 mm/minute versus 200 mm/minute). These workers also used calcium chloride as the coagulant at a concentration of approximately 0.02M. Wang and Hesseltine (1982) have shown that chloride coagulants produce firmer tofu than sulfate coagulants. Lu et

Table 10. Composition of whey[a]

Sample	Variety	Volume	Protein		Calcium		Copper		Iron	
			%	Total g	%	Total mg	ppm	Total g	ppm	Total g
1	Amsoy 71	355± 13	0.29± 0.05	1.03± 0.17	0.58± 0.01	199.67± 3.49	0.68± 0.05	232.66± 17.70	0.60± 0.04	207.27± 20.23
2	Amsoy 71	342± 8	0.34± 0.02	1.20± 0.03	0.35± 0.03	118.97± 11.75	0.35± 0.03	119.01± 11.64	0.38± 0.02	129.11± 4.55
3	Amsoy 71	307± 12	0.34± 0.02	1.13± 0.01	0.54± 0.01	166.92± 7.01	0.68± 0.04	209.64± 12.36	0.52± 0.10	156.10± 21.21
4	Amsoy 71	321± 15	0.36± 0.00	1.18± 0.04	0.58± 0.00	176.83± 3.01	0.73± 0.06	224.88± 18.84	0.67± 0.05	202.66± 10.46
5	Vinton	318± 19	0.35± 0.01	1.14± 0.05	0.56± 0.01	171.87± 9.52	0.75± 0.06	229.15± 6.88	0.89± 0.11	281.78± 7.22
6	Vinton	327± 19	0.36± 0.01	1.19± 0.08	0.58± 0.03	187.06± 18.25	0.73± 0.06	233.77± 13.55	0.84± 0.07	286.23± 1.33
7	Vinton 81	315± 30	0.38± 0.01	1.19± 0.11	0.58± 0.02	176.55± 22.13	0.72± 0.04	221.67± 28.52	0.72± 0.18	205.65± 3.15
8	Vinton 81	329± 14	0.32± 0.02	1.12± 0.10	0.56± 0.04	180.38± 20.08	0.72± 0.03	232.72± 11.40	0.76± 0.02	252.45± 26.01

[a]Mean±SEM based on three replications for minerals and four replications for volume and protein.

al. (1980) have also reported that tofu prepared using calcium chloride as the coagulant was firmer than that produced using other coagulants. Thus, the larger firmness values found by Kamel and DeMan (1982) probably reflect the use of calcium chloride as the coagulant.

Table 11. Textural characteristics of tofu[a]

Sample	Variety	Hardness Kg	Fracturability Kg	Cohesiveness	Elasticity mm
1	Amsoy 71	0.24±0.08	0.11±0.01	0.26±0.01	3.74±0.05
2	Amsoy 71	0.45±0.09	0.18±0.03	0.25±0.02	5.16±0.79
3	Amsoy 71	0.33±0.04	0.10±0.01	0.22±0.03	3.78±0.19
4	Amsoy 71	0.34±0.05	0.12±0.00	0.22±0.02	3.82±0.35
5	Vinton	0.39±0.04	0.16±0.02	0.24±0.02	4.48±0.30
6	Vinton	0.44±0.07	0.18±0.02	0.22±0.02	4.71±0.56
7	Vinton 81	0.47±0.11	0.17±0.03	0.20±0.03	4.71±0.56
8	Vinton 81	0.45±0.03	0.16±0.01	0.22±0.02	4.80±0.32

[a]Mean±SEM based on four replications.

Skurray et al. (1980) found hardness of tofu prepared from sixteen soybean varieties to average 17.75 kg. It is difficult to interpret this value since these authors report hardness values in kilograms of force, but stated that hardness was measured by the area of the peak obtained by 90% compression of a 3 cm cube of tofu. Calcium sulfate at a concentration of 0.019M was used as the coagulant in their studies.

Lu et al. (1980) found a hardness of 1.18 kg for tofu coagulated with 0.014M calcium sulfate. Hardness was measured using the compression anvil of an Instron to test tofu samples which were 1x1x1/2 inches. Other groups have measured hardness of tofu using methods other than compression with the Instron Universal Testing Machine. Thus, comparison of these values to the results reported here is difficult. Using a curdmeter, Saio (1979) reported a hardness of 40 g for tofu coagulated with a calcium sulfate concentration of 0.012M. Smith et al. (1960) used a penetrometer to measure tofu firmness. These authors reported mean penetrations of 43 mm and 49 mm for tofu prepared from Japanese and U.S. soybeans, respectively.

The mean value for cohesiveness of tofu for the data reported in this dissertation is 0.23. This is similar to the cohesiveness of 0.21 found by Skurray et al. (1980) in sixteen samples of tofu prepared from different soybean varieties.

Textural characteristics of tofu are influenced by a large number of factors. The effect of the type of coagulant chosen has already been mentioned. Coagulation temperature and coagulant concentration also affect the texture of the tofu obtained, with higher temperature and concentrations producing firmer tofu (Wang and Hesseltine, 1982; Saio, 1979). Length of heating of soymilk also affects tofu texture (Hashizume et al., 1978).

Other factors which are more difficult to control also have an effect on tofu textural characteristics. Agitation of the coagulated curd results in increased tofu firmness through disruption of the gel

network, with subsequent release of whey (Saio, 1979; Wang and Hesseltine, 1982). Thus, differences in extent of stirring on addition of the coagulant or disruption of the curd during transfer to the press box could have a substantial effect on texture of tofu.

Variation in the ratio of 7S:11S soy protein fractions has been shown to have an effect on texture of tofu (Saio et al., 1969b). Saio et al. (1969b) found tofu prepared from a soy protein preparation containing larger amounts of 11S protein to be firmer and more chewy. Conversely, tofu prepared with a smaller proportion of 11S protein relative to 7S protein was softer and more adhesive.

Saio et al. (1969b) have also related the phytic acid content of tofu to tofu textural characteristics. These workers reported that softer tofu was produced in the presence of larger amounts of phytic acid. Other work, however, has failed to show a relationship between phosphorus content of tofu and tofu quality (Skurray et al., 1980).

No significant differences were found among any textural characteristics measured with the exception of fracturability. This characteristic of tofu has not been previously reported in the literature. Lee et al. (1983) concluded that the mechanical behavior of tofu was regulated by two mechanisms. The first of these is the deformability and compressibility of the solid matrix; the second is the buildup and release of hydrostatic pressure in the sample as it is compressed. They concluded that at low strains rheological behavior of tofu can be explained by a relatively elastic deformability of the original structure, followed by stiffening of the structure as a result

of matrix compaction. At this stage, entrapped liquid also contributed to the accelerated increase in the force through the development of hydrostatic pressure. At larger deformations, liquid expression and mechanical rupture to the solid matrix were thought to assume dominant roles in the deformation mechanism. Tofu fracturability reflects the force required to rupture the gel network and release the entrapped fluid. With smaller amounts of trapped fluid, lower fracturability would be expected. The fracturability can then be used as an indication of the amount of trapped fluid and indirectly of the firmness of the tofu. The smaller amount of variability associated with the fracturability measurement (CV=21% versus 32%) allows detection of smaller differences in firmness.

Relationships among components

Significance among relationships was tested at $p < 0.05$ unless otherwise noted.

Soybeans versus tofu Significant correlations between components of soybeans and tofu and tofu textural characteristics are shown in Table 12. Positive correlations between the same component in soybeans and tofu were noted for phytic acid (r=0.94), copper (r=0.80), and iron (r=0.93). The relationship found between soybean and tofu lipid concentrations was not significant. But when these relationships are examined with composition expressed on a dry basis, a strong correlation (r=0.93) is found for soybean and tofu protein levels. The variation in moisture in tofu obscures relationships in soybean and tofu protein concentrations that are apparent when these relationships

are examined on a dry basis.

Wang et al. (1983) found a relationship between soybean and tofu levels of both protein and lipid. These workers stated that high protein soybeans are necessary to produce tofu having a high protein percentage, although they acknowledge that this is somewhat dependent on the method of tofu preparation used. Smith et al. (1960) reported that the protein and lipid contents of soybeans were roughly related to the protein and lipid contents of tofu, but that a lack of uniformity in the data existed. Causes of variation include factors such as variable protein extractability from soybeans resulting from differences in soybean hydration, grinding time and temperature, variation in extent of filtration in soymilk preparation, or precipitation of protein in the coagulation step. These factors have been previously examined when protein partitioning was discussed.

