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Effects of glycinin, b-conglycinin, their subunits and storage conditions on tofu sensory characteristics

Hui-Ping Chen
Iowa State University

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Chen, Hui-Ping, Ph.D.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Effects of glycinin, β-conglycinin, their subunits and storage conditions on tofu sensory characteristics

by

Hui-Ping Chen

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CHAPTER 1. INTRODUCTION

Whole soybeans have been an important source of protein, fat and flavor in the Asia for thousands of years. It is one of the world's least expensive and highest quality protein source that is available in large quantities. Mature, dry soybeans contain about 40% protein which is very high when compared to most other foods. Tofu is one of the most popular soy products and is widely consumed in Asia. It has found increasing acceptance in other parts of the world. In United States, production of tofu is increasing due to an increase of Asian immigrants and acceptance by the general population.

Soybean variety has been reported to affect the yield and quality of tofu in addition to processing conditions. However, previous works employed lab-scale mini-process and the conclusions obtained based on this kind of process need to be verified under large scale tofu processing conditions.

Various physical, chemical and biological changes take place in soybean during storage depending on the storage conditions, storage time and condition of soybean to be stored. If these physical and chemical changes exceed a certain threshold, the soybean is considered deteriorated. Thus, the acceptability and suitability of soybean are directly related to the various physico-chemical changes which occur during storage.

Many researchers have attempted to determine what role soybean variety plays in influencing the quality and acceptability of tofu, which processing factors are the most important in tofu production, how processing conditions influence the functional properties of soy proteins in soy products. There is an increasing demanding by soybean growers to know which factors will produce higher yield and better quality products for human consumption. However, tofu researchers have not been able to obtain complete answers to those questions.

Little information about the role of soy glycinin and β-conglycinin subunits in influencing certain characteristics of tofu quality is available. Additionally, most of the tofu studies have employed laboratory-scale process conditions. The results and models obtained from these studies need to be tested in large scale tofu processing conditions. The conditions used in large-scale commercial production of tofu vary from laboratory production.

Additional work is needed regarding soybean storage stability and the effects of soybean storage on sensory characteristics of final products. Based on the literature review, one can observed that sensory evaluation, by both instrumental analysis and sensory evaluation, of soy products, especially for tofu, has been an neglected area.

This study was initiated to investigate (1) correlations between the characteristics of raw soybeans with quality of tofu made on pilot-plant scale; and (2) the influence of soybean storage time on quality and consumer acceptability of tofu; and (3) the effects of variety on the textural characteristics of tofu.

CHAPTER 2. LITERATURE REVIEW

Structural Characteristics of Soybean Glycinin and β-Conglycinin

Structure of glycinin and β-conglycinin

The principal storage proteins in soybeans are glycinin and β-conglycinin. Together they account for about 70% of the total seed protein and they are both located in protein bodies. Glycinin is characterized by molecular weight of 350-360 kDa and sedimentation coefficient of 11S±1S. β-Conglycinin has a molecular weight of 180 kDa and sedimentation coefficient 7S. The acid-precipitable fraction of soybean seed protein (at pH 4.8) is composed of 34% glycinin, 27% β-conglycinin, and 39% remainder which are γ-conglycinin, Kunitz trypsin inhibitor, basic-β-conglycinin globulin, and other proteins (Iwabuchi and Yamauchi, 1987; Sato *et al.*, 1986).

β-Conglycinin has a trimeric structure and consists of three subunits: α, α', and β. The molecular weights of these subunits are 57 kDa, 58 kDa, and 42 kDa for α, α', and β subunits, respectively (Hirano *et al.*, 1987). The protein is a glycoprotein. Affinity for concanavalin A has improved the isolation success for β-conglycinin. Glycosylation gives a higher than actual molecular weight on sodium dodecylsulfate (SDS)-polyacrylamide gels due to the associated water (Murphy, 1984).

Glycinin is generally present in a higher concentration and is a richer source

of sulfur amino acids than β-conglycinin in soybean seeds. It accounts for more than 20% of the seed dry weight (Nielsen *et al.*, 1989). It is devoid of sugar and does not undergo the ionic strength dependent association/dissociation phenomena. According to the current understanding, it is a hexamer with subunits situated at the vertices of a trigonal antiprism, *i.e.*, the six subunits form two threefold rings, which are superimposed and are twisted by 60° against one another (Plietz *et al.*, 1987). Each of the six subunits consist of two polypeptide components, one with an acidic and the other with a basic isoelectric point. The acidic and basic polypeptides are associated in a non-random manner through disulfide bridges. The basic polypeptide components are all about 20 kDa, while the acidic polypeptide components are more variable in apparent molecular weight and range between 38 and 48 kDa (Nielsen, 1984). So far, eight acidic (A_{1a}, A_{1b}, A_2, A_3, A_4, A_5, A_6, and A_7) and five basic (B_{1a}, B_{1b}, B_2, B_3, B_4) subunits of the glycinin have been identified in soybean seeds by SDS-polyacrylamide gel electrophoresis (Kagawa and Hirano, 1988).

Varietal influence on quantity, subunit composition, and characteristics of soybean glycinin and β-conglycinin

The physical, chemical, and functional properties of glycinin and β-conglycinin have been an area of considerable research interest, since they are the two major protein fractions in the soybean seed. Glycinin and β-conglycinin play very important roles in the functionality of soy protein foods. Therefore, changes in the amounts of these two proteins in soybean seeds would yield different food properties.

A report by Hughes and Murphy (1983) investigated the content of glycinin in 10 varieties of soybeans grown in a uniform environment. Significant differences

in glycinin content between varieties were identified. Total protein content for the 10 varieties ranged from 39.40% to 44.10%. The content of glycinin per total protein was observed between 31.40% and 38.30%. Also, high concentrations of total protein did not necessarily correlate with high glycinin concentration. In another study (Murphy and Resurreccion, 1984), the amounts of glycinin and β-conglycinin were measured in 12 soybean varieties by using rocket immunoelectrophoresis to investigate the influence of varietal and environmental difference on soybean glycinin and β-conglycinin content. They reported that when 10 varieties were grown in a uniform environment in 1980 and 1981, the glycinin content was 46.90-54.40% and 46.70-37.20%, respectively. The average glycinin content was 51.00%. β-conglycinin content for the 2 years examined averaged 18.50%, with a range of 16.80-20.10% and 16.50-20.90%, respectively. Varieties Vinton and Weber from several growing seasons and different environments had glycinin content with a range of 11.80% and 14.50%, respectively, whereas β-conglycinin content of these soybeans varied by 5%. However, there seemed to be no relationship between glycinin and β-conglycinin content in these soybeans. The results also showed that environmental influences seem to have a much greater impact on glycinin concentration in soybeans than on β-conglycinin content. Genetics also has an influence on the expression of these two proteins but to a lesser extent than environment.

Soybean glycinin content reported in literature has varied among the researchers. This is not only due to the difference in soybean variety they used but also the methods employed to purify glycinin. The glycinin content (50%) reported by Murphy and Resurreccion (1984) was higher than the 34% which was reported by Iwabuchi and Yamauchi (1987). One explanation is Murphy and Resurreccion (1984) employed

conditions of sodium dodecyl sulfate gel electrophoresis for purifying glycinin, which may induce an alteration of molecular conformation. The immunological reactivity probably changes depending on the glycinin conformations and whether it is native or denatured, since the antigenicity of glycinin is more conformation dependent than β-conglycinin (Iwabuchi and Yamauchi, 1984).

Functional properties of soy proteins such as gelation and emulsification have been found to have a close relationship with the subunit composition, and have attracted much attention from protein chemists (Utsumi *et al.*, 1984; Mori *et al.*, 1986; Nakamura *et al.*, 1985;). Cultivar differences in gelling characteristics of soybean glycinin were examined for five cultivars having different subunit compositions (Nakamura *et al.*, 1984). The gelling characteristics of glycinin differed significantly among cultivars, arising from the differences in the nature of protein itself as well as protein concentration. They observed that glycinin of three of the cultivars studied formed a gel within half the time required for the other two cultivars. The faster gelling cultivars contained A_4 (which is linked with B_3 subunit to form an intermediary subunit through some bonds other than disulfide bonds) and the slower gelling cultivars did not contain A_4. Additionally, the hardness of the gel was different among cultivars, depending on the percentage of A_3, which is the largest molecular weight constituent acidic subunit of glycinin. Turbidity of the gel had a tendency to increase with increasing content of sulfhydryl groups of glycinin. Hence, the possibility exist that glycinin gels from different soybean cultivars can vary in their hardness, depending on the subunits composition. Recently, Nishinari *et al.* (1991) conducted a rheological study by dynamic viscoelastic measurements on the effect of the A_4 subunit on the gelation characteristics of soymilk. It was found that tofu gels prepared

from cultivars without the A_4 (A_4 being indicated as A_5 by Nishinari *et al.*, 1991) subunit were harder and more solid-like than those prepared from cultivars with the A_4 subunit. However, the effect of the composition of glycinin, such as A_3 subunit, should also be taken into account for a better understanding of the viscoelasticity of tofu gels. Since it has been shown that other components in soybean seeds such as phytic acid, lipids, and lecithin, also affect the gelation process of soy proteins (Miura and Yamauchi, 1984), more work needs to be accomplished in order to get the best picture of gelation mechanism of soy protein in soy products. Furthermore, this study (Nishinari *et al.*, 1991) used only 5 g of soybeans from each cultivar to prepare the soymilk and one set of coagulation condition (coagulant type, concentration, and coagulation temperature). It is possible to get different results from the same cultivars when different conditions (such as larger scale processing, different types and concentrations of coagulant,and different temperatures) are employed.

Separation and Quantification of Glycinin and β-Conglycinin Subunits

There are several techniques such as gel electrophoresis, fast liquid chromatography (FPLC), and the recently introduced method, capillary electrophoresis (CE) available for protein separation and quantitative analysis. Each of the techniques have their respective merits.

Application of ion-exchange high performance liquid chromatography (HPLC) to the assessment of subunit heterogeneity in plant glycinin storage globulins was successfully performed by Lambert *et al.* (1987) in their work. This technique has been shown to give good resolution and reproducibility. One major advantage of the high performance liquid chromatography (HPLC) method is the ease with which

resolved species can be collected and thereby made available for further analysis if required.

Capillary electrophoresis (CE) represents a new, alternative format for electrophoresis. Capillary electrophoresis is recognized as a potentially important new analytical separation technique because it brings speed, quantitation, reproducibility, and automation to the inherently highly resolving technique of electrophoresis. There are some recent studies employing CE to separate proteins and peptides (Deyl and Struzinsky, 1991; Grossman *et al.*, 1989; Nielsen *et al.*, 1989; McCormick, 1988; Liu *et al.*, 1988).

The subunits of soy glycinin and β-conglycinin are commonly separated by electrophoresis in SDS polyacrylamide gels. This method is rapid but not entirely satisfactory, because it can not resolve the acidic polypeptide components (*e.g.* A_{1a}, A_{1b}, A_2, A_4) that migrate together in the gel at molecular weight 37,000 nor the basic subunits (Moreira *et al.*, 1979). Two dimensional electrophoresis permits better resolution, but it is time-consuming (Fontes *et al.*, 1984).

Composition and Functional Properties of Soy Proteins as Affected by Heat Treatment and Other Processing Conditions

Effect of heat on soy proteins

Soy protein-containing products are generally subjected to a severe heat treatment to inactivate antinutritional factors. Studies on thermal processing effects on soy proteins have been reviewed by Kilara and Sharkasi (1986). Heating of proteins can result in several reactions such as association, dissociation, aggregation, and gelation.

In general, protein gelation refers to the transformation of the protein in the soluble state into a gellike structure, in which the individual protein molecules interact with each other to form a three-dimensional network. Several factors such as pH, ionic strength, temperature, and reducing agents have been shown to influence the gelation properties of several globular proteins (Oe *et al.*, 1986; Utsumi and Kinsella, 1985)

Soy glycinin heated to 95°C and β-conglycinin heated to 85°C gave firm gels on cooling to 4°C. Both proteins undergo partial unfolding between 25 and 55°C. Glycinin aggregation (intersubunit and/or intermolecular interactions) begins at 70°C and these soluble aggregates interact to form interconnected strands at 95°C. In β-conglycinin, partial unfolding persists through 70°C and both aggregates and strands form at 85°C. These strands formed at 95°C in glycinin and 85°C in β-conglycinin are referred to as being in a pregel state (Hermansson, 1986; Mori *et al.*, 1986; Fisher *et al.*, 1990). The carbon-13 NMR studies of soy glycinin and β-conglycinin (Fisher *et al.*, 1990) indicated that upon gelation, in both proteins, the amino acid residues contributing the conformation appear to come from the aliphatic and aromatic regions, while additionally the carbohydrate and glutamate moieties were involved in β-conglycinin gel structure. In addition, the carbon-13 NMR spectra of these two proteins during gelation process support the theory, proposed by others (Hermansson, 1986; Mori *et al.*, 1986), that gelation of both soy glycinin and β-conglycinin is a four-step process involving (1) unfolding, (2) aggregation, (3) strand formation, and (4) strand ordering/gelation.

Many researchers have attempted to elucidate the molecular forces involved in forming and stabilizing of gel network structures of soy glycinin and β-conglycinin (Babajimopoulos *et al.*, 1983; Mori *et al.*, 1982; Nakamura *et al.*, 1984). Mori *et al.*,

1986) stated that junction points of the constituent strands within soy glycinin and β-conglycinin gel network structure consist of both disulfide bonding and noncovalent bonds such as hydrophobic interaction and/or hydrogen bonding formed on subsequent heating after the initial network structure is established, thereby stabilizing the network structure and increasing gel hardness. From the data obtained from carbon-13 NMR spectra of glycinin and β-conglycinin under conditions of gelation and heat and chemical (6 M urea) denaturation (Fisher *et al.*, 1990), it can be concluded that much of their native structure depends on aliphatic and aromatic interactions. Hydrophilic interactions involving carbohydrate and glutamate are important to β-conglycinin secondary structure. These interactions are diminished upon heat and chemical denaturation but reappear upon gelation, indicating that these interactions are important in formation of β-conglycinin gel. The involvement of covalent forces in maintaining gel structure in glycinin can not be directly studied by NMR due to the low cysteine content of glycinin. However, the spectra of chemically denatured glycinin could lead them to conclude that covalent forces such as disulfide bonds, also help maintain glycinin structure in part by keeping sections of the acidic and basic polypeptides in close proximity regardless of external perturbations. Since there is little or no cysteine found in β-conglycinin (Coates *et al.*, 1985) and no disulfide bonds (Thanh and Shibasaki, 1977), it is unlikely that disulfide bonds is involved in β-conglycinin gel formation.

In summary, the molecular forces involved in forming and stabilizing of gel network structures of soy glycinin and β-conglycinin are following: both disulfide bonds and noncovalent bonds such as hydrophobic interactions and/or hydrogen bonds are involved in formation and stabilization of glycinin gel network structure; noncovalent

bonds including both hydrophobic interactions and hydrophilic interactions (which involve carbohydrate and glutamate moieties) and/or hydrogen bonds are involved in formation and stabilization of β-conglycinin gel network structure.