The significant relationship between soybean phytic acid and tofu calcium ($r=0.90$) indicates binding of this mineral by phytic acid. Saio et al. (1968) found greater retention of calcium in an 11S soy protein preparation as the amount of added phytic acid in the preparation increased. In addition, other work has clearly demonstrated the binding of calcium by phytic acid (Kroll, 1984; Vohra et al., 1965; Graf, 1983). Saio et al. (1969b) also showed calcium retention in tofu prepared from 11S soy protein to increase with increasing amounts of added phytic acid. These workers also noted that the addition of larger amounts of calcium sulfate coagulant were required for coagulation of soybeans containing larger amounts of

Table 12. Correlation coefficients among tofu components and textural characteristics and soybean components

Tofu Bean	Yield	Solids	Protein	Lipid	Phytic Acid	Calcium
Solids	0.43	-0.31	-0.04	-0.67	0.09	0.32
Protein	0.70	-0.26	0.62	-0.74*	-0.24	0.18
Lipid	-0.30	-0.13	-0.59	0.48	0.10	-0.21
Phytic Acid	-0.50	0.49	0.33	0.03	0.94*	0.90*
Calcium	0.70	-0.38	0.40	-0.66	-0.35	-0.03
Copper	0.68	-0.51	-0.03	-0.43	-0.89*	-0.60
Iron	-0.35	0.63	0.56	0.34	0.55	0.34

*$P < 0.05$.

Copper	Iron	Fracturability	Hardness	Cohesiveness	Elasticity
0.27	-0.25	0.30	0.55	-0.54	0.31
0.53	-0.16	0.56	0.61	-0.64	0.42
-0.52	-0.01	-0.30	-0.42	0.63	-0.21
-0.50	0.33	0.67	0.70	0.06	0.82*
0.64	-0.34	0.40	0.42	-0.55	0.25
0.80*	-0.52	-0.45	-0.35	-0.45	-0.60
-0.59	0.93*	0.73*	0.46	0.28	0.67

phytic acid. This implies that soybean phytic acid was associated with higher tofu calcium concentrations although this was not stated directly (Saio et al., 1969b).

The binding of calcium by phytic acid probably accounts for the relationship between soybean phytic acid level and tofu elasticity ($r=0.82$). With greater amounts of soybean phytic acid resulting in higher concentrations of tofu phytic acid, larger amounts of calcium are retained in the tofu cake. High tofu calcium is associated with textural changes including higher values for elasticity. In contrast, Saio et al. (1969b) reported that tofu having a higher yield (and which was presumably softer) was produced from soybeans containing larger amounts of phosphorus. These workers have attributed this to the presence of native phytic acid, although phytic acid itself was not measured. Saio et al. (1969a) have hypothesized that when phytic acid is present, added calcium coagulant is bound preferentially by phytic acid rather than by the soy protein. In the presence of larger amounts of phytic acid, the effective amount of coagulant is decreased and the resulting tofu has a softer texture.

Soybean iron content was positively correlated with tofu fracturability ($r=0.73$). This may be related to the fact that a relatively low percentage (3.1%) of soybean iron was lost in the whey which was separated from the tofu curds during pressing. A lower retention of whey in the tofu cake would result in a relatively larger tofu iron concentration. Smaller amounts of whey retained in the tofu would also result in less fracturable tofu.

Soybean copper was significantly correlated with tofu phytic acid (r=-0.89). Examination of a scatter plot of these data (Figure 2), however, indicates the presence of a single sample having phytic acid and copper values substantially different from the remaining samples. When this data point is omitted the correlation falls to -0.41. Analysis of a larger number of samples is needed before any firm conclusions can be made regarding the significance of the relationship between soybean copper and tofu phytic acid values.

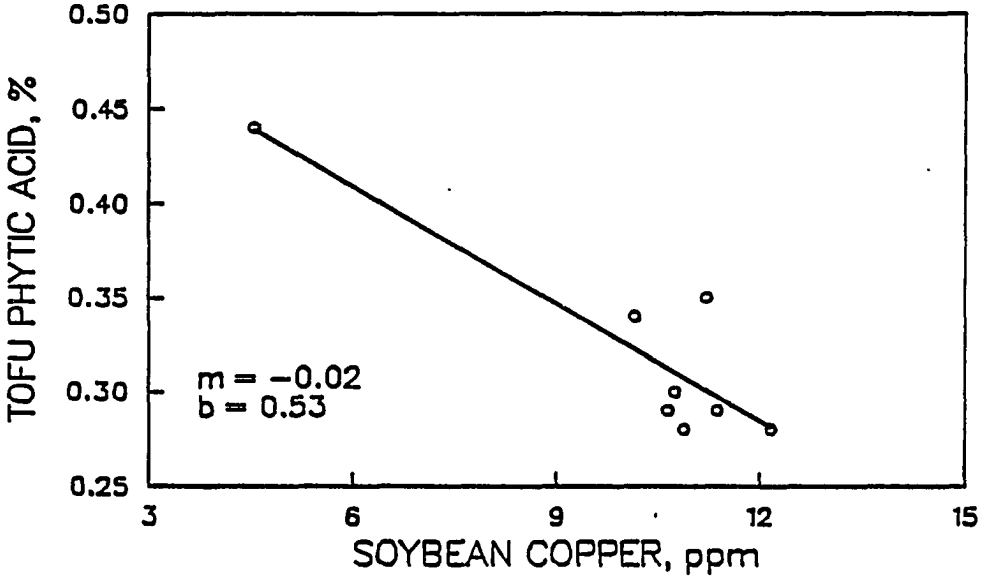

Figure 2. Relationship between soybean copper and tofu phytic acid levels

Soybeans and soymilk Negative relationships existed between protein and lipid concentrations both in soybeans (r=-0.54) and in

soymilk (r=-0.77). These relationships have been reported previously (DeMan et al., 1975; Krivoruchco et al., 1979; Wang et al., 1983).

Of greater interest are the relationships between protein and calcium concentrations which were found in both soybeans and in soymilk. The relationship was stronger (r=0.92) in soymilk than in soybeans (r=0.80), but was highly significant in both cases ($p<0.01$). This may reflect calcium binding by the soy protein. Using equilibrium dialysis, Appu Rao and Narasinga Rao (1975b; 1976b) have demonstrated interaction of calcium and 11S soy protein. These workers have also shown interaction of calcium with the 7S fraction of soy protein (Appu Rao and Narasinga Rao, 1976b). Binding of calcium was thought to involve the imidazole of histidine. Kroll (1984) has also shown calcium binding by soy protein isolate and has attributed this to interaction of calcium not only with histidine residues but also the carboxylic groups of aspartic and glutamic acids.

A negative correlation existed between the phytic acid and copper contents of soybeans (r=-0.83) and of soymilk (r=-0.88). A similar but weaker correlation was found between soybean copper and soybean iron concentrations (r=-0.50) while a positive relationship (r=0.74) between soymilk phytic acid and soymilk iron levels was seen. When scatter plots of these data are examined, however (Figures 3 and 4), it can be seen that a single sample (Amsoy 71/Hudson/1981) fell far outside of the range of other samples in copper and iron content. Thus, significant correlations were forced when the remaining data points may

not justify this. Correlation coefficients for soybean copper and iron concentrations and for soybean copper and phytic acid levels are 0.25 and -0.45, respectively, when the outlying values are excluded.

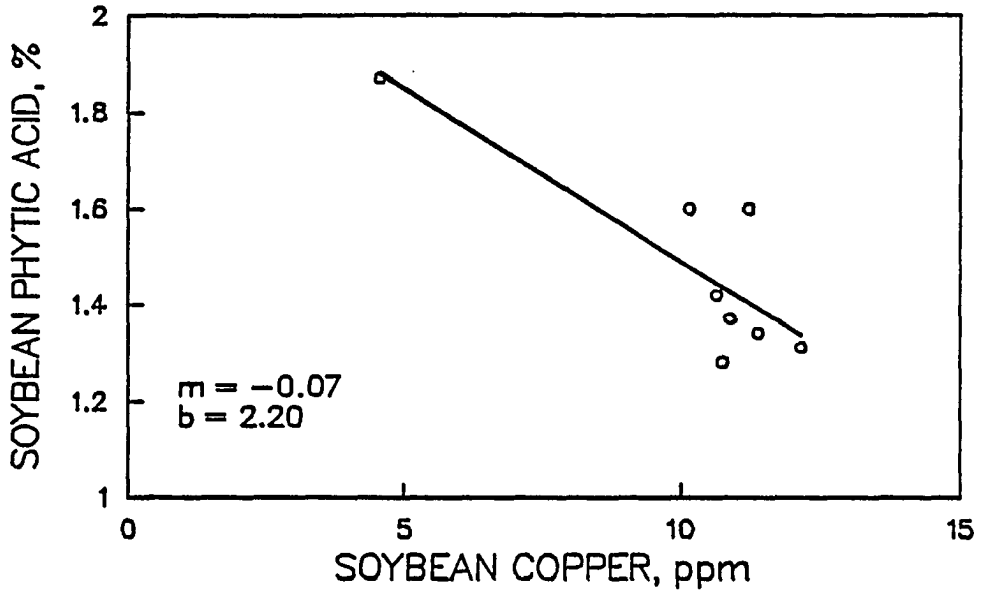

Figure 3. Relationship between soybean copper and phytic acid levels

There is a lack of relationship between calcium and phytic acid contents and between phytic acid and protein concentrations of soybeans and soymilk. In light of the well established mineral binding by phytic acid and phytic acid/protein binding, some type of relationship might have been expected. Cosgrove (1966) reported that beans (Phaseolus vulgaris) contained 4.5 moles of phytic acid per mole of protein, suggesting a consistent relationship between phytic acid and

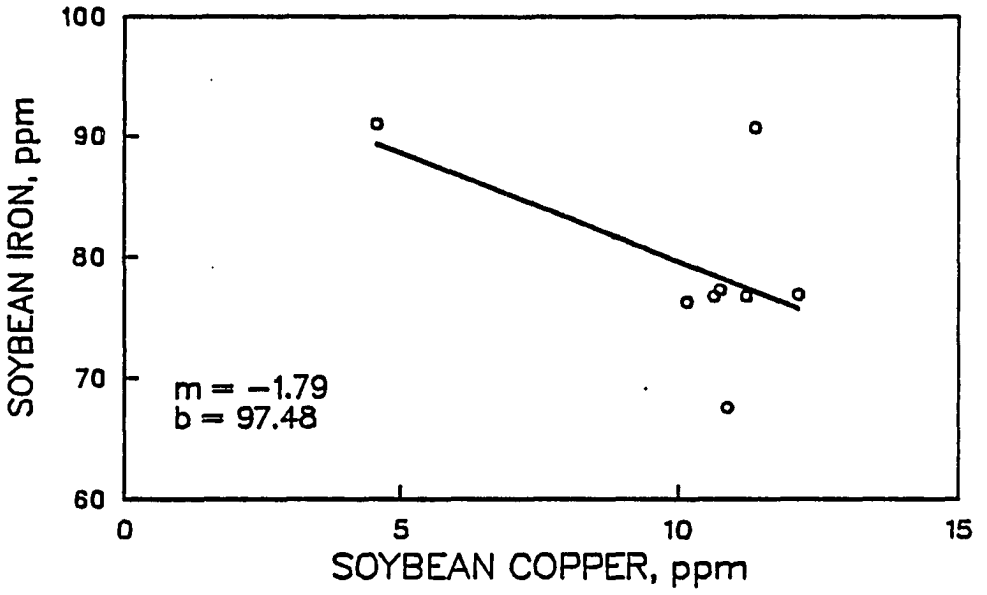

Figure 4. Relationship between soybean copper and iron levels

protein content. Lolas and Markakis (1975), however, measured the phytic acid and protein levels in Phaseolus vulgaris and found no consistent relationship between these two components.

Tofu Significant relationships among textural characteristics and the components of tofu are shown in Table 13. A strong negative relationship was found between percentage solids of tofu and tofu yield indicating that increased yield is primarily the result of increased water retention in the tofu gel. A relationship between moisture content and tofu yield was also noted by Wang et al. (1983). As a result of this association, it was expected that a relationship between tofu percentage solids and some textural characteristics would be

found. A higher moisture content of tofu should be associated with softer, more fracturable tofu. Wang et al. (1983) found a negative correlation (r=-0.65) between moisture level and hardness of tofu. Kamel and DeMan (1982) stated that the moisture content of the curd is the most important factor in determining the rheological parameters of tofu. It is interesting that no relationship was found between percentage solids of tofu and any textural characteristic. A significant negative correlation was found between tofu yield and tofu cohesiveness, however (r=-0.75). Cohesiveness reflects the extent of internal bond development. It seems reasonable, therefore, to expect a negative relationship between moisture in tofu, which would disrupt the internal bond formation, and cohesiveness.

A positive relationship (r=0.75) was found between tofu protein content and fracturability of tofu. Although it seems reasonable that a relationship of this type would exist, it has not been previously reported. No reference to the presence or absence of a relationship between any textural characteristic and tofu protein content was found in the literature.

Neither has a relationship between lipid content of tofu and texture of tofu been previously reported, although a correlation was found in this study. Tofu cohesiveness was very strongly related (r=0.92) to tofu lipid content. No rationale for this association is evident. It may be that there is some qualitative difference in the nature of protein found in high lipid containing tofu which contributes to cohesiveness. Perhaps some protein that is important in

Table 13. Correlation coefficients among components and textural characteristics of tofu

	Solids	Yield	Protein	Lipid	Phytic Acid	Calcium	Copper
Solids	---	-0.84*	0.53	0.66	0.73*	0.49	-0.58
Yield		---	-0.09	-0.84*	-0.73*	-0.38	0.75*
Protein			---	-0.07	0.34	0.58	-0.12
Lipid				---	0.33	-0.11	-0.70
Phytic Acid					---	0.82*	-0.66
Calcium						---	-0.41
Copper							---
Iron							
Fracturability							
Hardness							
Cohesiveness							
Elasticity							

*$P < 0.05$.

Iron	Fracturability	Hardness	Cohesiveness	Elasticity
0.82*	0.37	0.14	0.54	0.44
-0.60	-0.02	0.16	-0.75*	0.18
0.49	0.75*	0.58	-0.03	0.70
0.62	-0.22	-0.54	0.92*	-0.16
0.56	0.59	0.53	0.29	0.74*
0.28	0.67	0.73*	-0.03	0.83*
-0.70	-0.22	0.01	-0.78*	-0.36
---	0.49	0.18	0.53	0.47
	---	0.90*	-0.20	0.96*
		---	-0.52	0.90*
			---	-0.11
				---

emulsification of soybean lipid in soymilk preparation is of particular importance in determination of tofu cohesiveness. Saio et al. (1969) found higher cohesiveness of tofu in the presence of larger proportions of 11S soy protein relative to 7S protein. However, Aoki et al. (1980) reported greater emulsification ability of the 7S protein fraction. Although greater emulsifying ability of 7S protein and greater cohesiveness of 11S tofu does not negate the possibility of the hypothesized relationship, it does make the relationship seem less likely. Alternatively, phospholipids such as lecithin which are important in emulsification may affect tofu cohesiveness in some manner.

Phytic acid in tofu was positively associated with tofu elasticity ($r=0.74$). It can be seen from the scatter plot of tofu elasticity versus tofu phytic acid (Figure 5), that although a general tendency in this direction is apparent, the relationship is probably less strong than is indicated by the correlation coefficient due to the single sample which is very high in phytic acid. Despite the bias, it seems evident that a positive relationship between phytic acid and elasticity exists. Similar relationships can be seen for tofu phytic acid concentrations and hardness and fracturability relationships (Figures 6 and 7). Saio et al. (1969a), however, reported that the addition of phytic acid decreased hardness in tofu prepared from both 7S and 11S soy protein fractions. This same group of workers (Saio et al., 1969b) have also stated that hardness of tofu is decreased at greater total phosphorus/nitrogen ratios and have attributed this to the presence of

native phytic acid, although phytic acid per se was not measured. These workers have hypothesized that when phytic acid is present, added calcium coagulant is bound preferentially by phytic acid rather than by the soy protein. In the presence of larger amounts of phytic acid, the effective amount of coagulant is lessened, and the resulting tofu has a softer texture.