Several recent studied have been trying to investigate, from different approach, the factors that govern the rheological properties of soy protein gels (Wang and Damodaran, 1990; Wang and Damodaran, 1991; Kang *et al.*, 1991; Nishinari *et al.*, 1991). They stated that: (1) the hardness of soy protein gels is fundamentally related to the weigh-average molecular weight (size) and the hydrodynamic shape of the polypeptides in the gel network rather than to their chemical nature such as the amino acid composition and distribution; (2) β-sheet structure might be essential for protein-protein interactions and network formation in protein gel, therefore, it might stabilize and control the hardness of the gels; (3) the glycinin/β-conglycinin ratio affects the texture of the gels; and (4) tofu gels prepared from soybean cultivars without the A_4 subunit are harder than those prepared from cultivars with the A_4 subunit.

However, even with these results, we are not be able to draw the conclusion that which factor(s) play(s) the most important role in the rheological properties of soy protein gels. Furthermore, all of these studies, but for the one by Nishinari *et al.* (1991), were based on pure protein systems rather than real soy food systems where the other constituents such as lipid, carbohydrate, water, etc. which may affect the final texture characteristics of soy products.

Functional properties of soy proteins

The properties determining the behavior of proteins in foods during processing, storage and consumption are collectively called functional properties (Kinsella *et al.*, 1985). Solubility and water imbibing capacity (WIC) of soy proteins are important functional properties in food formulations. Quite a few studies have been done recently on the WIC of soy protein isolates. Sorgentini *et al.* (1991) studied the WIC of soy protein isolates as affected by protein heat denaturation and reported that WIC of commercial soy protein isolates is the sum of the contributions of the dispersable and insoluble protein but the insoluble fraction (heat denatured glycinin and β-conglycinin) contributed the most to the WIC of the total isolate. This finding is in agreement with Lopez de Ogara *et al.* (1992) who also reported that significant correlation was found between gel viscosity and WIC of soy isolates. Additionally, structure properties of soy isolates are also influence the WIC of soy isolates. Isolates with highly denatured proteins, high surface hydrophobicity, low solubility in 0.2 M NaCl solution and low SH exhibited the highest water imbibing capacity (Wagner and Anon, 1990).

Influence of water content on properties of soy proteins

Thermal denaturation of soy protein at different water contents was investigated (Kitabatake *et al.*, 1990). Their results indicated that the soy protein was not denatured at temperatures even above 100°C when the water content was low. Oates (1991) also reported that initial moisture content of β-conglycinin soy protein affected its thermal behaviors. In this study, measurements of the relative change in the specific heat of β-conglycinin on denaturation were determined at a range of moisture

contents (5-50%) by differential scanning calorimetry. The specific heat functions associated with denaturation of the β-conglycinin globulin were found to be dependent on initial moisture content. These values increased with increasing water content. In another study, the effect of constant rates of increasing temperature on the protein denaturation of defatted soy flour was investigated at various moisture contents (Yoshii *et al.*, 1990). They reported that the protein solubility was remarkably dependent on the moisture content of the isolated soy protein, and solubility decreased significantly as moisture content increased.

Tofu Production

Introduction

Whole soybeans have been an important source of protein, fat and flavor in the Asia for thousands of years. It is one of the world's least expensive and highest quality protein source that is available in large quantities. Mature, dry soybeans contain about 40% protein which is very high when compared to most other foods. Soybeans contain about 20% oil which is very high as compared to that of most cereals and vegetables. Soybean oil is about 85% unsaturated and is cholesterol-free which makes it highly desirable in the human diet. Whole soybeans are a good source of protein, fiber, calcium, iron, zinc, phosphorus, magnesium, thiamin, riboflavin, niacin, and folacin. Carbohydrate in soybeans, although not all utilized by the human system, adds to the total calorie contribution. The increase in soyfood consumption, which is expected to continue throughout this decade, is attributed to the number of factors, including economics, health, ethics, and the environment (Messina and Messina, 1991). Recently, the potential role of soybeans in cancer prevention has

received attention (Messina and Barnes, 1991).

Soybeans contain, in relatively high concentrations, several compounds with demonstrated anticarcinogenic activity. Among those thus far identified are isoflavones, protease inhibitors, phytic acid, saponins, phytosterols, and phenolic acids. Among the hypothesized anticarcinogens in soybeans, isoflavones and protease inhibitors are found in the highest concentration relative to most other commonly consumed foods (Messina and Messina, 1991).

Tofu is one of the most popular soy products. It is a high protein product widely consumed in Asia but has found increasing acceptance in other parts of the world. In United States, production of tofu is increasing due to an increase of Asian immigrants and acceptance by the general population. The traditional way of tofu preparation involves first preparation of a soymilk (*i.e.*, slurried soybeans) which is boiled, filtered and then treated at high temperature, with the coagulant which precipitates the proteins with the concomitant release of curds and whey; the curds are filtered off and molded into shape under pressure. Good quality of tofu is judged in terms of appearance, texture, aroma, taste, and mouthfeel, and high yield. Soybean variety has been shown to influence yield and quality of tofu due to variations in composition, *e.g.*, protein, oil, ash, and phosphorous content, among others. Tofu manufacturers prefer soybean varieties that produce tofu with high yield and good textural properties. The texture of tofu must be coherent, smooth and firm but not hard and rubbery. Due to its bland nature, the textural properties of tofu play an important role in influencing quality and consumer acceptability.

Processing conditions that affect the yield and quality of tofu

Manufacturing variables can profoundly influence the yield and the quality of tofu. These variables include time, temperature, and type of soaking soybeans prior to making soymilk, the water to soybean ratio in the soymilk, time and temperature of heating the soymilk, temperature and extent of stirring during coagulation, and pressure applied to soybean and during processing into tofu.

In traditional method of tofu making, soybeans were soaked in water for about 12 hours at room temperature. Studies have been done on other types of soaking. Johnson and Wilson (1984) reported that percent solids of the ethanol-soak soymilk were significantly lower. Because soymilk solids have been shown to be an important factor in affecting tofu texture, ethanol-soak or other solvent-soak methods are currently not being used in tofu production. Water uptake of soybeans during soaking was determined by recording the weight increase in beans with respect to time (Hsu *et al.*, 1983). Temperature was found to influence the rate of water uptake, with higher rates associated with higher temperatures. The rate and maximum amount of absorption showed little correlation with the protein content, density, and size of the bean. Rahma and Mostafa (1988) studied the effect of either soaking of soybean in water as well as 2% NaOH, or heating at 85°C, 100°C, and 120°C and autoclaving for 5 and 10 minutes on the chemical composition and functional properties of the produced flour. They reported that soaking of soybeans in water at room temperature for 12 and 24 hours showed a marked decrease in total protein, non-protein nitrogen, and total ash and reducing sugars. The decreasing rate of these components increased with the increasing soaking time. The same trend was observed pertaining soybeans soaked in 2% NaOH. The longer times of soaking reduced the protein

solubility index in both treatments.

The ratio of beans:water was shown to be very critical in terms of the amount of protein extracted and the properties of tofu (Beddows and Wong, 1987a). In the range 9-14:1, 10:1 gave the best result. Extraction at 8:1 and making up to 10:1 gave much poorer results, but maceration at 8:1 followed by a further extraction at 2:1 gave a better quality tofu. The time of maceration was also shown to have a pronounced effect on the amount of protein extracted.

In tofu making, soybean milk is generally heated for about 3 minutes after boiling. Beddows and Wong (1987b) investigated the effect of various heat treatments on the yield, protein content and physical form of tofu. They found better yields and quality were obtained when the macerated bean slurry was filtered prior to heating and a defined rate of heating was used with stirring.

The stirring speed during coagulant addition was found to be critical and the optimum for protein conversion, net yield, and texture is 240-280 rpm (Beddows and Wong, 1987c). When the same amount of soymilk is coagulated, the hardness of tofu increased with increasing stirring speed. Also, increasing the coagulating temperature increases the hardness of tofu, and the optimum coagulation temperature is 75-80°C (Beddows and Wong, 1987b).

The pressure applied to soybean curd during processing into tofu has a profound effect on the moisture content, yield, and some textural parameters of tofu (Gandhi and Bourne, 1988). As the pressure increased from 0.186P to 0.744P the moisture content decreased from 82% to 60% and yield decreased from 2.0 kg to 1.2 kg per kg whole dry soybeans. Hardness, brittleness, chewiness, and gumminess increased linearly with increasing pressure. Springiness, cohesiveness, and adhesiveness were

hardly affected by increasing pressure. Beddows and Wong (1987c) reported the optimum pressure applied to curd is 4-6 g/cm^2.

Effect of coagulants and concentrations

It has been recognized that the selection and addition of a salt at the proper level is the most important step in the preparation of soybean curd. A number of coagulants have been used in preparation of tofu. It appears that calcium sulfate is the most commonly employed salt in soybean curd preparation. The use of calcium sulfate, however, causes some problems. This salt is practically insoluble, therefore, the addition of this salt requires skill, otherwise, the quality of soybean curd may vary from time to time.

Other coagulants which have been used in the literature are: calcium chloride, calcium lactate, calcium acetate, calcium citrate, calcium gluconate, glucono-delta-lactone (GDL), acetic acid, and magnesium chloride (Saio *et al.*, 1979; Lu *et al.*, 1980; Tsai *et al.*, 1981; Kamel and deMan, 1982; deMan *et al.*, 1986). Each coagulant resulted in tofu with special textural characteristics. For example, Shen *et al.* (1991) used GDL in their tofu study. The results indicated that yield of pressed GDL tofu was 20% higher than calcium sulfate tofu and the hardness of packed GDL tofu increased with protein content in soymilk.

Insufficient amounts of salt may result in incomplete precipitation of soy protein and make the subsequent filtration difficult, whereas excess amounts of salt make the texture of soybean curd hard and unpalatable. Beddows and Wong (1987c) reported that calcium sulfate at 9-10 mM produced tofu with an even texture and a good retention of cut shape; the protein and solids content was at the maximum. Sun and

Breene (1991) also stated that product yield, solids recovery, protein recovery, and textural quality were optimal at 0.02N calcium sulfate for all five varieties used in the study. However, both studies were mini-process, only 250 g (Beddows and Wong, 1987c) and 32.5 g (Sun and Breene, 1991) of soybeans were used to make tofu. It was subject to further verification by large scale tofu production.

Influence of variety on yield and quality of tofu

Soybean variety has been reported to affect the yield and quality of tofu in addition to processing conditions. Smith *et al.* (1960) found that Japanese varieties were more desirable than U.S. varieties. But Smith *et al.* (1960) reported that the most important differences between Japanese and U.S. soybeans, as viewed according to Japanese custom, were in texture and color of the tofu produced from them. Although yield and composition of tofu varied with soybean varieties, the average yield from U.S. soybeans was the same as that from Japanese beans. However, the same authors cautioned that the differences in composition of beans probably reflected the effect of location as much as varietal differences. Tsai *et al.* (1981) studied on the yield and quality characteristic of tofu made from imported U.S. soybeans and local Ta-lien soybeans grown in Taiwan. The yields of tofu made from those two sources showed a similar yield and were higher than that of defatted soy flakes. However, there are several problems which should be mentioned: (1) they failed to state how many grams of soybean was used in plant-scale tofu-making; (2) although they employed both laboratory and plant operation for preparation of tofu, the characteristics of tofu were measured on product prepared from different operational sizes without stating if the two scales were similar to each other in terms of

tofu properties (for example, tofu made by plant-scale was used for moisture content determination while tofu made by laboratory scale was used for determination of solids of tofu); (3) in plant scale production, soybeans were soaked in water for only 4-7 hours (presumed to be at room temperature) which is not long enough to let the soybeans reach 100% hydration according to Hsu *et al.* (1983). Due to these unclear points and the methods used for measurements of tofu properties, the results obtained from this study are difficult to interpret. Kamel and deMan (1982) analyzed 11 varieties of Ontario-grown soybeans for protein, phosphorus, and moisture content and studied the properties of tofu produced from them. Moisture content of the curd was probably the most important variable in influencing the rheological properties of the curd. The variation was probably related to processing conditions rather than to the composition of the beans investigated. Wang *et al.* (1983) studied the effect on the yield and quality of tofu of 5 U.S. and 5 Japanese soybean varieties grown under the same environmental conditions. The differences among the 10 varieties were not attributable to the country of origin. Significant varietal differences were noted for protein and oil content of the beans. Tofu prepared from each soybean variety showed no significant difference in yield of dry product but showed significant difference in the yield of fresh tofu. The difference is, therefore, due to its water content. Significant variation among soybean varieties was noted in the hardness of resultant tofu. But the hardness of tofu was found to be negatively correlated to its water content. Based on their results, soybean variety does not seem to play an important role in tofu processing. However, their results were drawn from irreproducible mini-tofu conditions in which only 30 g soybean were used. Therefore, significant differences in yield and texture of tofu among varieties could also due to processing conditions,

and not the water content as the authors suggested.

Snyder and Kwon (1987) stated that soybeans having large uniform size, light colored hilum, thin seed coat and high protein were preferred for soymilk and tofu production. Recently, Lim *et al.* (1990) used 9 light hilum soybean varieties to study the characteristics of soybeans and soymilk that affect the yield and quality of tofu coagulated with calcium sulfate. They reported that the yield of tofu was not affected by the size of soybeans. Soybean varieties high in protein, fat, and phosphorus contents produce tofu with higher protein, fat, and phosphorus contents. They also proposed two models for predicting the yield of tofu. According to the model one, soymilk with higher pH and total solids gave a higher yield of tofu. According to model two, soybeans high in protein and ash and low in phosphorus give a higher yield of tofu. However, these models were proposed based on small-scale process conditions. Their models need to be validated in tofu factories.

A mini-process was described for making tofu from 50 g quantities of soybeans of soybeans (Sun and Breene, 1991). It was used to compare yield and quality among 5 Minnesota grown varieties (Vinton, Corsoy, Hardin, Stine 2510, Stine 2810). Vinton had the highest protein and the lowest oil content. Negative relationships existed between protein and fiber ($r = -0.71$) and protein and oil ($r = -0.87$) content. Hydration of soybeans occurred rapidly and similarly in all 5 soybean varieties. Negative linear regression relationship were found between calcium sulfate concentration and both yield ($r = -0.90$ to 1.00) and percent protein ($r = -0.96$ to 1.00) in tofu for all varieties. Vinton was the most sensitive to changes in calcium sulfate concentration. However, additional work is needed to verify that this lab scale mini-procedure could predict the behavior of different soybean varieties and processing variables under large scale

tofu processing conditions

Effect of storage conditions on quality of soybeans

Various physical, chemical and biological changes take place in soybean during storage depending on the storage conditions, storage time and condition of soybean to be stored. If these physical and chemical changes exceed a certain threshold, the soybean is considered deteriorated. Thus, the acceptability and suitability of soybean are directly related to the various physico-chemical changes which occur during storage.

Narayan *et al.* (1988) observed that the kernel weight and density decreased whereas hardness increased when soybean stored in Jute bags under ambient conditions for 1, 2, 3 and 4 years, The color changed from creamy yellow to brown. Among chemical characteristics, moisture content, fat, water-soluble nitrogen (WSN), nitrogen solubility index (NSI), sugars, trypsin inhibitor activity, available lysine, pigment and lipoxygenase activity of seeds decreased during storage whereas non-protein nitrogen (NPN), extent of browning, free fatty acid (FFA) content and peroxide value increased. Ash content and phytic phosphorus increased initially and thereafter decreased with increasing storage period.