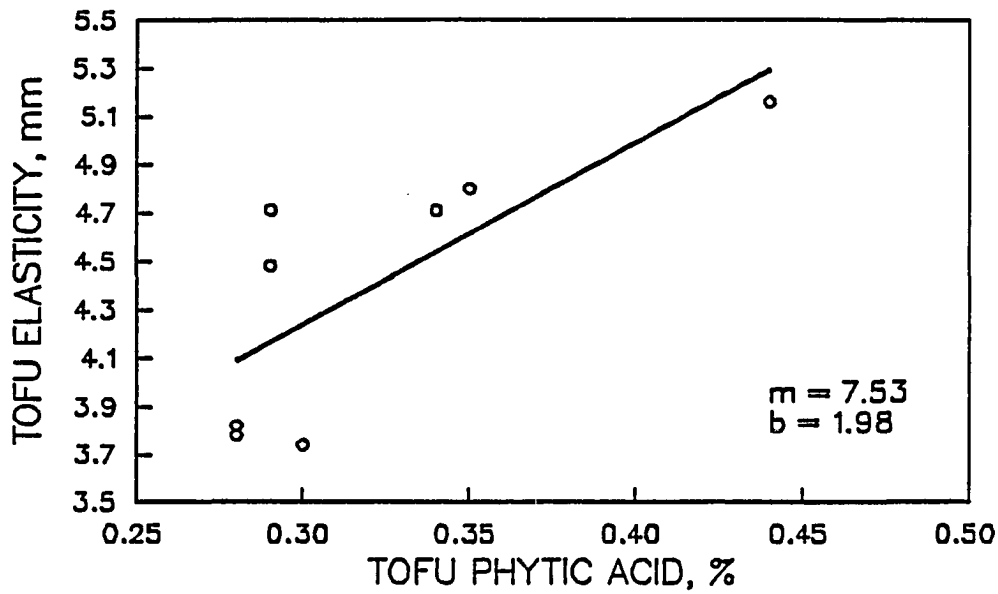

Figure 5. Relationship between tofu phytic acid level and tofu elasticity

Kamel and DeMan (1982) have also calculated a correlation coefficient for tofu phosphorus and hardness and found a value of 0.54, which was not significant. This is quite similar to the correlation of

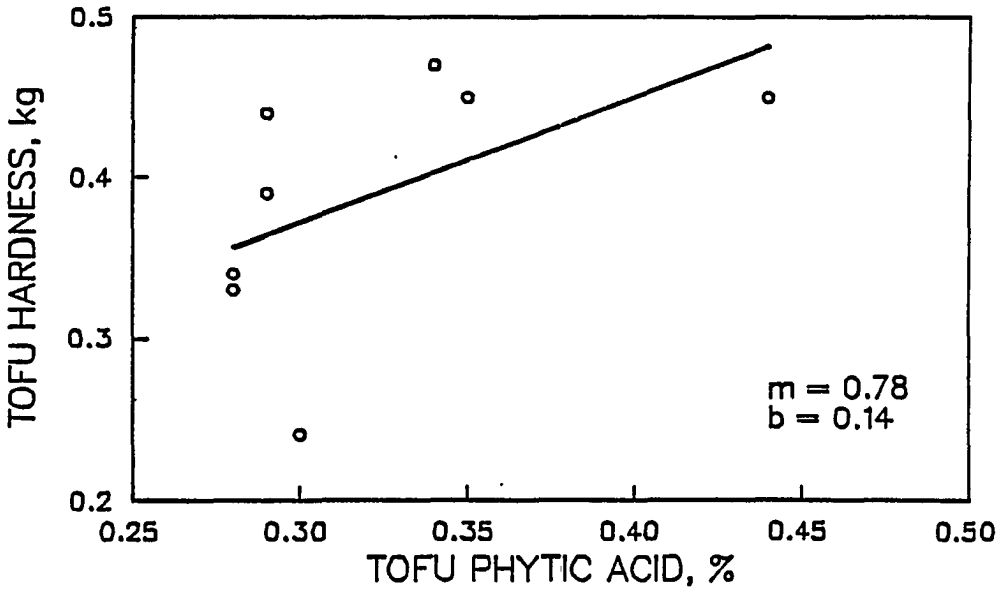

Figure 6. Relationship between tofu phytic acid level and tofu hardness

0.53 for phytic acid and hardness reported herein. Skurray et al. (1980) concluded that there was no relationship between tofu phosphorus content and a subjective quality assessment of tofu, which included textural considerations.

An explanation which would encompass all these effects begins with the acceptance of the basic hypothesis of Saio et al. (1969a; 1969b). The preferential binding of calcium by phytic acid seems likely and is supported by the strong correlation between tofu phytic acid and tofu calcium (r=0.82). in a coagulation system where the tofu coagulant was carefully balanced, high levels of phytic acid could result in failure

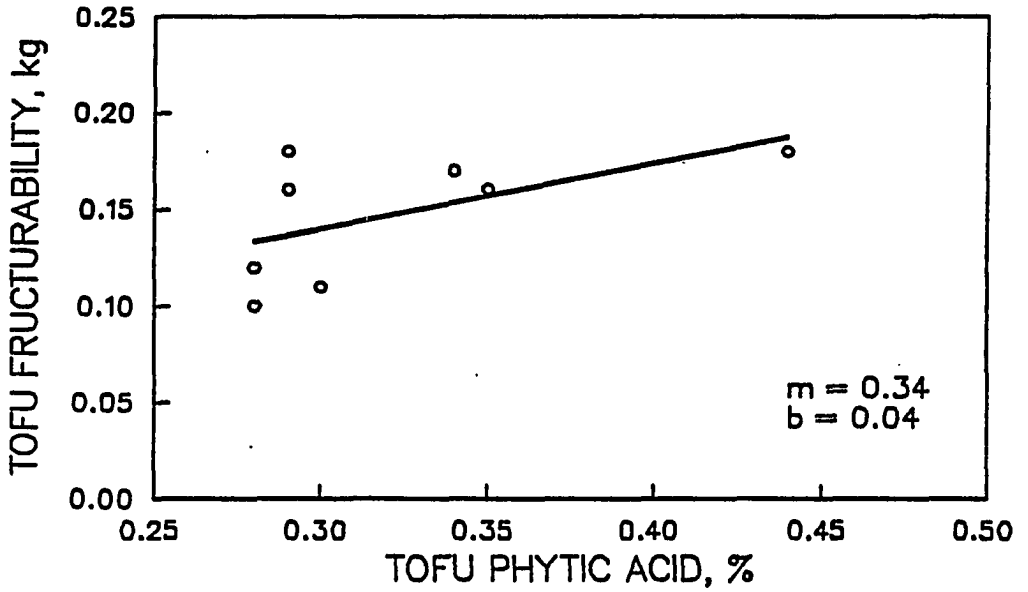

Figure 7. Relationship between tofu phytic acid level and tofu fracturability

to coagulate. This occurred a number of times in the preparation of tofu from the Amsoy 71/Hudson/1981 sample. This sample contained a level of phytic acid considerably higher than the remaining samples. It was found that to assure coagulation of this sample, a slightly longer stirring period was necessary for dispersion of the coagulant. More agitation is likely to have resulted in firmer tofu (Saio, 1979; Wang and Hesseltine, 1982). Thus, while phytic acid does exert an effect on tofu texture, the effect is dependent upon the conditions of coagulation and may appear erratic. Wang et al. (1983) reached a similar conclusion. They stated that variation in phytic acid content

may affect texture of tofu, but this effect may be overcome by other variables.

The calcium concentration of tofu was significantly correlated with both tofu hardness (r=0.73) and tofu elasticity (r=0.82). Although the effect of increasing tofu hardness with increasing levels of calcium coagulant is well documented (Saio, 1979; Tsai et al., 1981; Kantha et al., 1983), there is a limited amount of data in the literature relating tofu hardness to calcium content of the finished tofu. Although the calcium coagulant concentration was the same for all samples, making the range of calcium concentrations small, the data presented here indicate that hardness of tofu is related to the amount of calcium retained in the tofu cake. The retention of calcium in tofu is also related to the phytic acid content of tofu. In the presence of larger amounts of phytic acid in tofu, larger amounts of calcium are retained in the cake (r=0.82). In contrast to the work reported here, Tsai et al. (1981) found that the rheological properties of tofu which they measured (softness, chewiness, and jelly strength) were not related to tofu calcium or magnesium content. These workers used a rheometer to measure tofu textural characteristics, which may account for the difference in results. Also, the tofu preparation method they employed seemed to be rather variable with respect to soybean hydration time, bean:water ratio and heating time used in soymilk preparation. Variation in these factors could obscure the effect of tofu calcium concentration on tofu texture.

A negative relationship was found between tofu copper concentration and tofu cohesiveness (r=-0.78). When the partitioning of copper among the products obtained from tofu preparation is examined (Table 5), it can be seen that a relatively large amount of copper was found in whey (23.8%). Tofu samples which retained a larger amount of whey (i.e., had a higher yield would also retain a larger amount of copper. Thus, when the copper content of whey is considered, along with the significant relationship between yield and tofu cohesiveness (r=-0.75), the negative association between tofu copper and cohesiveness can be explained. The positive relationship (r=0.75) between tofu copper concentration can tofu yield and be explained in a similar way.