Saio *et al.* (1980) conducted a model storage study in which the quality changes were investigated in two soybean varieties stored one year at temperature of 15°C, 25°C, and 35°C and relative humidity levels of 60%, 70%, and 80%. They found that the color of beans darkened; the acid values of extracted crude oil and the acidity of beans increased as deterioration progressed. The nitrogen solubility index decreased rapidly at high temperature and high relative humidity of storage. The color of

soybean milk darkened and its pH decreased slightly. The hardness of tofu-like gel formed from stored beans decreased. The levels of solids, nitrogenous constituents, sugar, and ash in the water extracts definitely increased. Electron spin resonance spectra of stored beans suggested that the combination of phosphatidylcholine with the proteins in soybean milk was strengthened. Temperature and relative humidity are both related to overall changes during storage, but relative humidity seems to be more important. Similar work was done by Yanagi *et al.* (1985). Soybeans were stored at temperature of 15°C and 30°C and relative humidity levels of 50%, 70%, and 80% for 10 months. They found that the moisture of beans stored under various conditions gradually reduced for the first 6 months and maintained a consistent state after that. The final moisture content was lower under lower humidity storage than under higher humidity storage at 30°C. At high humidity and high temperature, the acid values increased markedly suggesting that neutral fat in fresh bean was hydrolyzed to free fatty acid. Germination activity reduced with the advancing months of storage. The NSI decreased gradually with the prolonged months of storage at higher humidity. There are a few studies that have been done on protein denaturation of soybeans and meals as a function of storage time and conditions. Saio's study (Saio *et al.*, 1982) showed that as storage time under improper conditions increased, protein extractability decreased rapidly. Extractability was influenced by high ionic strength and pH but was most affected by the presence of dithiothretol. Changes in β-conglycinin and glycinin extractabilities were investigated by ultracentrifugation and electrophoresis. The results indicated that extractabilities of β-conglycinin and glycinin components decreased, indicating an even more rapid decrease of the glycinin component. Whole beans were more resistant to deterioration during storage than

meals, and full-fat meals deteriorated more rapidly than defatted meals.

Soymilk and tofu properties are influenced by soybean storage conditions. Thomas *et al.* (1989) stored soybeans at two temperatures, 20°C and 30°C, and two relative humidities, 65% and 85%. The amount of protein extracted into soymilk decreased by about 14% of the initial extractability in all cases after eight months of storage. Tofu made from beans that were stored at 85% relative humidity became less uniform in microstructure toward the end of the storage period. The volume of whey produced increased with bean storage time. Effect of soybean storage on the sensory qualities of the products (soymilk, tofu, and soynuts) was reported by Narayan *et al.* (1988). Color of tofu and soymilk, crispness and taste of soynuts were lost with increase in storage time of beans. Overall organoleptic score of all the products was found to decrease with increase in storage time of beans. Additionally, total solids and protein in soymilk, as well as in tofu, decreased with the storage time.

Effect of soybean storage conditions on lipid composition of soybeans

Soybean storage conditions have an effect on lipid composition of soybeans and the soy products made from stored beans. Changes in lipid composition of soybeans and extracted oil during storage were analyzed (Nakayama *et al.*, 1981). After soybean stored for six months at 35°C, 45% of the total phospholipids had decomposed, and 72% of the total phospholipids originally extracted with the oil was lost. Phosphatidylcholine and phosphatidylethanolamine decreased significantly, and phosphatidic acid and lysophosphatidylcholine increased in soybeans during storage. No difference was found between soybeans and extracted oil in fatty acid composition of phosphatidic acid. Phospholipase D activity was found in an acetate buffer extract of

homogenized soybeans. Clark and Snyder (1991) reported that lipid peroxidation was thought to occur in seeds and was responsible for seed deterioration during storage, causing reduction in vigor or germination. Hydroperoxides can break down to form secondary oxidation products which have off-flavors and are undesirable in vegetable oil used for food. High-performance liquid chromatography was used to separated the crude lipid and results indicated that there was a lag in hydroperoxide production for the first nine months storage followed by a steady increase for remaining 22 months.

Effect of soybean maturity on chemical composition and storage stability

Raw soybeans contain many biologically active factors. The Trypsin inhibitors and the lipoxygenases are major factors responsible for poor protein digestibility and beany off-flavor, respectively. Their activities have been reported to undergo some changes during maturation as well as storage.

Hack and Williams 82 soybeans from four maturation stages were studied for storage stability and process quality (Steinberg *et al.*, 1989). In comparison to mature beans, trypsin inhibitor, urease and lipoxygenase activities were lower in immature seeds, but free fatty acid content was higher and the oil extracted was greener. During six months storage at 23-25°C, lipoxygenase decreased and free fatty acid increased at a faster rate in immature than in mature soybeans. Crude oil and protein contents were similar, regardless of maturation or storage time. Both β-conglycinin and glycinin proteins increased with maturation but the β-conglycinin/glycinin ratio decreased. There was no change in protein content during storage.

Statement of Problems and Objectives of Present Study

As described in the literature review, many researchers have attempted to determine what role soybean variety plays in influencing the quality and acceptability of tofu, which processing factors are the most important in tofu production, how processing conditions influence the functional properties of soy proteins in soy products. There is an increasing demanding by soybean growers to know which factors will produce higher yield and better quality products for human consumption. However, tofu researchers have not been able to obtain complete answers to those questions.

Little information about the role of soy glycinin and β-conglycinin subunits in influencing certain characteristics of tofu quality is available. Additionally, most of the tofu studies have employed laboratory-scale process conditions. The results and models obtained from these studies need to be tested in large scale tofu processing conditions. The conditions used in large-scale commercial production of tofu vary from laboratory production.

Additional work is needed regarding soybean storage stability and the effects of soybean storage on sensory characteristics of final products. Based on this review, one can observed that sensory evaluation, by both instrumental analysis and sensory analysis, of soy products, especially for tofu, has been an neglected area.

Therefore, the main objectives of my study are (1) to separate and determine the amounts of glycinin, β-conglycinin, and their subunits in 7 soybean cultivars; (2) to correlate the characteristics of raw soybeans with quality of tofu made on pilot-plant scale; and (3) to investigate the influence of soybean storage time on quality of tofu and consumer acceptability of tofu. This study could provide information on how different cultivars (which may have different protein subunit composition) correlate

with the characteristics of tofu and what are the most important constituents in soybean to be utilized when selecting a variety of bean for tofu making. Evaluation for sensory characteristics of tofu would help in evaluating the effects of different factors on tofu characteristics. The combination of results of the instrumental and the sensory analysis will provide a basis for preferred sensory characteristics of tofu. These concepts will be used to identify better soybean varieties for production of marketable tofus.

CHAPTER 3. MATERIALS AND METHODS

Soybeans

Seven varieties of soybeans were selected for this study. They are: Vinton 81, LS 201, A892641, Pioneer 9202, NK S2026, NK S2303, and Latham 650. Varieties LS 201 and A892641 were provided by Dr Walter Fehr of Agronomy Department at Iowa State University. Varieties Pioneer 9202, NK S2026, NK S2303, and Latham 650 were provided by respective seed companies. Variety Vinton 81 was provided by West Central Coop. Varieties Northrup-King 2026 (NK 2026), Northrup-King 2303 (NK 2303), and Latham 650 are commonly grown in Iowa for animal feed. Pioneer 9202 is of low protein content cultivar and A8926 is of low linoleic acid cultivar. Variety LS 201 is larger in size than other varieties except Vinton 81. Variety Vinton 81 is usually grown for human food uses and is used as standard in this study. All varieties were grown on the Iowa State University research farm except Vinton 81. They were planted in spring of 1991 and harvested in fall of 1991. They were stored at 0-5°C in sealed bags in the cooler until use.

Protein Analysis

Analytical grade reagents were obtained from commercial sources and used as received. Sodium lauryl sulfate (SDS), N,N,N',N'-tetramethyl ethylenediamine

(TEMED), B-mercaptoethanol (βME), 2[N-morpholino]ethanesulfonic acid (MES), Sepharose 6B-Cl (fractionation range for globular protein 10-4000 kDa), Concanavalin A-Sepharose 4B, Agarose (type 1:low EEO), methyl α-D-manno-pyranoside (mannoside), N-*tris*[hydroxymethyl]methylglycine (tricine), bovine serum albumin (lyophilized), Freund's complete and incomplete adjuvant, urea (electrophoresis reagent), acrylaimde (electrophoresis reagent), *bis* acrylaimde (electrophoresis reagent), Dalton Mark VI molecular weight standard and Coomassie Brilliant Blue R were purchased from Sigma Chemical Co. (St. Louis, MO). The Dalton Mark VI molecular weight standard contained a lyophilized mixture of lysozyme (14.3 kDa), β-lactoglobulin (18.4 kDa), trypsinogen (24 kDa), pepsin (34.7 kDa), egg albumin (45 kDa), and bovine albumin (66 kDa). The chemicals purchased from Fisher Scientific Co. (Fair Lawn, NJ) were *tris*[hydroxymethyl]aminomethane (THAM), sucrose, glycine, acetic acid, sodium azide, calcium lactate, K_2HPO_4, NaCl, sodium potassium tartrate, NaOH, phenol red and ammonium sulfate. Ammonium persulfate (APS), bromophenol blue, KH_2PO_4, NaH_2PO_4 and $CuSO_4.5H_2O$ were from J. T. Baker Chemical Co. (Phillipsburg, NJ). The methanol used in the staining and destaining was of practical grade and was purchased from Barton Solvents (Des Moines, IA). The Biogel Wrap was from BioDesign Inc. (Carmel, NY). The microemulsifying needles were from Thomas Scientific (Swedesboro, NJ). The catheter needles were from Travenol Laboratories Inc. (Deerfield, IL) and the Gel Bond film support medium was from FMC BioProducts (Rockland, ME).

Protein purification

Vinton 81 variety soybeans were ground in a coffee and spice mill (Moulinex, France) and were defatted by hexane extraction (soy flour : hexane = 1 : 1.5, v/v) for 2 hours at room temperature. Then the solids were allowed to settle and the solvent was decanted. This process was repeated six times; at the end, the hexane extract was very light yellow. Desolventization of the soy flour was by air drying. The defatted flour was stored at 0-5°C in plastic bottles until needed.

Isolation of glycinin and β-conglycinin was according a modified version of the method published by Thanh and Shibasaki (1976). Defatted soy flour (40 g) was stirred for 3 hours in 800 mL of T buffer (0.03 M Tris at pH 8, containing 0.02% NaN_3 and 10 mM βME). The solubilized protein was separated from the insoluble matter by filtering through eight layers of cheese cloth, followed by centrifuging. Throughout this procedure a Beckman model J2-21 centrifuge fitted with a JA-17 fixed angle rotor (Beckman Instruments Inc., Palo Alto, CA) was used. The conditions of operation were 10,000 rpm at 20°C for 10 minutes. The pH of the supernatant was adjusted to pH 6.1-6.4, stirred for 1 hour, and centrifuged to separated out the precipitated crude glycinin. The precipitated crude glycinin was dissolved in the minimum amount (30 mL) of Wolf's buffer (0.0026 M KH_2PO_4, 0.0325 M K_2HPO_4, 0.4 M NaCl, 0.01 M βME, 0.02% NaN_3 at pH 7.4) and was used for further purification. The supernatant was adjusted to 5.5, stirred for 1 hour and centrifuged. The supernatant was adjusted to pH 4.8, stirred for 30 minutes and centrifuged to separate the precipitated crude β-conglycinin. The precipitated crude β-conglycinin was redissolved in the minimum amount of Wolf's buffer (40 mL), adjusted to pH 7.0, stirred for 30 minutes and centrifuged. The supernatant was used as the crude β-conglycinin fraction.

The crude glycinin was purified on a Sepharose 6B-Cl column (3.2×85 cm) with Wolf's buffer. Approximately, 20 mL of crude glycinin was loaded onto the column per run. The glycinin containing peak was concentrated by ultrafiltration. The retentate was eluted on a Concanavalin A-Sepharose 4B (3.2×22 cm)column (Kitamura *et al.*, 1974) to remove any β-conglycinin contaminants present. The β-conglycinin bound to the Concanavalin A column was eluted out by washing with 0.01 M mannoside in Wolf's buffer. The column was reequilibriated in Wolf's buffer before reuse. The purified glycinin was concentrated by ultrafiltration and stored at 0-5°C until needed.

The crude β-conglycinin was purified by elution on a Concanavalin A-Separose 4B column (3.2×22 cm) with Wolf's buffer. A 30 mL sample was loaded per run. After the contaminants were washed off, the eluent was changed to 0.01 M mannoside in Wolf's buffer to elute the bound β-conglycinin. The eluted β-conglycinin was concentrated by ultrafiltration and stored at 0-5°C until needed. The column was reequilibriated in Wolf's buffer before reuse. After the elution of four samples of β-conglycinin, the column was washed in 0.05 M mannoside to regenerate the capacity of the column.

Ultrafiltration of glycinin and β-conglycinin were carried out at 20 psi pressure of nitrogen in a ultrafiltration apparatus (Nuclepore, Pleasanton, CA) fitted with a magnetic stirrer. With glycinin and β-conglycinin samples, the ultrafiltration membranes used had molecular weight cut-off at 50 kDa and 10 kDa, respectively.

Antibody production

The method of antibody production was adapted from Mayer and Walker (1980). Purified glycinin and β-conglycinin were used for this purpose and three month old

male goats were used, one for each protein. Prior to the first inoculation of the antigen, a sample of blood (5 mL) was removed from the jugular vein of the goats and checked for the presence of any antibody to either of these proteins. The antigen (0.25 mg) together with 0.5 mL Freund's complete adjuvant was made up to 1 mL in 0.7% NaCl solution to be injected into the goat each time. Prior to injection, the innoculum was emulsified between two systems connected by a microemulsifying needle. This protein emulsion was inoculated subcutaneously once every two weeks for four times. After that Freund's incomplete adjuvant was used in place of the complete adjuvant. Boostings were done on a monthly basis and the amount of protein injected was reduced to 0.1 mg/injection per goat. Starting at the age of 5 months, blood samples were tested by an Ouchterlony diffusion gel for the presence of antibody at a good titer. The dry Ouchterlony plates were hydrated in Wolf's buffer and the antigen was placed in the center well while the antibody was placed in the peripheral wells. Immunodiffusion was carried out for 16 hours at room temperature in a moist atmosphere. Unreacted materials were washed out by shaking in 0.15 M NaCl for 30 minutes. It was then stained in Coomassie blue for 2 hours and then destained in a solution consisting of acetic acid:methanol:water (1:5:4). Once the antibody titer was high, blood samples were withdrawn from the jugular veins of the goats, starting at 150 mL and gradually increasing to 250 mL volume of blood per goat, every two weeks. When over 50 mL of blood was withdrawn per time, a catheter was used to bleed the goats.