Strong relationships among tofu fracturability, elasticity, and hardness were also found, although cohesiveness was not related to any other textural characteristic measured. Lee et al. (1983) have described the rheological behavior of tofu as a combination of the deformability and compressibility of the solid matrix and the buildup and dissipation of hydrostatic pressure. Fracturability and elasticity probably represent a combination of these two factors while hardness is more accurately described in terms of the compressibility of the solid matrix alone. Kamel and DeMan (1982), however, found no relationship between elasticity and hardness of tofu when these characteristics were measured using an Instron at 75% deformation and a crosshead speed of 20 mm/minute.

Soybeans versus soymilk Significant relationships among components of soybeans and soymilk are shown in Table 14. Each component in soymilk was significantly correlated with the same component in soybeans with the exception of lipid. In general, the correlations were quite high (r=0.95) indicating that for most components, soybean composition is a good predictor of the composition of soymilk prepared from those soybeans. Bourne et al. (1976) also failed to find a relationship between soybean and soymilk lipid levels (r=0.258). Neither could these authors find a consistent relationship between soybean and soymilk protein levels (r=0.59). Bourne et al. (1976) stated that low protein soybeans also produce low protein soymilk but that high protein soybeans may yield either low or high protein soymilk. A lack of consistent relationship between soybean and soymilk protein is not evident based on data collected in this study, however. Figure 8 shows a clear relationship between soybean and soymilk protein levels. Wang et al. (1983) stated that the soybean protein content was "expected" to directly affect soymilk protein content, although this relationship was not tested.

Bourne et al. (1976) utilized a "hot grind" technique in soymilk preparation. It is possible that this technique produced more variable results for protein extraction than were obtained with the traditional soymilk preparation method used in the study reported herein. In addition, extraction of soybean protein in soymilk is dependent on a number of factors such as bean:water ratio and grinding time (Johnson and Snyder, 1978). A statistically significant but low relationship

Table 14. Correlation coefficients among components of soybeans and soymilk

Milk \ Bean	Solids	Protein	Lipid	Phytic Acid	Calcium	Copper	Iron
Volume	-0.48	0.36	-0.45	-0.60	0.16	0.62	0.12
Protein	0.35	0.96*	-0.47	0.14	0.86*	0.20	0.27
Lipid	-0.39	-0.77*	0.61	-0.31	-0.66*	-0.19	0.12
Phytic Acid	0.23	-0.12	0.15	0.99*	-0.20	-0.88*	0.44
Calcium	0.34	0.94*	-0.43	-0.04	0.96*	0.40	-0.06
Copper	-0.10	0.41	-0.42	-0.84*	0.45	0.99*	-0.60
Iron	0.03	0.07	0.22	0.71*	-0.07	-0.78*	0.81*

*$P < 0.05$.

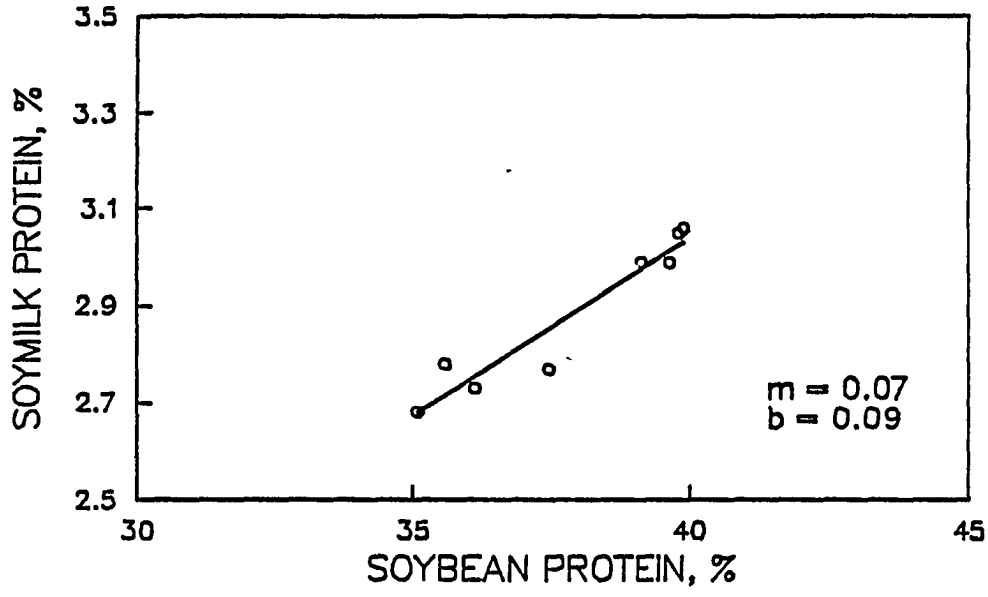

Figure 8. Relationship between soybean and soymilk protein levels

(r=0.31) between soybean moisture and soymilk protein levels was found by DeMan et al. (1975). Such a relationship was not found in the data reported in this dissertation, however.

Soymilk lipid was inversely related to soybean protein concentration (r=-0.77). The negative relationship between soybean concentrations of protein and lipid has been previously discussed. The negative association between soybean protein and soymilk lipid reflects this relationship in soybeans.

Strong positive correlations between soybean and soymilk calcium and protein concentrations were found. Similar associations were also found for protein and calcium levels in both soybeans and soymilk.

Again, calcium binding by soy protein seems likely and has been reported by a number of investigators (Appu Rao and Narasinga Rao, 1975a; Appu Rao and Narasinga Rao, 1976b; Kroll, 1984).

Copper and phytic acid in soybeans and soymilk were negatively correlated (r=-0.84 and r=-0.88). Similar relationships were found between phytic acid and copper in both soybeans and in soymilk. When scatter plots of these relationships are examined, however, it can be seen that the associations are probably weaker than the correlation coefficients indicate (Figures 9 and 10). With the outlying value omitted, these correlations fall to -0.59 and -0.53, respectively. Similarly, the association between soybean phytic acid and soymilk iron (r=0.71) is less strong than indicated by the correlation coefficient (Figure 11).

It is interesting that the relationships of phytic acid to copper and iron were negative and positive, respectively, in light of phytic acid/mineral affinity data. Vohra et al. (1965) found phytic acid to have the greatest affinity for copper of any mineral tested. Iron binding by phytic acid was fairly low, however.

Soymilk versus tofu Correlation coefficients for comparison between components of soymilk and tofu are shown in Table 15. Significant relationships were found between soymilk and tofu concentrations of lipid, phytic acid, and copper. Although no previous reports of the relationships between soymilk and tofu concentrations of these components have been published, these relationships are not unexpected in light of the relationships between soybean and soymilk

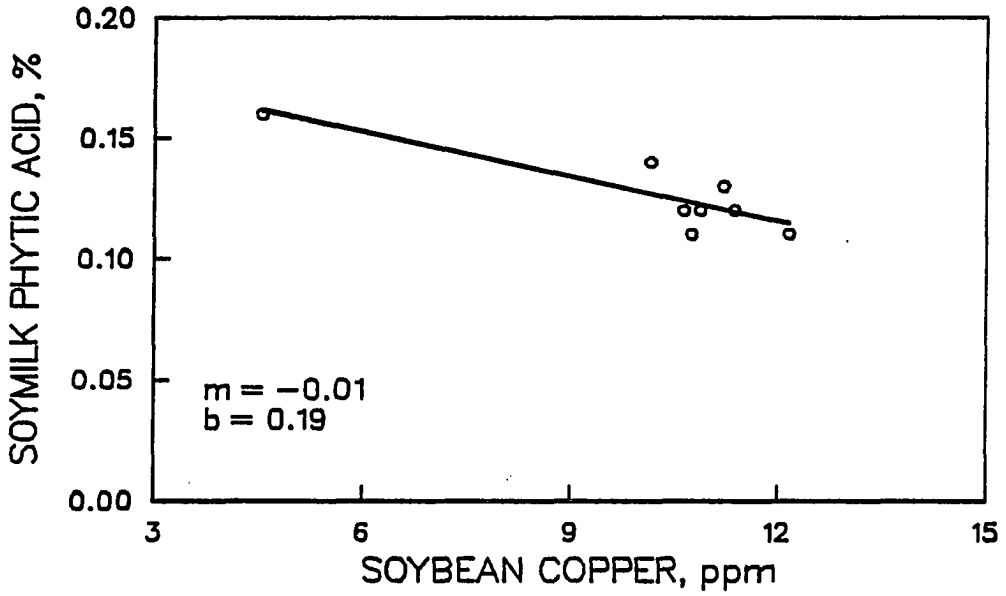

Figure 9. Relationship between soybean copper and soymilk phytic acid levels

components and those between soybean and tofu components. Although no relationship between soymilk and tofu protein was found on a fresh basis, when this association was tested on a dry basis, a significant correlation between soymilk and tofu protein concentration was found ($r=0.92$). Variation in moisture content of tofu obscures the relationship between soymilk and tofu protein when it is examined on a fresh basis. The relationships between soymilk and tofu concentrations of protein are reasonable in light of the relatively small losses (3.6%) of soybean protein in whey (Table 5).

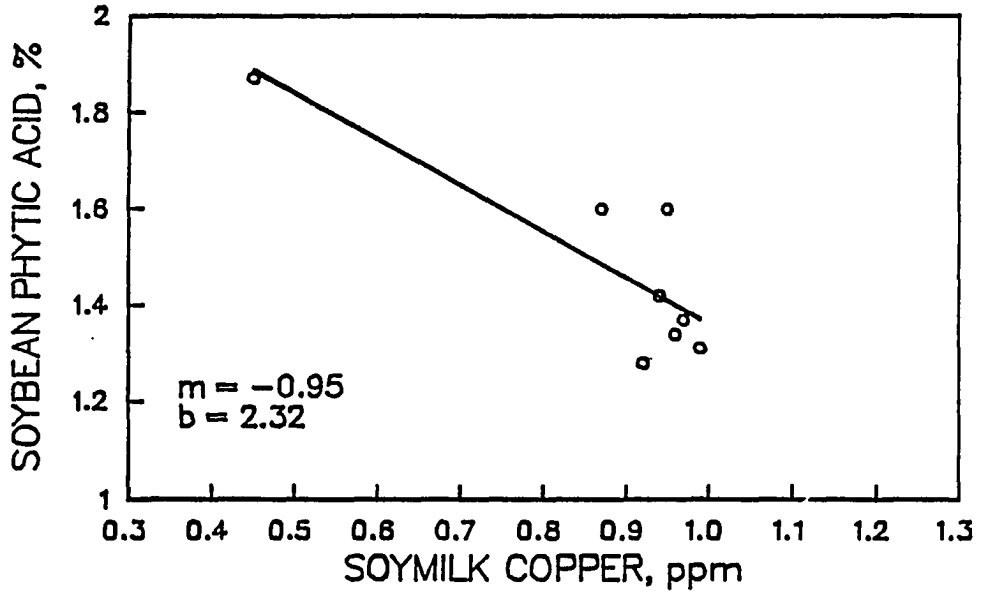

Figure 10. Relationship between soymilk copper and soybean phytic acid levels

The phytic acid content of soymilk was directly related to the calcium concentration of tofu (r=0.85). This relationship reflects retention of soymilk calcium in tofu (Table 15) and binding of calcium by tofu phytic acid. As has been discussed previously, this phenomenon is probably also related to the relationship between soymilk phytic acid and tofu elasticity (r=0.80). Retention of larger amounts of calcium in tofu is associated with tofu textural changes (Table 13).

An inverse relationship between soymilk copper content and tofu iron level was found (r=-0.92). A negative association of this type was also noted in a number of other products examined in this study

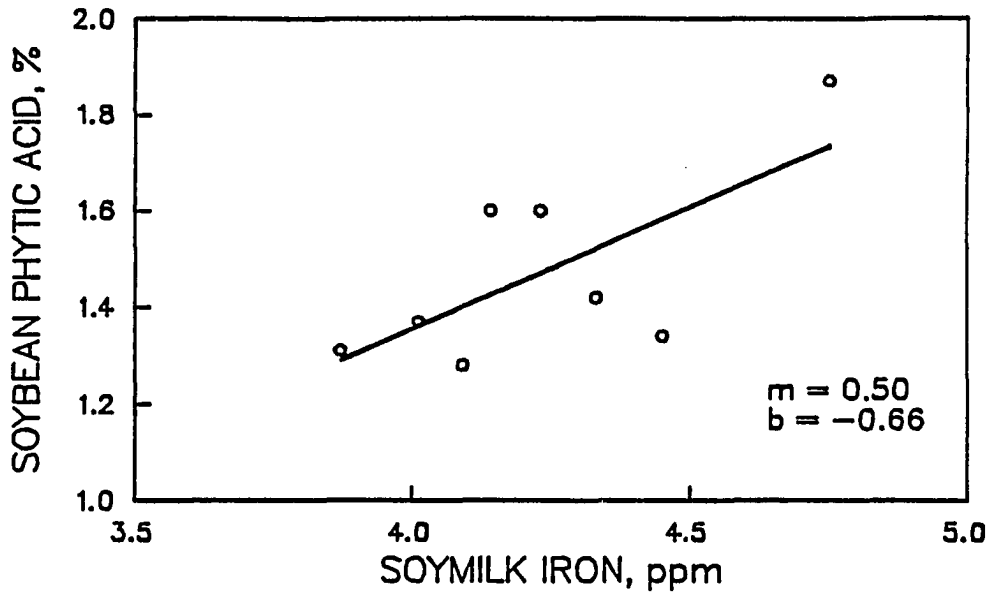

Figure 11. Relationship between soymilk iron and soybean phytic acid levels

(soybeans, soymilk, soybean/soymilk and soybean/tofu). Copper/iron relationships between products other than soybeans reflect the relationship in soybeans. The explanation for the association between copper and iron is not clear.

Tofu phytic acid content was positively correlated with soymilk iron level (r=0.72). Tofu yield was also associated with soymilk copper (r=0.72) while tofu copper and soymilk iron were inversely related (r=-0.75). Closer examination of these relationships (Figures 12, 13 and 14), however, indicates that the associations may be less strong than indicated by the correlation coefficients. Had more data

Table 15. Correlation coefficients among tofu components and textural characteristics and soymilk components

Soymilk \ Tofu	Yield	Solids	Protein	Lipid	Phytic Acid	Calcium
Volume	0.29	-0.05	0.43	-0.01	-0.53	-0.30
Protein	0.57	-0.16	0.69	-0.63	-0.06	0.30
Lipid	-0.49	0.24	-0.48	0.80*	-0.07	-0.47
Phytic Acid	-0.53	0.48	0.27	0.10	0.95*	0.85*
Calcium	0.63	-0.28	0.57	-0.65	-0.25	0.15
Copper	0.72*	-0.60	-0.09	-0.46	-0.92*	-0.61
Iron	-0.39	0.48	0.51	0.24	0.72*	0.63

*$P < 0.05$.

Copper	Iron	Fracturability	Hardness	Cohesiveness	Elasticity
0.12	0.14	-0.06	-0.21	0.02	-0.19
0.33	-0.02	0.73*	0.72*	-0.51	0.61
-0.51	0.38	-0.55	-0.75*	0.77*	-0.52
-0.55	0.35	0.65	0.66	0.10	0.80*
0.53	-0.28	0.48	0.49	-0.50	0.36
0.80*	-0.61	-0.48	-0.37	-0.44	-0.62
-0.75*	0.68	0.79*	0.58	0.34	0.84*

points falling between the outlying value and the remaining samples been included, a better estimate of the true relationship between these components would be possible.