The serum was obtained from the blood by ringing the blood and allowing it to clot at room temperature overnight. The serum was separated by centrifuging at 10,000 rpm at 15°C for 15 minutes. The serum was decanted off and stored at 0-5°C

until about 1 L of serum was collected. The serum (1 L) was thawed. All subsequent steps in isolating the antibody were maintained in an ice bath (2-5°C). The antibody was precipitated by a 50% $(NH_4)_2SO_4$ cut (291 g $(NH_4)_2SO_4$ per L serum). The precipitate was washed about seven times (until the washings were clear) with 1.75 M $(NH_4)_2SO_4$, centrifuging between washing to separate the precipitate from the supernatant. At the end of the washings, the precipitate was dissolved in minimum quantify of 10 mM NaH_2PO_4 at pH 7 (30 mL) and dialyzed against water overnight at 4-5°C. It was centrifuged at 10,000 rpm at 2°C for 10 minutes to remove the lipoproteins that had precipitated. The supernatant was dialyzed against 10 mM NaH_2PO_4 at pH 8 to get the antigen. This purified antibody was stored at -30°C until needed.

Rocket immunoelectrophoresis

This method of electrophoresis was used to quantitate the amount of glycinin and β-conglycinin in a whole soy protein extract. It was also used to check for the absence of β-conglycinin in the glycinin sample and vice versa.

A gel cast was assembled to make a 18 × 9 × 0.15 cm gel with two glass plates, 3 acrylic strips and a Gel Bond film which was aligned so that the hydrophilic side faced the gel. Agarose (300 mg) was heated while stirring on a water bath, in rocket electrophoresis buffer (0.025 M tricine, 0.08 M Tris, 0.34 mM calcium lactate and 0.02% NaN_3 at pH 8.6) (29 mL) to 90-95°C, to solubilize the agarose. The mixture was cooled to 55°C. Both the gel cast and the syringe were warmed to about 50°C under a heat lamp to prevent gelling of agarose from sudden cooling upon contact. The gel was allowed to set at room temperature for 30 minutes. The gel was removed

from its cast, and 35 wells were made (1 cm from the bottom edge) using a steel tube of diameter 3 mm (Pratt and Witney cutting tools and gases) and the cut out gel was sucked out of the wells. The gel was adhered to the stage of the electrophoresis unit (Model 1405 electrophoresis cell, BioRad Laboratories, Richmond, CA) using a few drops of water. The wells were loaded with standard and unknown protein samples (4-5 μL) in a random order. The protein being tested and the standard used were determined by the antibody incorporated into the gel. Rocket electrophoresis buffer (total 500 mL) was placed in both the buffer chambers, contacts were established between the buffer and the gel using filter paper and the electrophoresis unit was set up for electrophoresis. The electrophoresis was at 180 mV for 16 hours. At the end of the electrophoresis run, the gel was removed from the unit, dried between filter papers and absorbent paper (10 minutes), pressed under a weight of 1 kg and rinsed in 0.1 M NaCl for 10 minutes. The gel was rinsed in water two times for 5 minutes each, press again and dried under a heat lamp for 15 minutes. It was stained for 15-20 minutes in 0.5% Coomassie blue in strong destain and then destained in strong destain until the background was clear. A calibration plot of height of rocket vs μg standard protein was used to determine the concentration of the protein being tested, in the samples analyzed.

Determination of protein concentration

The soluble protein concentration was determined by the biuret method where a $Cu^{2}+$ ion in a alkaline solution, complexes with peptide linkage to give a red color that has an absorbance maximum at 540 nm. The biuret reagent consisted of 1.5 g/L $CuSO_4.5H_2O$, 6 g/L sodium potassium tartrate and 30 g/L NaOH. Several

dilutions of a standard protein solution (10 mg/mL bovine serum albumin) were made in water to a final volume of 1 mL. The protein solutions being analyzed were also diluted accordingly to a final volume of 1 mL. The biuret reagent (4 mL) was added to the protein, mixed well and allowed to stand for 30 minutes. The absorbance was measured at 540 nm in a Gilford spectrophotometer 250 (Gilford Instrument Laboratories Inc., Elgin, IL). Using a Beer's law plot, the concentration of the proteins was determined.

SDS-Urea polyacrylamide gradient gel electrophoresis

Seed samples were ground to a fine powder and then a 0.15 g sample was suspended in a 20 mL of sample buffer (50 mM Tris-HCl, pH 8.0, 0.2% SDS, 10 mM β-mercaptoethanol, and 5 M urea). Samples were extracted 3 hours at room temperature and then centrifuged to remove seed debris.

Sodium dodecyl sulfate-urea polyacrylamide gel electrophoresis (SDS-urea PAGE) was used to separate glycinin and β-conglycinin and to quantify the relative amount of their subunits. The method was adapted from Pacheco *et al.* (1984) with some modifications. A discontinuous 10 to 18% acrylamide gradient gel was used. The stacking gel was a non-restrictive gel with 4% acryl-*bis*, 0.058% SDS, 0.083% TEMED, 0.21% ammonium peroxydisulfate (APS), 2.75 M urea and 0.437 M Tris at pH 8.0. The resolving gel was a linear gradient with acryl-*bis* concentration varying from 10-18% with 0.058% SDS, 0.083% TEMED, 0.13% APS, 0.437 M Tris at pH 8.0, 3.75 M urea for 10% gel and 5.45 M urea for 18% gel. A 14.5 $\times$ 13.5 $\times$ 0.15 cm slab gel was prepared with a 9 cm resolving gel and a 4.5 cm stacking gel in which 15 wells made by the use of a comb. The sample was prepared for loading onto

the gel by boiling in a water-bath for 10 minutes together with tracking dye and βME (protein : tracking dye : βME = 18 : 5 : 1 by volume). The tracking dye consisted of 5% SDS, 1.0 g/mL sucrose, 0.05 M MES and adequate Bromophenol blue to give dark blue coloration. The slab was assembled on a Sturdier slab gel electrophoresis unit model 400 (Hoefer Scientific Instruments, San Francisco). The samples were loaded into the wells and the apparatus was assembled for electrophoresis with tank buffer in both upper (300 mL) and lower (200 mL) buffer chambers. The tank buffer consisted of 0.3% Tris, 1.44% glycine, and 0.1% SDS. The pH of tank buffer was 8.3. The samples (10-20 μl) were applied to the gels, and they were run at a voltage of 100 v for 1 hour and then at 125 v for 5 hours. Upon completion of electrophoresis, the gel was removed from the slab and stained in staining solution (0.225% Coomassie blue in a solution which consisted of water : ethanol : acetic acid = 50 : 47 : 7 volume basis). The staining was performed while shaking gently for overnight. Then the stained gel was destained for more than 24 hours in 2-3 changes destain until the background was very light. The gel was gently shaken in the destaining solution to enhance the destaining process. The destain solution consisted of water : ethanol : acetic acid = 17 : 2 : 1 volume basis. When it was necessary to store the gel for shorter period of time, it was kept in destain solution closed in a plastic box. For long term storage and quantification, the gel was dried between two layers of Biogel Wrap for overnight at room temperature.

The relative amounts of each fraction of glycinin and β-conglycinin were quantified by image analysis of dried, bonded gel.

Storage Study

One level of relative humidity (84%) and one temperature (35°C) were used for storage study. The required humidity was maintained in a temperature-controlled incubator containing saturated NaCl solution (Rockland, 1960). All soybeans were in cloth bags and stored in the incubator under above conditions for total 110 days. Soybean samples were taken out at 22 days intervals for various analyses.

Tofu Processing

The traditional method of tofu processing was adapted from Johnson (1984) with some modifications. Soybeans (900 g) were soaked in tap-water for 12-14 hours at room temperature, washed and ground twice in a Cherry-Burrell vibroreactor with 6 liters of tap-water. The slurry was cooked at 95°C for 7 minutes, with an additional 1 liter of tap-water in a steam-jacketed kettle. The cooked slurry was filtered through a coarse mesh sack followed by a fine mesh sack. A cider-press was used to provide sufficient pressure to press any remaining soymilk from water-insoluble residue (okara). After initial pressing, 1 liter of tap-water was used to wash the okara. The okara was then re-pressed. The volume of the collected soymilk was measured and a 20 ml soymilk was removed to measure the solids content by using refractometer. The solids content of soymilk was adjusted to 5% by the addition of tap-water. The required coagulant ($CaSO_4.2H_2O$, food grade) amount was determined by the soymilk solids level and the final soymilk volume (Johnson, L. D, 1984). The soymilk was then transferred back to the steam-jacketed kettle and heated to 85°C. At 85°C the stirring rate was increased, the coagulant slurry added and the stirring was brought

to a halt in 10 seconds. After allowing the curds to form for 3 minutes, the resulting coagulum was cut and 2000 ml of whey removed. The curd was poured into a cheese cloth lined, stainless steel box with perforations on all sides placed in a larger bucket. The curd was then pressed for total 15 minutes. The tofu block was weighed and stored at 4°C in a water filled plastic container.

Chemical Analysis of soybeans

For moisture content, a whole soybean sample was ground to very fine particles using a Magic Mill III (Model No. 100, Salt Lake City, UT.) and moisture content was determined according to the AOAC method (1984).

The lipid content of soybean was determined by extraction with hexane (Goldfish method) for 4 hours.

The protein content of beans was determined by the Kjeldahl method (AOAC, 1984) using the factor 5.71 to convert nitrogen to protein.

Nitrogen solubility index of raw beans was determined according to AACC method (1983).

Instrumental Analysis of Tofu Texture and Color

Instrumental analysis of tofu texture and color were done on the same day as sensory analysis. The sample size was 2.0×2.0×2.0 cm.

A texture profile analysis described by Bourne (1968) was run on 3 tofu cubes at room temperature using an Instron (Model 1122) Universal Testing Machine (Instron Corporation). Instron was equipped with a compression anvil. The applied compression force was measured using a 50 kg compression load cell and recorded

using a chart speed of 100 mm/minute with a 5 kg load equalling full scale. The tofu cube was compressed 80% with a cross-head speed of 100 mm/minute for 2 cycles.

The Hunter color system parameters L, a and b were measured on HunterLab spectrocolorimeter (Hunter Associates Laboratory, Inc., Reston, VA) for all the tofu samples.

Tofu Sensory Analysis

Panelists were graduate students and staff members of Food Science and Human Nutrition Department at Iowa State University. A few training sections were conducted so that the panelists could become familiar with the attributes and terms for tofu evaluation. The training sections had 12 people and the total number of 18 panelists participated in the sensory evaluation throughout the study. The sensory evaluation was conducted on tofu samples stored for about 20 hours at 5°C after being made. The samples were cut into 2.0×2.0×2.0 cubes and then heated by steaming in a steamer over kitchen stove for 2 minutes. The samples were then served to the panelists under fluorescence day light. The instruction score sheet used for this panel is shown in Figure 3.1. There were 4 or 5 tofu samples at each sensory panel and tofu made from fresh Vinton 81 was used as standard at each session.

Statistical Analysis

All treatments were run in triplicates for all varieties of soybeans. Statistical analysis was carried out using the Statistical Analysis System package developed by the SAS Institute, Inc. (Box 8000, Cary, North Carolina). Analyses of variance (ANOVA) were conducted and differences between the group means were analyzed by

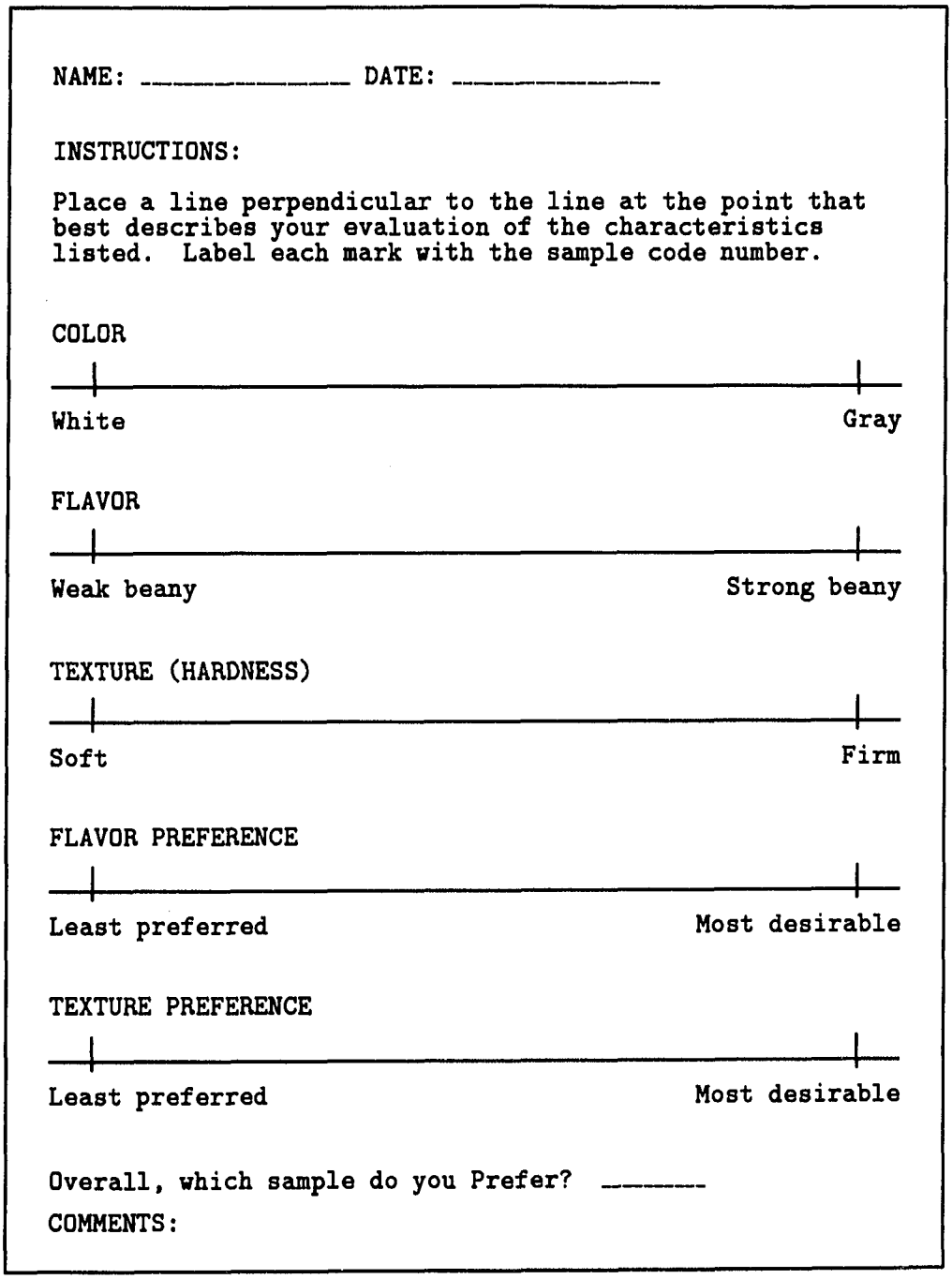

Figure 3.1: Tofu sensory evaluation sheet

Fisher's Least Significant Difference (LSD) test. The apparent correlations among various measurements were determined based on linear regression analysis by the selection of the statistically best fitted and correlated significant for all statistical procedures.

CHAPTER 4. RESULTS AND DISCUSSION

Separation and Quantification of Glycinin and β-Conglycinin Subunits

There are several techniques such as gel electrophoresis, fast protein liquid chromatography (FPLC), and the recently introduced method, capillary electrophoresis (CE) available for protein separation and quantitative analysis. Each of the techniques have their respective merits. We attempted to fractionate soy storage protein subunits by these methods.

When we employed FPLC technique to separate glycinin subunits, we encountered several problems during analysis. First, the procedure was time-consuming requiring more than 90 minutes to run one sample (which included column equilibration and regeneration (20 minutes), elution of unbound material after injection (10 minutes), and development of linear gradient (60 minutes)). Secondly, the pumps in the FPLC system were always contaminated by the urea buffer system which created clean up problems. The pumps had to be taken apart and cleaned almost every working day. Thirdly, the preparation of samples for HPLC involved several steps and was not a efficient procedure. The glycinin had to be purified, then dissociated into component subunits by Tris buffer. Iodoacetamide (IAA) was add to block any free SH groups and finally, excess IAA was removed by gel filtration.

We used CE (ISCO Model 3140 Electropherograph) to run our soy protein sam-

ples under several conditions. We were not able to resolve the soy proteins. Although capillary electrophoresis has some features that are attractive, challenges remain including standardization of separation conditions for different type of compounds, the development of preparative systems, and increased sensitivity for trace-level assays. Improvements in capillary electrophoresis of proteins in general are currently under way. Only after capillary electrophoresis systems become available to a broader range of researchers will the full spectrum applications begin to be explored and the true quantitative potential of the technique be realized.

The 10-18% SDS-PAGE was not able to separate A_4 peptide from the rest of acidic peptides. However, when 6M urea was included in 10-18% SDS-polyacrylamide gradient gels, glycinin acidic polypeptide components A_3 and A_4 could be resolved from the other acidic polypeptides (Figure 4.1). Similar observation was seen by others (Fontes *et al.*, 1984; Kitamura *et al.*, 1984). Therefore, SDS-urea polyacrylamide gradient (10-18%) gel electrophoresis was employed throughout this study to separate and quantify glycinin and β-conglycinin subunits. The major advantage of the method, in addition to resolution of A_3 and A_4 polypeptides, was that proteins can be evaluated without prior fractionation and purification of glycinin and β-conglycinin.

Physico-Chemical Properties of Selected Soybeans and Textural Characteristics of Tofu Made From These Beans

The seven varieties beans were selected in this study. They were: Vinton 81, LS 201, Pioneer 9202, A8926, NK 2026, NK 2303, and Latham 650. The variety selection was based on two factors: all varieties, with exception of Vinton 81, were grown on

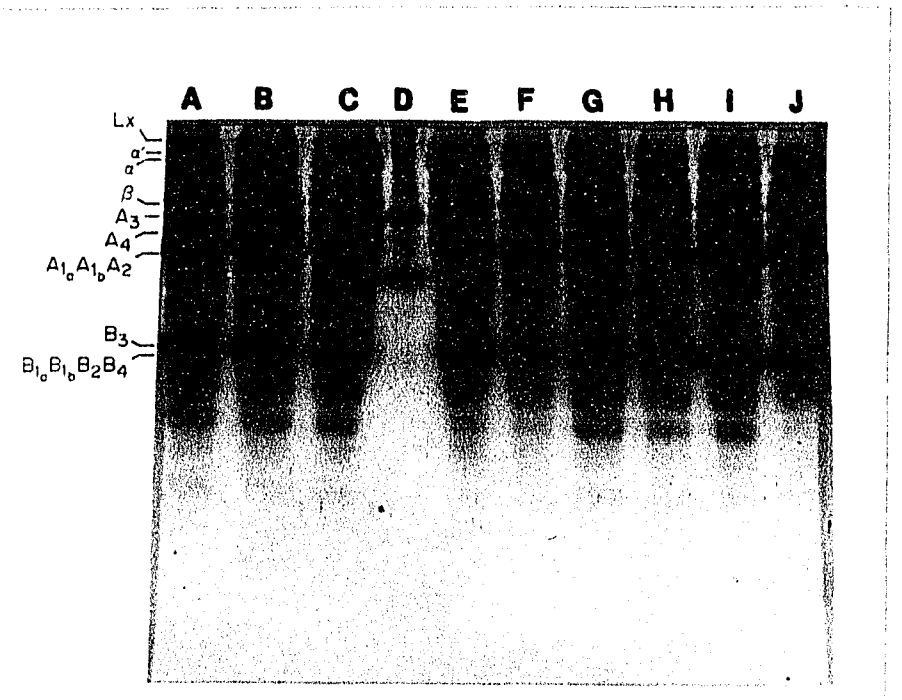

Figure 4.1: Sample of urea SDS-PAGE gel of soy proteins [a]

[a]Lane A LS 201; Lane B, A8926; Lane C, Latham 650; Lane D, M.W. Standard; Lane E and F, NK 2303; Lane G and H, NK 2026; Lane I, Vinton 81; Lane J, Pioneer 9202.

the same environmental conditions; and the end-uses of beans are different. Vinton 81, LS 201, and Pioneer 9202 are commonly grown for human foods and the other are usually used for oil production. Therefore, the difference in usage of these beans may relate to the their different textural characteristics of tofu made therefrom; the size of beans were different among varieties. Vinton 81 and LS 201 are relatively larger seeds. Previous research has demonstrated that the size of the beans was related to the yield of tofu (Snyder and Kwon, 1987).

The seven soybean varieties were different in their proximate chemical composition (Table 4.1) and soy protein composition and distribution (Table 4.2). The initial moisture and lipid contents, but not NSI, were significantly different ($p<0.05$) among varieties. The buffer-extractable glycinin, but not β-conglycinin determined by rocket electrophoresis, was significantly different ($p<0.05$). The buffer-extractable total protein determined by biuret was not significantly different among varieties. The relative amounts of glycinin subunits (A_3, A_4, $A_{1a}A_{1b}A_2$) measured by image analysis of dry bonded urea SDS-PAGE gels were significantly different ($p<0.05$) among varieties. The measured (by Instron) hardness, fracturability, cohesiveness, and Hunter L, a, b values of tofus made from these varieties showed significant differences ($p<0.05$). Among food beans, the relative amount of α' was negatively correlated ($p<0.05$) with fracturability ($r=0.788$) and the glycinin/β-conglycinin ratios (R) were correlated with hardness ($r=0.900$) and fracturability ($r=0.901$) of tofus. The A_4 peptide was present in Vinton 81 and LS 201. The presence of A_4 peptide in Vinton and LS 201 apparently may contribute to the higher values in hardness and fracturability of tofus made from these two beans (Table 4.3). In addition, there was no significant differences ($p<0.05$) in the yield of tofu among seven varieties.

Table 4.1: The proximate composition of seven soybean varieties [a]

Variety	Moisture %	Lipid %	Protein %
A8926	10.73±0.15 ^{x}	21.74±0.19 ^{xy}	39.10±0.01 ^{xz}
LS 201	10.80±0.06 ^{x}	20.41±0.25 ^{yu}	38.45±0.11 ^{xz}
Latham 650	10.76±0.03 ^{x}	21.94±0.22 ^{xy}	36.92±0.10 ^{xz}
Pioneer 9202	10.91±0.05 ^{x}	23.92±0.12 ^{zv}	34.97±0.13 ^{z}
Vinton 81	12.17±0.17 ^{y}	18.68±0.25 ^{u}	42.96±0.11 ^{y}
NK 2026	8.79±0.03 ^{z}	22.80±0.49 ^{xv}	36.26±0.13 ^{yz}
NK 2303	8.91±0.13 ^{z}	24.98±0.59 ^{z}	38.29±0.12 ^{yz}

[a]Mean±SEM is based on three replications; values sharing common superscripts are not significantly different at α=0.05 level.

Table 4.1 (Continued)

Variety	NSI	Bean Size #/100g
A8926	53.81±3.27 ^{x}	444
LS 201	41.28±3.03 ^{yz}	358
Latham 650	48.23±2.67 ^{xyz}	558
Pioneer 9202	47.87±6.38 ^{xyz}	482
Vinton 81	39.41±1.21 ^{z}	410
NK 2026	47.37±8.86 ^{xyz}	502
NK 2303	46.91±1.04 ^{xy}	540

Table 4.2: Soy protein composition and distribution of seven soybean varieties [a] [b]

Variety	Total Protein mg/ml	Glycinin %	β-Conglycinin %
A8926	16.69±1.34 ^{x}	37.10±1.35 ^{xy}	22.69±3.93 ^{x}
LS 201	14.08±0.23 ^{x}	51.84±1.45 ^{x}	25.17±1.19 ^{x}
Latham 650	18.40±0.96 ^{x}	36.15±8.64 ^{xyz}	21.83±1.99 ^{x}
Pioneer 9202	15.95±1.07 ^{x}	28.50±0.04 ^{xy}	19.06±1.75 ^{x}
Vinton 81	16.51±1.10 ^{x}	36.72±1.25 ^{z}	17.39±3.22 ^{x}
NK 2026	16.02±0.94 ^{x}	37.49±0.93 ^{yz}	19.96±1.26 ^{x}
NK 2303	18.58±0.31 ^{x}	33.29±8.03 ^{x}	17.39±0.99 ^{x}

[a]Mean±SEM is based on three replications; values sharing common superscripts are not significantly different at α=0.05 level.

[b]Total buffer-extractable protein is determined by biuret method; glycinin and β-conglycinin are determined by rocket immunoelectrophoresis; all subunits are determined by image analysis of urea SDS-PAGE gels.

Table 4.2 (Continued)

Variety	α' %	α %	β %
A8926	33.96±5.11 ^{x}	37.25±3.66 ^{x}	28.79±1.54 ^{x}
LS 201	29.35±3.04 ^{x}	36.92±1.78 ^{x}	29.71±4.31 ^{x}
Latham 650	32.92±3.44 ^{x}	40.99±0.75 ^{x}	24.26±3.03 ^{x}
Pioneer 9202	34.92±0.55 ^{x}	42.47±1.39 ^{x}	22.61±0.84 ^{x}
Vinton 81	32.72±2.77 ^{x}	40.11±0.55 ^{x}	27.18±3.32 ^{x}
NK 2026	37.14±3.38 ^{x}	38.90±2.02 ^{x}	23.96±1.43 ^{x}
NK 2303	31.72±1.18 ^{x}	43.59±2.44 ^{x}	24.69±1.59 ^{x}

Table 4.2 (Continued)

Variety	A_3 %	A_4 %	$A_{1a}A_{1b}A_2$ %
A8926	17.86±0.12 ^{xy}		82.14±0.12 ^{x}
LS 201	21.27±0.79 ^{yz}	9.62±0.73 ^{x}	69.12±1.16 ^{y}
Latham 650	21.36±0.15 ^{yz}		78.64±0.15 ^{xy}
Pioneer 9202	20.46±2.01 ^{yz}		79.54±2.01 ^{xy}
Vinton 81	20.98±0.74 ^{yz}	10.02±0.07 ^{x}	73.96±3.28 ^{xy}
NK 2026	15.67±1.03 ^{x}		84.33±1.03 ^{x}
NK 2303	23.80±2.40 ^{z}		76.17±2.49 ^{xy}

Table 4.3: Textural characteristics of tofu at time 0 [a]

Variety	Fracturability	Hardness	Cohesiveness
Vinton 81	1.42±0.09 ^{x}	3.05±0.22 ^{x}	0.35±0.02 ^{x}
LS 201	1.20±0.17 ^{xy}	2.48±0.28 ^{xy}	0.32±0.02 ^{xy}
A8926	1.05±0.14 ^{yz}	2.71±0.40 ^{xy}	0.40±0.03 ^{xy}
NK 2303	1.00±0.12 ^{yzu}	2.35±0.23 ^{xy}	0.36±0.02 ^{xy}
Pioneer 9202	0.95±0.12 ^{yzu}	2.26±0.25 ^{y}	0.37±0.03 ^{xy}
Latham 650	0.85±0.07 ^{zu}	2.22±0.22 ^{y}	0.35±0.04 ^{xy}
NK 2026	0.71±0.07 ^{u}	1.46±0.09 ^{z}	0.34±0.03 ^{y}

[a]Mean±SEM is based on three replications; values sharing common superscripts are not significantly different at α=0.05 level.

Effect of Soybean Varieties on the Yield and Quality of Tofu

There were statistically significant differences in hardness and fracturability, but not cohesiveness, of tofus made from the seven varieties. There were no significant differences in the yield of tofu among the varieties. The varietal effect on the yield, quality of tofu, and soymilk has been an area of interest among food scientists. Wang *et al.* (1983) studied the effect on the yield and quality of tofu of 5 U.S. and 5 Japanese soybean varieties grown under the same environmental conditions. Significant varietal differences were noted for protein and oil content of the beans (Wang *et al.*, 1983). Tofu prepared from each soybean variety showed no significant differences in yield of dry product but showed significant difference in the yield of fresh tofu. The differences were, apparently, due to water content. Significant variations among soybean varieties were noted in the hardness of resultant tofus. But the hardness of tofu was found to be negatively correlated to its water content. Wang *et al.* (1983) reported no significant correlation between the protein content of the soybeans and the yield of tofu. Therefore, based on their results, soybean variety did not seem to play an important role in tofu processing. However, these results were drawn from non-production scale conditions in which only 30 g of soybeans were used. Therefore, the significant differences in the yield and texture of tofu among varieties could have been due to their processing conditions, and not the water content as the authors suggested.

Recently, Kang *et al.* (1991) investigated the effect of varietal on texture and mechanical properties such as elasticity, hardness and fracturability of soy proteins. Their results demonstrated that: the heating temperature had an effect on elasticity; the protein concentration affected hardness and toughness; and glycinin/β-

conglycinin ratio affected fracturability. The thermal behavior of protein gels may be responsible for contributing to the textural quality of foods made from soybeans, such as tofu. However, in these cases, not only the thermal behavior of soy proteins but also the interaction of proteins with other ingredients may influence their texture qualities. Kang *et al.* (1991) concluded that beans with higher protein concentration may produce the harder tofu. The order of initial protein concentration of beans was Vinton 81 > A89 > LS 201 > NK 2303 > Latham 650 > NK 2026 > Pioneer 9202 (Table 4.1), and the order of the hardness of tofus made from these varieties respectively was Vinton 81 > A89 > LS 201 > NK 2303 > Pioneer 9202 > Latham 650 > NK 2026 (Table 4.3). Thus, Kang *et al.* (1991) conclusion regarding the relationship between protein concentration and the hardness of protein gels held true for most varieties used in this study. The variety Pioneer 9202 had the lowest protein concentration but the tofu made from Pioneer 9202 beans was not the softest among seven varieties. In addition, glycinin/β-conglycinin ratio did correlate ($p<0.05$) to the fracturability of tofus made from food beans. This finding was in agreement with Kang *et al.* (1991). However, the ratio, R, did not correlate to any textural characteristics of tofus made from beans used for oil production. Furthermore, the correlations of protein concentration and glycinin/β-conglycinin ratio with the hardness and fracturability of tofu apparently did not hold for all of stored beans and this will be discussed later.