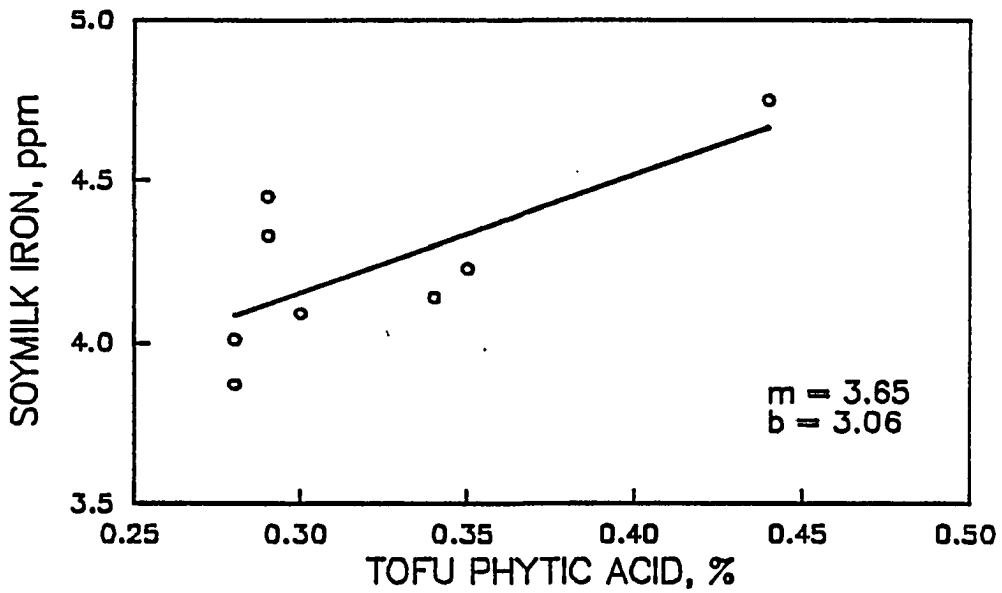

Figure 12. Relationship between tofu phytic acid and soymilk iron levels

A number of relationships between soymilk composition and tofu texture emerged. Protein content of soymilk was positively related to both tofu fracturability (r=0.73) and tofu hardness (r=0.72), supporting the statement of Wang et al. (1983) that high protein soybeans are necessary for high protein tofu. Soybeans containing larger amounts of protein yield soymilk containing higher protein

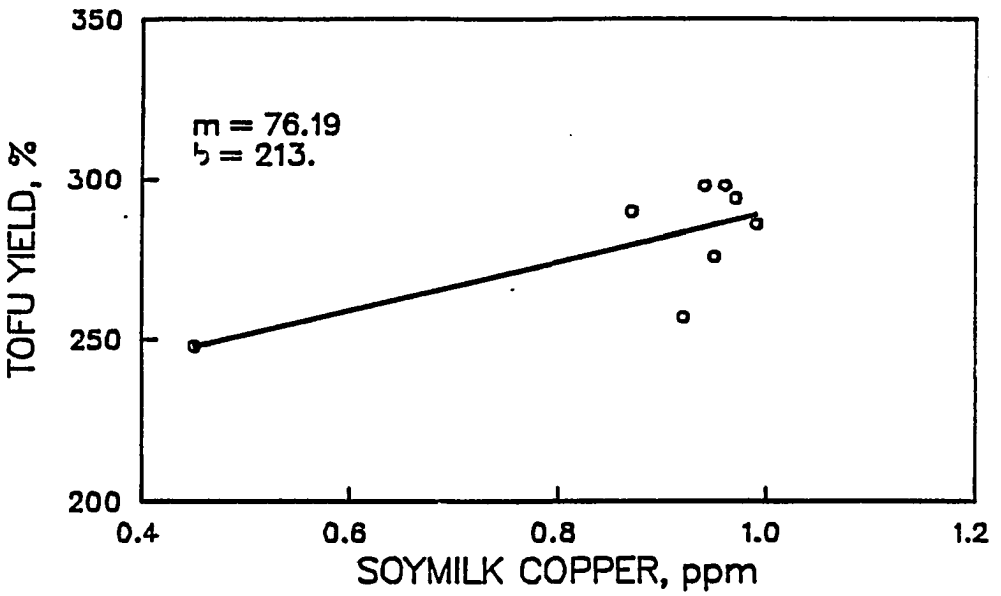

Figure 13. Relationship between soymilk copper level and tofu yield

concentrations (Table 12). Since harder, more fracturable tofu contains relatively less moisture (Wang et al., 1983; Kamel and DeMan, 1982) and relatively more protein (higher percentage solids) the assertion of Wang et al. (1983) is supported. However, the data reported in this dissertation are difficult to reconcile with the statement of Shurtleff and Aoyagi (1975) that higher solids (and presumably higher protein) soymilk results in slower coagulation of the soymilk and a softer tofu curd.

It has been shown that higher levels of soymilk protein are associated with higher levels of soymilk calcium (r=0.92). The effects of higher levels of calcium on tofu hardness are well documented (Saio,

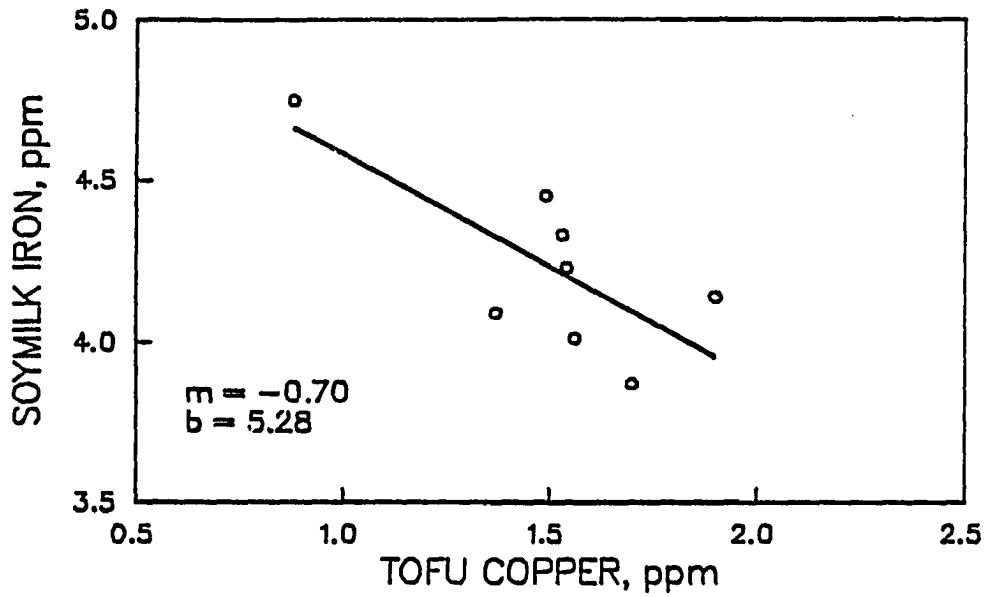

Figure 14. Relationship between tofu copper and soymilk iron levels

1979; Tsai et al., 1981; Kantha et al., 1983; Wang and Hesseltine, 1982). It seems questionable, however, whether the concentration of native calcium would be of importance when relatively large amounts of calcium sulfate were added as the coagulant. Soymilk contained approximately 0.1 mg calcium/ml or was 0.0025M in calcium. This is one-tenth the concentration of added calcium sulfate (0.025M). It is probable that soymilk contains a more highly soluble form of calcium than calcium sulfate. Thus, native soybean calcium could be more effective than calcium sulfate in precipitation of soy protein.

The lipid content of soymilk was negatively related to hardness of tofu (r=-0.75) but positively correlated with tofu cohesiveness. The

negative relationship reflects the inverse correlation of soymilk protein and lipid levels (r=-0.77) and the association between soymilk protein and tofu hardness previously discussed. The positive relationship between lipid and tofu cohesiveness was also noted when tofu lipid content and cohesiveness were compared and has been discussed in that section.

The iron content of soymilk was related to both tofu fracturability (r=0.79) and tofu elasticity (r=0.84). This association of iron with textural characteristics of tofu was also observed when correlations of soybean components with tofu texture were discussed and may be related to the partitioning of iron between tofu and whey as has been previously noted.

Effects of soybean variety on tofu texture

The effect of soybean variety on composition of soybeans is well documented. Variation in protein content (Cartter and Hopper, 1942; DeMan et al., 1975; Krivoruchco et al., 1979; Papedopetros and Katsikas, 1981) and in calcium content of soybeans (Cartter and Hopper, 1942; Taira et al., 1977) related to soybean variety has been reported. Of soybeans analyzed in the work reported here, beans of the Amsoy 71 variety contained significantly less protein and calcium when compared to both Vinton and Vinton 81 variety soybeans. The means of protein percentages in Amsoy 71, Vinton, and Vinton 81 soybeans were 36.05, 39.83, and 39.37, respectively. Levels of calcium in these samples were 1.30, 1.99, and 1.85 mg calcium/g in Amsoy 71, Vinton, and Vinton 81 soybeans, respectively.

These differences in protein and calcium content of soybeans resulted in analogous differences in composition of soymilk prepared from those soybeans. The protein content of soymilk was lowest in soymilk produced from the Amsoy 71 soybean variety (2.74% versus 3.05% and 2.99% for Vinton and Vinton 81 soybeans, respectively). Smith et al. (1952) have reported an effect of soybean variety on nitrogen dispersability, which may also contribute to the differences in soymilk protein seen here.