Snyder and Kwon (1987) recommended that soybeans having large uniform size, light-colored hilum, thin seed coat, and high protein content were preferred for soymilk and tofu production. But the basis of that statement has not been verified by research. Lim *et al.* (1990) examined nine soybean varieties of widely different

seed size and found that bean size had no significant effect on yield of tofu. In our study Vinton 81 and LS 201 had a larger seed size (Table 4.1) than the other varieties. However, the tofu yields were not necessarily higher than others (Figure 4.2). Lim *et al.* (1990) developed two models. In model 1, soymilk with higher pH and total solids produced a higher yield of tofu. This model may explain the observation that for most varieties, the yield decreased as storage time increased due to the decrease in pH of the soymilk during storage. In Lim *et al.* (1990) model 2, soybeans high in protein and ash and low in phosphorous, resulted in higher yields of tofu. Our results are in agreement with this second model. NK 2303, Vinton 81, and NK 2026 had of higher protein contents (41 to 43%) and this resulted in higher yields (217 to 220%) compared to Pioneer 9202 (179%), the lowest protein content (37.6%) (Table 4.2). However, the differences in the initial yields of tofus among varieties were not statistically significant ($p<0.05$).

In conclusion, the variety had significant effect on hardness and fracturability of tofu. This varietal effect could not be explained by the initial protein content of the beans alone. But, there was no significant varietal effect on yield of tofu under the conditions used in our study. Some of the models suggested by previous researchers could not be successfully applied to the results obtained from this study. Therefore, laboratory-scale tofu process conditions, which were employed by most of the tofu studies in the literature, can not replace large-scale commercial production conditions in order to predict variety performance as tofu bean.

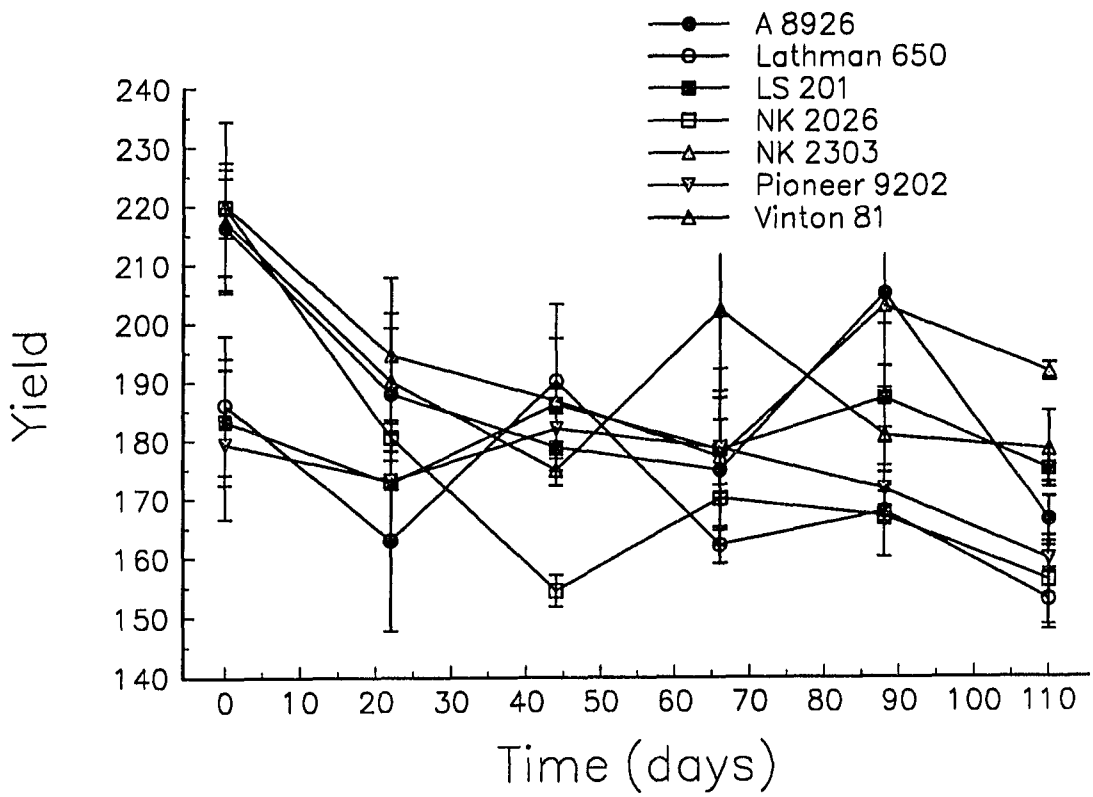

Figure 4.2: The changes in the yield of tofu during soybean storage

Varietal Effect on Soy Protein Composition and the Correlation of Soy Proteins and Texture of Tofu

There were statistically significant differences in relative percentages of A_3, A_4, and $A_{1a}A_{1b}A_2$. The A_4 peptide was present in Vinton and LS 201 only. There was no significant difference in terms of amounts of α'%, α%, and β% among the varieties. The presence of A_4 peptide in Vinton and LS 201 apparently may contribute to the higher values in hardness and fracturability of tofus made therefrom (Table 4.3). The hardness and fracturability of tofu made from these two varieties were significantly higher than the rest of the varieties, except for A89. These observations were in contradiction with the results reported by Nishinari *et al.* (1991). They reported that tofu gels prepared from cultivars without the A_4 subunit were harder and more solid-like than those prepared from cultivars with the A_4 subunit. However, only 1.5 ml soymilk was used for tofu gels preparation in their study.

As stated in above, Vinton, LS 201, and Pioneer 9202 were commonly used for tofu manufacture whereas the other varieties were used mainly for oil production. There were some significant correlations between soy protein subunits and texture characteristics of tofu for regular food beans. The relative amount of α' was negatively correlated ($p<0.05$) with fracturability ($r=0.788$) and the R was correlated with hardness ($r=0.900$) and fracturability ($r=0.901$) of tofu made from food beans. Therefore, for regular food beans, α'% and glycinin/β-conglycinin ratio apparently played an important role in the hardness and fracturability of tofu.

The molecular weights of subunits α and α' are 57 kDa and 58 kDa, respectively (Hirano *et al.*, 1987). Since their molecular weights are so close to each other that one would expect that their effects on the textural characteristics of tofu would be the

same if there was any. However, our results showed that only relative amount of α', but not α, correlated to fracturability of tofu made from food beans. The correlation between relative amount of α' and textural properties of tofu has not been observed by other studies before. The correlation between glycinin/β-conglycinin ratio and textural properties of protein gels has been reported in the literature (Kang *et al.*, 1991). Our results showed that this relationship appeared only in certain varieties, but not every variety. Therefore, we concluded that soy protein composition does have some effect on the textural characteristics of tofu . The ways of protein composition affect the texture depends on soybean variety.

Effect of Storage on Physico-Chemical Properties of Soybeans

The changes in the moisture content during 110 days of storage at 84% relative humidity and 35°C of LS 201, Latham 650, Pioneer 9202, A89, and Vinton 81 were not significantly different ($p<0.05$). This was in accordance with results reported by Yanagi *et al.* (1985) and Narayan *et al.* (1988a). The tendency was different for varieties NK 2026 and NK 2303. There was a significant increase ($p<0.05$) of moisture content initially, then moisture decreased after 22 days. This increase might be due to the initial low moisture content of NK 2026 and NK 2303, 8.77 and 8.93%, respectively. These soybeans gained moisture during the early period of storage. The changes in moisture content of soybeans during this storage study were different from those reported by Thomas *et al.* (1989). They conducted the storage study under two temperatures (20 and 30°C) and two levels of humidity (65 and 85%). Their results indicated that moisture content of the soybeans stored under 85% humidity increased with storage time whereas that of the soybeans stored at 65% humidity

gained moisture during first month storage, and remained constant for the rest of storage period. The tendency for an increase in moisture content of stored soybeans in the Thomas study might be due to the initial moisture content of soybeans of 6.7% which was significantly lower than NK 2303 and NK 2026 of this study.

The extractable lipid content of all beans stored at 35°C and 84% relative humidity decreased linearly ($p<0.05$) with the increase in storage period (Table 4.4), although the extent of decrease was different for different varieties of soybeans. The results of the different kinetic models study indicated that the decrease in total extractable lipid upon soybean storage followed an apparent zero order mechanism. This tendency was in agreement with others (Melton *et al.*, 1981; Narayan *et al.*, 1988a). Melton *et al.* (1981) reported that the total lipid content of defatted soy flours decreased linearly during storage. It has been reported that soybean storage conditions have an effect on lipid composition of soybeans and soy products made from stored beans (Nakayama *et al.*, 1981; Narayan *et al.*, 1988a; Clark and Snyder, 1991). The occurrence of some chemical reactions during soybean storage such as hydrolysis of lipids to fatty acids, oxidation of fatty acids, and formation of peroxides from unsaturated fatty acids, have been identified by thin layer chromatography (TLC) (Melton *et al.*, 1981), HPLC (Clark and Snyder, 1991), and other methods. Deterioration of lipids during storage may cause changes in sensory characteristics of tofu made from stored beans. These changes will be discussed later.

Nitrogen solubility index (NSI) measures nitrogen extracted with water under defined conditions. Figure 4.3 shows changes of NSI during 110 days accelerated storage of whole soybeans. In general, the NSI decreased with the prolonged periods of storage for all soybean varieties except variety LS 201. Statistical analysis showing

Table 4.4: Significant ($p<0.05$) correlation coefficients between lipid content and storage time

Variety	A8926	LS 201	Latham 650	Pioneer 9202
r	-0.409	-0.734	-0.942	-0.855
Variety	Vinton 81	NK 2026	NK 2303	
r	-0.916	-0.736	-0.918	

that there were significant correlation between NSI and storage time for varieties A89, Latham 650, NK 2303, Pioneer 9202, and Vinton 81. The correlation coefficients were -0.765, -0.706, -0.693, -0.614, and -0.750, respectively. These correlations were in accordance with the earlier findings of Saio *et al.* (1980), Saio *et al.* (1982), Yanagi *et al.* (1985), Narayan *et al.* (1988a) and Thomas *et al.* (1989). The storage conditions affected the NSI of soybean to different extents for different soybean cultivars. The largest NSI change was a decrease of 29.27% (from 47.87 to 18.60%) in Pioneer 9202. The smallest NSI change was a decrease of 8.63% (from 47.37 to 38.74%) in NK 2026. It should be noted that, for most of varieties, the NSI of beans increased nominally during the first 22 days of storage and then decreased. These data indicated that short term storage under high temperature and high humidity conditions increased soy proteins solubility in water.

Saio *et al.* (1980) suggested that the decrease in protein solubility during storage of soybeans was caused by lowering of the measured pH of the whole water extract. At high humidity (85%) and temperature (30°C), the acid values of the oil and organic acids increased (Yanagi *et al.*, 1985), showing that the neutral fat in fresh bean was hydrolyzed to free fatty acids (FFA). Oxidation of free fatty acids or/and other active biological processes during storage may result in the increase of organic acids and contributed to the drop in pH value of whole water extract (Narayan *et*

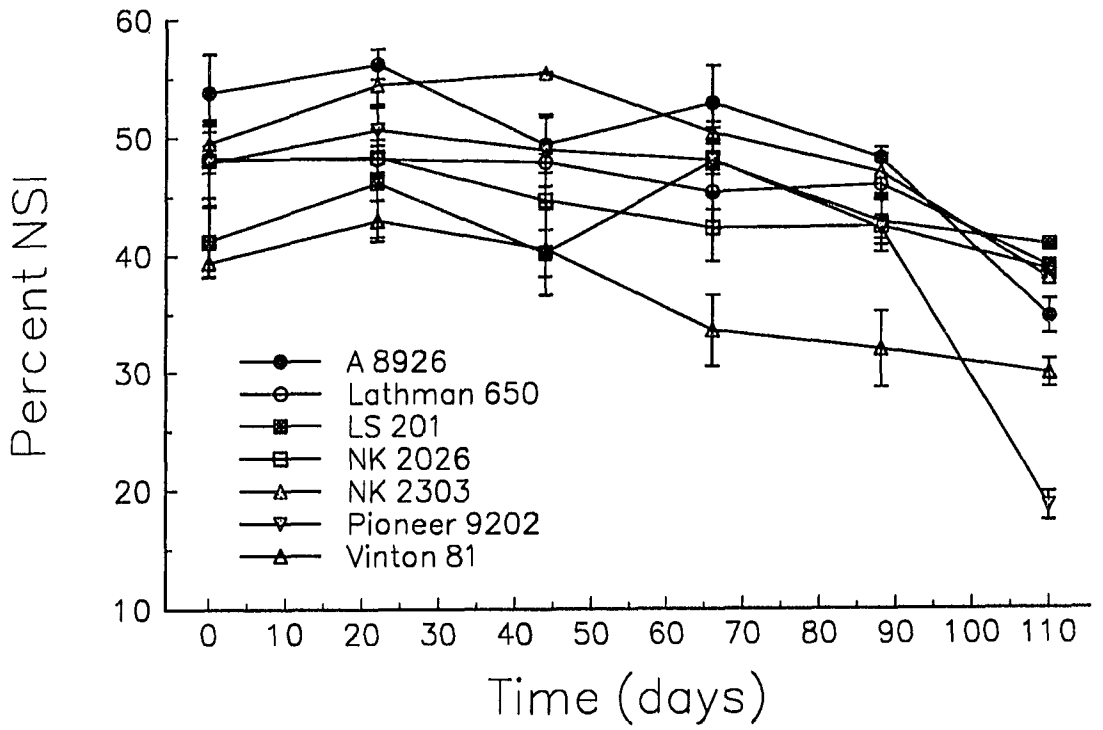

Figure 4.3: The NSI changes during soybean storage

al., 1988a). The results obtained by Thomas *et al.* (1989), however, do not support this hypothesis. In their study, the pattern of pH change did not reflect the trend of reduced protein solubility. Another possible cause for the decreased solubility may be divalent cations combining with soy protein (Thomas *et al.*, 1989). An increase in the cation:anion ratio would indicate increased levels of cations capable of interacting with the proteins. This hypothesis will need to be confirmed in a future study. The decreases in NSI could also be due to the formation of the Maillard reaction products, thereby reducing the solubility of nitrogen.

Statistical analysis showed that the total extractable lipid decreased linearly with storage time (Table 4.4). The off-flavor of tofu was first reported when beans had been stored for 88 days. The hydrolysis of neutral fat in the beans to free fatty acids and the oxidation of these free fatty acids, apparently, resulted in the decrease in total extractable lipid (Table 4.4) and the development of the off-flavor during beans storage. Therefore, the drop in pH of water extract (Saio *et al.*, 1980) might be one of the causes for decreasing NSI with time. Another possible cause for the decreased solubility in our storage study might be due to the occurrence of the Maillard reactions. Although the color of stored beans did not show any browness, the color of the tofu made from stored beans changed from bright yellowish or creamy yellow initially to slightly brownish to grayish toward the end of the storage period. In conclusion, the decrease in protein solubility during storage might be caused by lowering of the pH of the whole water extract and the occurrence of the Maillard reactions.