The calcium concentrations of soymilk prepared from Amsoy 71, Vinton and Vinton 81 soybeans were 0.07, 0.13, and 0.12 mg calcium/g, respectively. The value for soymilk prepared from Amsoy 71 soybeans was significantly lower than that for soymilk from either Vinton or Vinton 81 soybeans, reflecting the lower calcium level in Amsoy 71 soybeans. Soymilk calcium was strongly correlated with soymilk protein (r=0.92). The varietal effect on soymilk protein may thus account, in part, for the effects on soymilk calcium.

The varietal effect noted for protein and calcium in soybeans and soymilk was not apparent in tofu, however. In the case of calcium, this is not surprising due to the addition of the calcium sulfate coagulant. Some variation in tofu protein content based on variety might have been expected, however. Wang et al. (1983) has stated that high protein soybeans are necessary to produce high protein tofu. This has been confirmed by the data presented in this dissertation. Smith et al. (1960) have also found a rough relationship between soybean protein and tofu protein concentration although a lack of uniformity in

the data existed. In the data reported herein, variation in tofu composition contributed by environmental effects on soybeans obscures some varietal effects. When variation by soybean growing location and crop year are removed statistically using the type III sums of squares in the analysis of variance procedure, differences in both protein and phytic acid contents emerge. Tofu prepared from the Vinton 81 variety was significantly higher in both these components.

The effects of these differences in composition of soybeans and thus tofu have been previously examined in the discussion of relationships among components and textural characteristics. No direct effect of soybean variety on tofu texture was apparent due to the variability of the effects of soybean growing location and crop year. When these effects are removed statistically one varietal effect does become apparent. Amsoy 71 soybeans produced tofu which was significantly less fracturable then either that produced from Vinton or Vinton 81 soybeans. Mean fracturability values for Amsoy 71, Vinton, and Vinton 81 soybeans were 0.13, 0.17, and 0.17 kg, respectively. This is reflected in the relationships between tofu fracturability with both soymilk ($r=0.73$) and tofu ($r=0.75$) protein.

Other workers have investigated the effects of soybean variety on textural characteristics of tofu. Smith et al. (1960) found soybean variety to affect tofu "quality" (which included hardness). These authors reported the hardness (measured by penetrometer) of tofu prepared from a number of varieties of U.S. and Japanese grown soybeans. A statistical comparison of hardness values was not included

with their data. However, variation within samples from a particular source (U.S. versus Japan) were substantial, particularly for U.S. grown soybeans. Penetration values ranged from 36 to 48 mm in tofu from Japanese grown beans and from 35 to 78 mm in tofu from soybeans grown in the U.S. The mean penetration readings for tofu from each source were similar, however: 43 mm and 49 mm for Japanese and U.S. samples, respectively. Smith et al. (1960) also stated that sample differences probably reflect the effect of growing location as well as soybean variety.

Wang et al. (1983) also compared the performance of a number of varieties of U.S. and Japanese soybeans in tofu preparation. This group found a significant effect of soybean variety on tofu hardness (measured with an Instron Universal Testing Machine). Wang et al. (1983) were not able to show a difference based on the origin of the soybean varieties;; however, and they suggested that uniform tofu could always be produced if a given variety of soybeans and a selected set of coagulation conditions were employed.

Murphy and Resurreccion (1984) found significant differences in soybean glycinin and beta conglycinin content of soybeans based on environmental conditions and on soybean variety. These authors showed that environmental conditions exerted a greater influence than variety, however. Soybean glycinin content has been shown to influence the textural characteristics of tofu prepared from those soybeans (Johnson and Wilson, 1984; Saio et al., 1969a).

These results and the data reported herein show that while specification of soybean variety is valuable, it may not be adequate to produce tofu having consistent composition and textural characteristics unless some alterations in coagulation conditions are made.

The effect of soybean growing environment seems to be more important than variety on certain compositional characteristics of soybeans. This variation in soybean composition would affect tofu processing characteristics. The periodic failure to achieve coagulation that is reported to occur in tofu plants, and the differences in processing characteristics noted by tofu processors for soybeans of a given variety produced in different crop years (Wilson, L. A., Department of Food Technology, Personnal communication, 1985) are probably due to differences in soybean composition.

Therefore, specification of soybean variety may be advantageous in preparation of tofu having consistent textural characteristics, but this in itself, will not guarantee successful tofu preparation. A possible solution is soybean analysis to aid in evaluation of the suitability of soybeans for tofu preparation. To be most useful, the analysis should be as simple and as rapid as possible.

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate the effects of variation in composition of soybeans on the textural characteristics of tofu and on tofu composition. The effects of coagulation temperature and coagulant concentration on tofu texture and composition and the distribution of soybean components among soymilk, tofu, and whey were also examined.

Tofu was prepared from eight soybean samples of varying variety/growing location/crop year combinations with calcium sulfate being used as the coagulant. Texture measurements were made using the Instron Universal Testing Machine. Percentage solids were determined in soybeans, tofu, and okara. Soybeans, soymilk, okara, and tofu were analyzed for Kjeldahl nitrogen, crude lipid, copper, iron, calcium, and phytic acid. Kjeldahl nitrogen, copper, iron, and calcium were determined in whey. Trace minerals were determined by atomic absorption spectroscopy. Calcium was determined by EDTA titration and phytic acid was determined by phosphorus analysis following precipitation of ferric phytate.

Recoveries of soybean protein, phytic acid, and copper in soymilk were relatively high (> 50%), however, the recovery of soybean iron in soymilk was lower (39%). Retention of soymilk protein, phytic acid, and iron in tofu was also high (> 90%), however, losses of copper in whey were substantial (37%). The retention of soybean protein, iron, and copper make tofu a significant source of these nutrients although soymilk is a better source of dietary copper.

The composition of soybeans had a marked effect on the composition of soymilk. Thus soybean composition is a useful indicator of the composition of soymilk prepared from those soybeans. High correlations between the same component in soybeans and soymilk were found for protein, phytic acid, calcium, copper, and iron.

Soymilk composition was a predictor of tofu composition for some but not all components. Soymilk levels of lipid, phytic acid, and copper were significantly correlated with the same component in tofu. Soybean composition was also indicative of tofu composition for certain components, notably phytic acid, copper, and iron.

Components of soybeans that were found to be the best indicators of tofu texture were iron and phytic acid, which were related to tofu fracturability ($r=0.73$) and elasticity ($r=0.82$), respectively. The presence of these two components in soymilk was also related to tofu elasticity ($r=0.84$ and 0.80), and these were the components for which correlations with textural characteristics were the highest, although concentrations of soymilk protein (positively) and lipid (negatively) were also related to tofu texture. In finished tofu, lipid was best related to tofu cohesiveness ($r=0.92$) although copper (negatively), calcium, and phytic acid were also significantly correlated with textural characteristics of tofu.

The results support the conclusion that the composition of soybeans affects the composition of tofu and ultimately, tofu texture. Soybean variety is one factor that is readily controlled in tofu preparation, and variety may be manipulated in an attempt to affect

textural characteristics of tofu. In addition, it is recognized that a great many other factors such as climate, fertilizer application and other environmental factors influence soybean composition and thus tofu texture. For large scale tofu production, analysis of soybeans might well be justified. The components of greatest concern are probably protein, phytic acid and iron. Protein is of importance because it is related to tofu protein content (dry basis), and because high-protein soybeans yield high-protein soymilk which produces harder and more fracturable tofu. Both phytic acid and iron are also of importance due to their influence on tofu texture with higher soybean concentrations of phytic acid and iron resulting in more elastic and more fracturable tofu, respectively.

In addition to the effects of soybean composition, the conditions under which tofu is prepared can have an effect on tofu textural characteristics. Both coagulation temperature and coagulant concentration were found to influence tofu texture; however, the effect was much more pronounced in the case of coagulation temperature. Coagulation at a relatively high temperature (90°C) produced tofu which was harder and more fracturable than tofu prepared at lower temperatures. Although the effect of coagulant concentration on tofu texture was small in the range of coagulant concentrations tested (0.02M to 0.03M), larger textural differences would probably be apparent over a greater range of concentrations or using a different (more soluble) coagulant.

The observation that soybean composition affects the textural characteristics of tofu suggests that composition should be determined. It must be noted, however, that tofu texture is affected by coagulant concentration, coagulation temperature and probably other factors which may, unless carefully controlled, overshadow the influence of compositional effects when tofu is prepared.

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