Effect of Storage on Soy Proteins Evaluated By Rocket Immunoelectrophoresis and Urea SDS-PAGE

Total buffer-extracted protein was measured by the biuret method (AOAC, 1984) and the results are shown in Table A.1 in the Appendix. The amounts of glycinin and β-conglycinin were determined by rocket immunoelectrophoresis and the results are shown in Tables A.2 and A.3 in the Appendix. Table 4.5 showed that relative amount of glycinin decreased linearly with storage time for NK 2026, Pioneer 9202 and Vinton 81. The relative amount of β-conglycinin significantly ($p<0.05$) decreased with time for A89 and LS 201. The results of the different kinetic models study indicated that the decrease in glycinin and β-conglycinin upon soybean storage followed an apparent zero order mechanism. There were significant correlations between glycinin/β-conglycinin ratio with soybean storage time for A89 and LS 201. Saio *et al.* (1982) studied the protein denaturation of intact soybeans and soy flours as function of storage time. They reported that glycinin components decreased and 2S components increased in the relative proportion to extractable protein from soybean flours measured by ultracentrifugation and electrophoresis. They pointed out that whole beans were more resistant to deterioration during storage than flours. The quality seemed to be more readily retained in the native state when the intracellular membranes were intact.

Glycinin and β-conglycinin subunits were separated by using urea SDS-PAGE. The relative amounts of their subunits were measured by image analysis of dry bonded gels and the results are presented in Tables A.4-A.8 in the Appendix. Vinton and LS 201 were the only two varieties with the A_4 subunit band in their SDS-PAGE gels. The hardness and fracturability of tofu made from these two varieties were higher

Table 4.5: Significant ($p<0.05$) correlation coefficients between relative amounts of soy proteins and storage time

Variety	Glycinin	β-Conglycinin	R [a]
A8926		-0.513	0.635
LS 201		-0.633	0.856
Latham 650			
Pioneer 9202	-0.551		
Vinton 81	-0.617		
NK 2026	-0.685		
NK 2303			

[a] R = Glycinin/β-Conglycinin.

than the other varieties at time 0 (Table 4.3). The A_4 subunit band disappeared after these beans were stored for 22 days at 35°C and 84% relative humidity. The storage conditions affected the relative amounts of subunits by different extents for different varieties. Only β% changed significantly ($p<0.05$) over storage time in variety A89. In NK 2303 and Pioneer 9202, α'%, β%, A_3%, and the $A_{1a}A_{1b}A_2$% showed significant differences over the storage time. In Vinton, α'%, A_3%, and $A_{1a}A_{1b}A_2$% were significantly different during storage. In Latham 650, α'% and β% were significantly difference. LS 201 showed significant difference in β%, A_3 and $A_{1a}A_{1b}A_2$% whereas NK 2026 had relative amounts of glycinin subunits (A_3 and $A_{1a}A_{1b}A_2$) changed significantly. Statistically significant ($p<0.05$) linear correlations between relative amounts of A_3 and storage time were observed only in three varieties, NK 2303, Pioneer 9202 and Vinton. The correlation coefficients are presented in Table 4.6. The results of the different kinetic models study indicated that the decrease in A_3 upon soybean storage followed an apparent zero order mechanism. The results presented in Table 4.6 show that the relative amounts of A_3 in these three varieties decreased

Table 4.6: Significant ($p<0.05$) correlation coefficients between A_3 subunit percentage and storage time

Variety	Pioneer 9202	Vinton 81	NK 2303
r	-0.822	-0.608	-0.732

linearly during storage.

Thomas *et al.*(1989) reported that during 8 months of storage at 20 and 30°C and 65 and 85% relative humidity, the abundance of the acidic or basic subunits of soy proteins did not appear to change as demonstrated by gel electrophoresis. However, their conclusion drawn from qualitative analysis. There are not any data in literature on soy protein subunits changes during bean storage for comparison with our data.

Effect of Soybean Storage on Tofu: Instrumental Color and Texture

Color of tofu made from stored soybeans was evaluated using Hunterlab colorimeter. Tofu made from LS 201, NK 2303, Pioneer 9202, and Vinton 81 beans showed significant changes ($p<0.05$) in color with respect to storage time in Hunter L, a, b values. NK 2026 had significant changes ($p<0.05$) in L and a values. Varieties A8926 and Latham 650, however, had significant change in Hunter a value only. Table 4.7 presents the correlation coefficients between L, a, b values with the storage time. The correlation coefficients suggested that there was a general tendency for a decrease in lightness and an increase in the red attribute in tofu as storage time progressed for most varieties. Additionally, there was stronger linear correlation between Hunter a-value and storage time for all varieties which indicated that the redness (a value) of tofu increased linearly with increase in storage time of beans (Figure 4.4). In this study, tofu color changed from bright yellowish or creamy yellow

Table 4.7: Significant ($p<0.05$) correlation coefficients between Hunter L, a, b values and storage time

Variety	L	a	b
A8926		0.900	
LS 201	-0.359	0.884	-0.403
Latham 650		0.771	
Pioneer 9202	-0.604	0.737	-0.558
Vinton 81	-0.392	0.368	-0.309
NK 2026	-0.361	0.838	
NK 2303	-0.428	0.604	-0.520

at the beginning to slightly brownish to grayish toward the end of the storage period. This color change in most of varieties was similar to the results observed by Narayan *et al.* (1988b) with soynuts. They found as storage time of soybeans increased, the color of soynuts prepared changed from creamish yellow to brown. Saio *et al.* (1982) have reported darkening of soybeans stored at high humidity (85%). Thomas *et al.* (1989) reported that the increase in redness (a value) of soymilk made from beans stored at 65% relative humidity and 30°C was statistically significant.

The changes in color of tofu made from stored soybeans were attributed to the occurrence of enzymatic and non-enzymatic browning reactions during soybean storage period (Friedlander and Navarro, 1972; Narayan *et al.*, 1988). Enzymatic browning is a reaction between oxygen and a phenolic substrate, and non-enzymatic browning involves the phenomena of carmelization by direct heating and the interaction of proteins with reducing sugars (Maillard reactions). The Maillard-type sugar-protein interaction was first suggested as the main process involved in the spontaneous browning of stored soybeans (Milner and Tompson, 1954). The decrease in reducing sugars in stored beans was attributed to their participation in Maillard reactions (Fried-

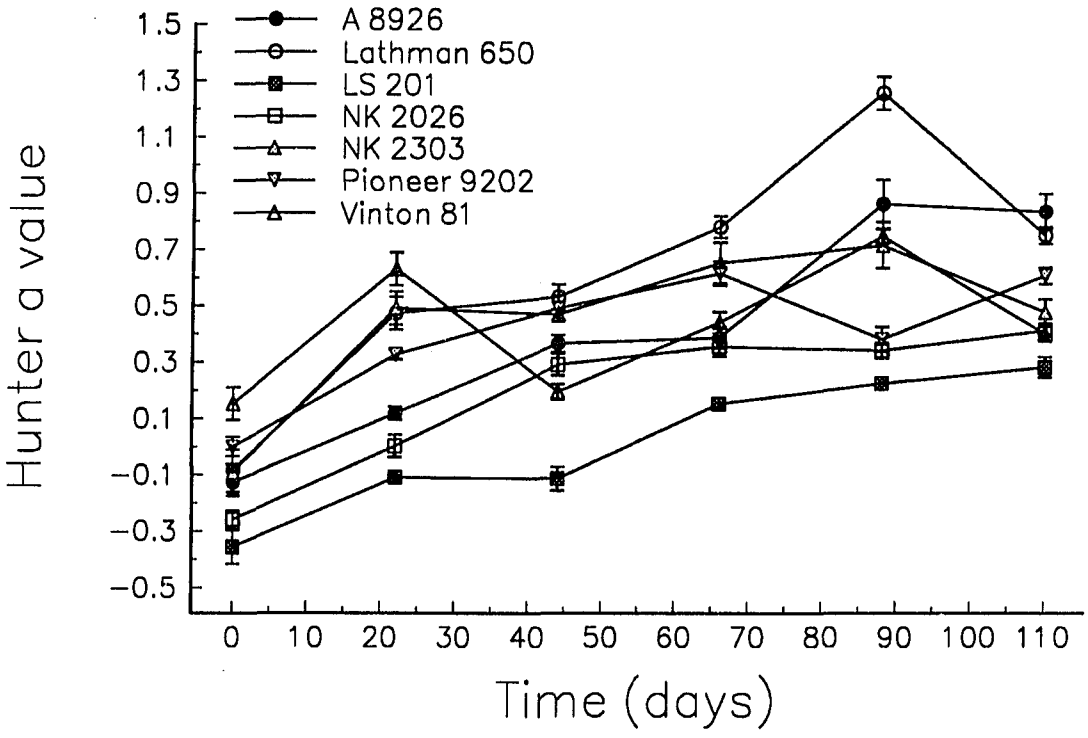

Figure 4.4: The changes in Hunter a value during soybean storage

lander and Navarro, 1972). In addition, Friedlander and Navarro (1972) reported that the browning process, in terms of phenolic acids formation, is a complex one and phenolic acid index could be an indicator long before any visible discoloration appears on the beans.

The texture characteristics, fracturability, hardness, and cohesiveness, of tofu samples were obtained from the texture profile analysis (TPA). According to Bourne (1982), the height of the force peak on the first compression cycle (first bite) was defined as hardness, which was the force necessary to attain a given deformation. Fracturability (originally called brittleness) was defined as the force of the significant break in the TPA curve on the first bite, which was the force with which the material fractures. The ratio of the positive force areas under the first and second compression was defined as cohesiveness, which is the strength of the internal bonds making up the body of the product.

The changes in hardness, fracturability and cohesiveness of tofu with storage time are reported in Figures 4.5-4.7, respectively. Tofu hardness, fracturability, and cohesiveness decreased significantly ($p<0.05$) upon prolonged storage of soybeans for all varieties, except NK 2026. For this variety the changes in the mechanical parameters did not show any statistically significant differences during storage. A negative relationship existed between the hardness, fracturability, and cohesiveness with storage time. The correlation coefficients are reported in in Table 4.8.

The hardness of tofu is very important factor in influence of acceptability of tofu by western consumers. In general, these consumers prefer the tofu with the texture being smooth, not too hard or too soft. The linear reduction in tofu hardness, fractability, and cohesiveness values suggests the longer the beans were stored, the

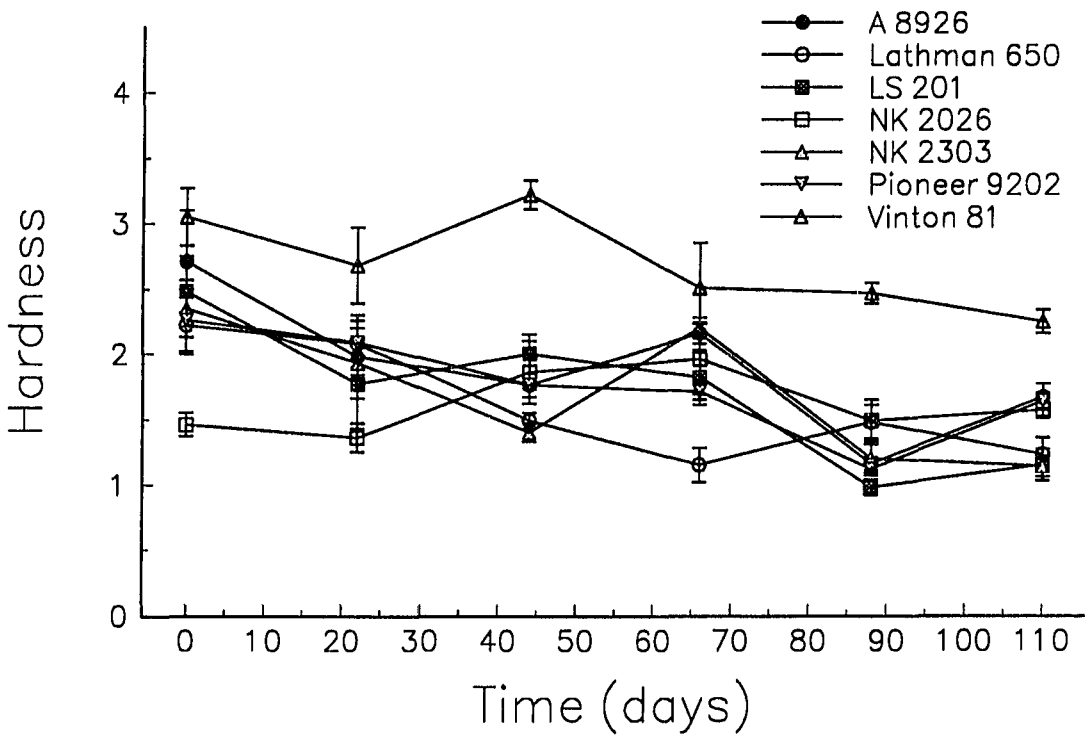

Figure 4.5: The changes in tofu hardness (Kg) during soybean storage

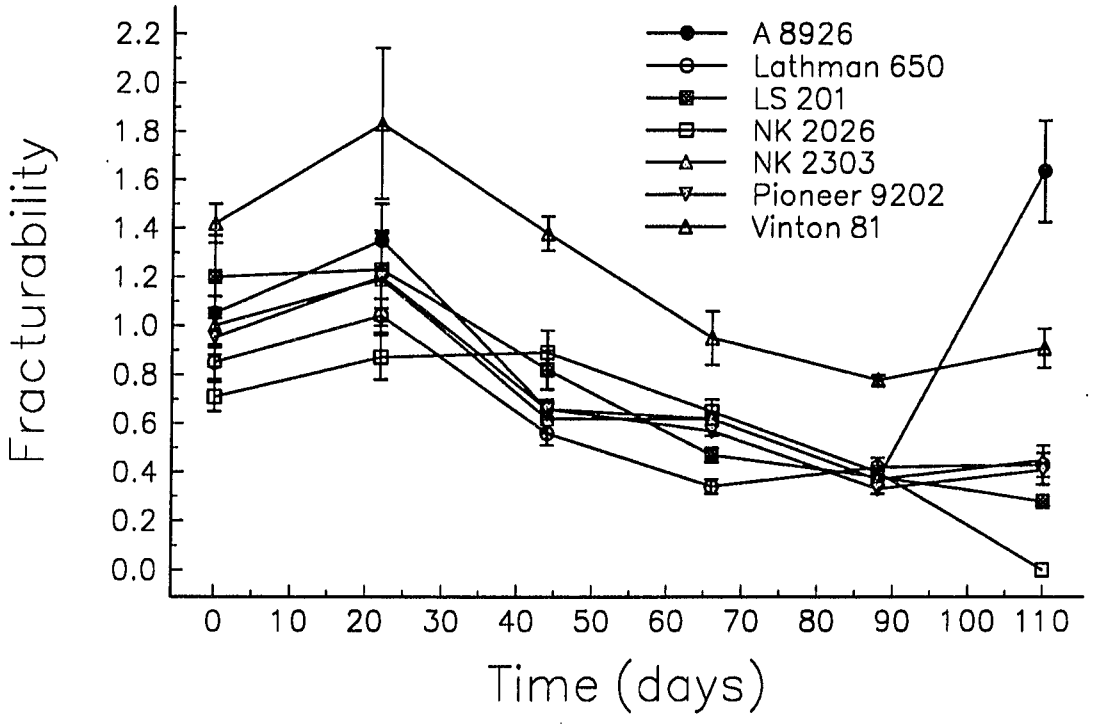

Figure 4.6: The changes in tofu fracturability (Kg) during soybean storage

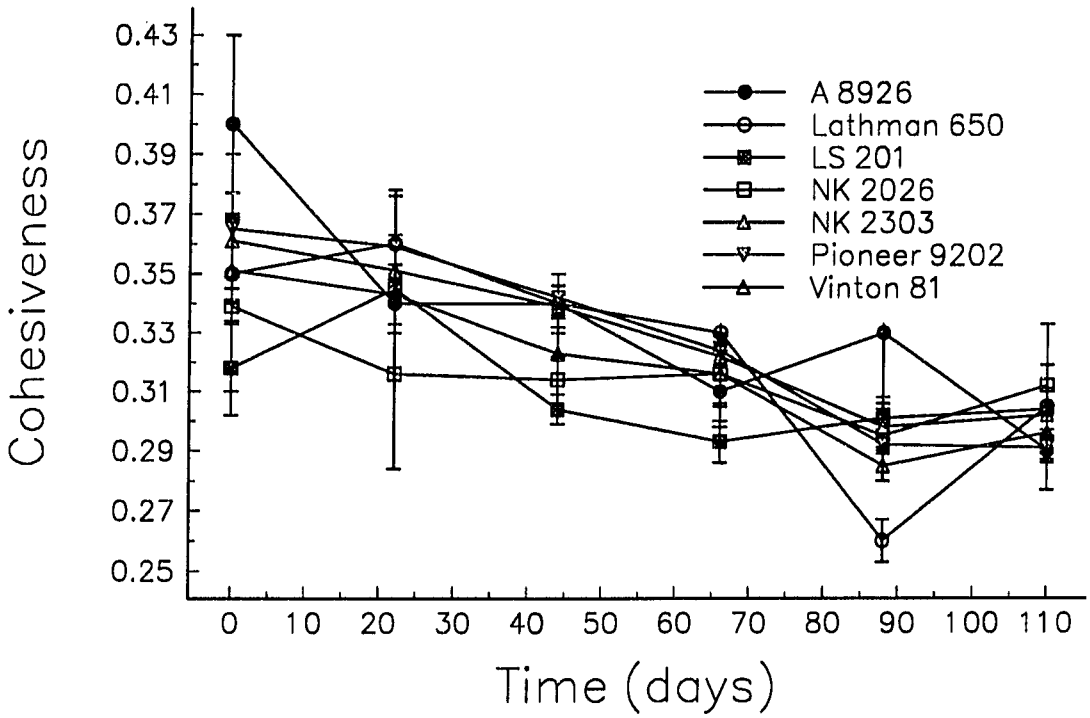

Figure 4.7: The changes in tofu cohesiveness during soybean storage

Table 4.8: Significant ($p<0.05$) correlation coefficients between tofu textural properties and storage time

Variety	Hardness	Fracturability	Cohesiveness
A8926	-0.510	-0.644	-0.505
LS 201	-0.606	-0.785	-0.346
Latham 650	-0.610	-0.702	-0.363
Pioneer 9202	-0.531	-0.649	-0.600
Vinton 81	-0.431	-0.586	-0.554
NK 2026			
NK 2303	-0.557	-0.628	-0.597

softer and moer fragile the tofu were. However, by the end of 88 days storage, the tofus made from most varieties were not acceptable any more. Based on the storage study, the cohesiveness of tofu was another important factor that influenced the acceptability of tofu. Cohesiveness of tofu represents the strength of the tofu internal matrix in holding the protein particles together. As beans underwent aging process, the cohesiveness of tofu made from stored beans decreased. After 88 days storage, the tofus were so fragile and crumbly that they were difficult to pick up without breaking apart.

The extent of network formation in tofu was supposedly responsible for the mechanical properties of tofu. Therefore, any conditions that either affect the formation of the network or affect the extent of network will affect the mechanical properties of tofu. The principle of network formation in tofu has been believed to be as follows: tofu is made by adding calcium sulfate (or other coagulants) to heated soybean milk, causing the soybean milk to be coagulated. This is due to decrease of the negative charge on the soy protein as a result of bonding of calcium ions to the negatively charged acidic amino acid residues of the protein molecules. The unfolded protein

molecules form a gel-like structure, in which the individual protein molecules interact with each other to form a three-dimensional network.

The texture properties of this three-dimensional network have been recognized to be a direct consequence of microstructure determined by chemical composition and physical forces (Furukawa *et al.*, 1979; Furukawa and Ohta, 1982). These authors suggested that the texture properties of a protein gel were governed by the degree of the network formation which was influenced by various factors. Scanning electron microscopy (SEM) images of soft or fragile protein gels revealed that the formation of the porous structure either was not yet adequate or partial collapse of this structure occurred. Since the three-dimensional network is formed by bonding of calcium ions to the negatively charged acidic amino acid residues of the protein molecules, the affinity of the binding sites in soy proteins for calcium ions will play an important role in the network formation. Krall (1984) reported that the affinity of the binding sites in soy proteins for calcium ions was shown to decrease as pH decreased over the pH range of 4-9 (Kroll, 1984). According to Thomas *et al.* (1989) and Saio *et al.* (1980), the pH values of soybean milk made from stored soybeans decrease during soybean storage. This decrease in pH values may result in decrease in affinity of the binding sites in soy proteins for calcium ions and there are less calcium ions binds to soy protein. Therefore, the weaker network structure formed among the proteins themselves may result in softer, more fragile (low cohesiveness value) texture of the tofu.

Another possible cause for the decrease in hardness, fracturability, and cohesiveness of tofu may be the occurrence of protein-lipid interactions in soybean seeds during prolonged storage. The protein-lipid interactions may weaken the soy protein

three-dimensional network by decreasing the probability of protein-protein interactions and inadequate formation of the porous structure. Previous researchers have suggested that the interactions of proteins with lipids have occurred in seeds, particularly in beans stored at high temperature and relative humidity, resulting in major quality changes (Saio *et al.* (1980). The protein-lipid interactions were enhanced by a decrease in the lipid chain length caused by the hydrolysis of lipids to fatty acids during storage (Catsimpoolas and Meyer, 1971). Alternatively, the current study showed soy protein solubility decreased over the storage period (Figure 4.3). This change may be the cause of the decrease in these textural parameters of tofu made from stored beans.

A decrease in hardness of tofus made from stored soybeans was observed by Saio *et al.* (1980). They reported that the decrease in hardness depended mainly on the decrease in concentration of soybean milk solids because of decreased extractability into soybean milk. In our study, all tofus were made from soybean milk which were adjusted to 5% of solids and the amount of adjustment did not changed significantly with time. Therefore, decreases in hardness of this study's tofu could not be due to the concentration of soybean milk solids.

The extent of network formation in tofu was supposedly responsible for the mechanical properties of the protein gel and the water-holding capacity. In many protein gels, an increase in mechanical strength has been assumed to be associated with an increase in water-holding capacity (Furukawa and Ohta, 1982). Therefore, we speculate that the water-holding capacity of proteins extracted from stored beans would decrease as storage time increased. Thomas *et al.* (1989) found that more whey was expelled by tofu curds made from beans stored at 85% relative humidity. This

suggested that the decreased water-holding capacity of the gels due to the decrease in protein content of the soymilk. There was a high negative correlation between the protein content of the milk and the volume of whey expelled. Furthermore, based on the current study, the changes with storage time of hardness, fracturability, and cohesiveness are so similar that one may be able to predict the textural quality of tofu by using only one of the three parameters.

Effect of Soybean Storage on the Yield of Tofu

Figure 4.2 shows the changes in tofu yields over storage period. NK 2026 was the only variety showing significant ($p<0.05$) decrease in tofu yield with storage time ($r=-0.71$). Although there was no statistically significant change in yield% for most of the varieties, there was a definite tendency of a decrease in tofu yield as function of soybean storage time (Figure 4.2). Since the same tofu processing conditions were applied for all varieties, the loss of tofu yield may be due to the decrease in water-holding capacity of protein in stored beans.

Effect of Soybean Varieties: Tofu Texture Characteristics and Physical-Chemical Properties of Soybeans During Storage

Correlation analyses were carried out between soybean physical-chemical properties, NSI, amount of soy proteins and composition with tofu texture characteristics hardness, fracturability, and cohesiveness in order to determine the important factors contributing to the textural properties of tofu. The statistical analysis of the results indicated that variety played a very significant role in the correlation analysis.

Figure 4.3 presents the NSI values which decreased over the storage time. The

rate of decline of NSI was significantly correlated ($p<0.05$) with the decrease in fracturability ($r=0.947$) and cohesiveness ($r=0.853$) of tofus made from stored Vinton beans. The NSI value significantly correlated with cohesiveness ($r=0.812$) of tofus made from stored Pioneer 9202 beans. Therefore, decrease in protein solubility during storage means the tofu will have low fracturability and be less cohesive. However, this conclusion only applied for certain varieties. For the other five varieties, there was no significant linear correlation between NSI and the measured textural characteristics of tofu.

When the correlation analysis between soy protein subunits and the tofu texture properties in the storage study was conducted, the varietal effect appeared very clearly. Each variety yielded different correlations. Table 4.9 summarizes the linear correlation coefficients for different relationships among protein subunits and textural characteristics. NK 2026 was the only variety which did not show any significant correlation between the amounts of soy protein subunits and instrumental texture characteristics of tofu. This was apparently in agreement with the observation that the hardness of the tofu from NK 2026 over the storage time did not change much. Although soy protein subunits were correlated to textural characteristics of tofu made from stored beans, the correlations varied from variety to variety.

The relative amounts of α and α' correlated hardness in A89 and fracturability in LS 201 tofus. The β subunit related to hardness of tofu made from stored Pioneer 9202. The correlations between β-conglycinin subunits (α, α' and β) and textural properties of protein gel or soy foods have not been reported in the literature previously and the correlations apparently depended on soybean variety. Table 4.9 showed that relative amount of A_3 was positively correlated to the hardness, fracturability

Table 4.9: Significant ($p<0.05$) correlation coefficients between tofu textural properties and soy protein subunits during storage

Variety	Relationship	r
A8926	α/hardness	-0.507
LS 201	α'/fracturability	-0.560
	$A_{1a}A_{1b}A_2$/fracturability	-0.669
Latham 650	β/cohesiveness	-0.517
Pioneer 9202	β/hardness	-0.631
	A_3/hardness	0.808
	A_3/fracturability	0.879
	A_3/cohesiveness	0.736
Vinton 81	$A_{1a}A_{1b}A_2$/fracturability	-0.613
	$A_{1a}A_{1b}A_2$/cohesiveness	-0.521
NK 2026		
NK 2303	A_3/hardness	0.709

and cohesiveness of tofu made from stored Pioneer 9202 and to the cohesiveness of NK 2303 tofus. This finding was in agreement with the observation reported by Nakamura *et al.* (1984). According to Nakamura *et al.* (1984), the hardness of the soybean glycinin gels was different among cultivars depending on the percentage of A_3, the largest acidic subunit of glycinin with higher percentages of A_3, yielding harder gels. However, these results were based on glycinin gel model systems and may be true for the varieties Pioneer 9202 and NK2303 (Table 4.9), but the same conclusion could not be drawn for LS 201, Latham 650, Vinton, and A89. Therefore, it appears that the results obtained from an isolated soy protein system could not accurately predict soy protein behavior in the real food system. Additionally, this particular relationship did not hold in any of the fresh beans.

There was no significant correlation between total protein by biuret method and the texture characteristics of tofu determined by rocket electrophoresis for any of the

seven varieties. The fracturability of tofus made from stored A89 and LS 201 beans significantly correlated ($p<0.05$) with their relative amount of β-conglycinin, r=0.840 in A89, 0.808 in LS 201, and the glycinin/β-conglycinin ratio (R), r=-0.901 in A89, and -0.930 in LS 201. The R value significantly correlated with hardness of tofu from stored LS 201 beans. The relative amount of glycinin correlated with cohesiveness of tofu from NK 2026 (r=0.883).

The glycinin/β-conglycinin ratio (R) was another factor which may predict the texture of the gels. Kang *et al.* (1991) reported that the ratio affected the mechanical properties of acid precipitated soy protein (APP) gels. Their results indicated that the gels with smaller R values were more fracturable and more elastic. In our storage study, the R values were significantly correlated with the fracturability of tofu made from A89 and LS 201 only. Thus, Kang *et al.* (1991) conclusion regarding the relationship between R value and fracturability of protein gels held true for certain varieties only. In addition, Kang *et al.* (1991) reported that the higher the R, the greater the gel hardness value when the heating temperature was above 93°C. However, in our study the change in this ratio did not correlate with the hardness of tofu for most varieties except LS 201. For LS 201, the R value was negatively correlated with hardness of tofu, in contrast with the Kang *et al.* (1991) observation in the APP system where the R value was positively correlated with hardness of gels. Thus, the conclusions drawn from the model soy protein system could not predict what would happen in a real food system.

In conclusion, the soy protein subunits appear to have certain relationships with the textural characteristics of tofu. The relationship depends on soybean variety and soybean storage conditions. However, Wang and Damodaran (1990) pointed out

the hardness or gel strength of typical globular protein gels is fundamentally related to the size and shape of the polypeptides in the gel network rather than to their chemical nature such as the amino acid composition and distribution. However, this hypothesis needs to be tested in the complex food system.

Tofu Sensory Analysis

The results of correlation analysis of sensory evaluation scores were showed in Table 4.10. The tofu color score and texture and flavor preference scores given by panelists showed statistically significant ($p<0.05$) changes with storage time for most varieties. The changes in flavor and texture of tofu over soybean storage time were detected by most of the panelists but the evaluation scores were not large enough to show any statistical significantly differences except the texture change in LS 201. The sensory evaluation results are shown in Tables A.9-A.13 in the Appendix. As soybean storage time increased, the tofu prepared from stored beans became softer in texture and grayish in color. For all varieties the off-flavors which described by panelists as bitter, painty aftertaste, oxidized flavor, and astringent taste were developed as beans storage time increased. Lipid degradation, by both enzymatic and autoxidative means, has been associated with the development of off-flavors in numerous food products. The off-flavors associated with soy products are directly attributable to oxidation of polyunsaturated lipids by lipoxygenase action. Lipases play an important role in producing bitterness in stored soybeans (Cheleev, 1960). Overall sensory quality of tofu made from all varieties of soybeans was found to decrease with increase in storage time of beans. These finding were consistent with the results observed by Narayan *et al.* (1988b) with soymilk, tofu, and soynuts.

Table 4.10: Significant ($p<0.05$) correlation coefficients between tofu sensory evaluation scores and storage time

Variety	Color	Flavor	Texture	Flavor Preference	Texture Preference
A8926	0.591			-0.408	-0.392
LS 201	0.414		-0.215		-0.189
Latham 650	0.565			-0.343	-0.393
Pioneer 9202	0.797			-0.470	-0.294
Vinton 81	0.317			-0.294	-0.229
NK 2026	0.511			-0.271	-0.252
NK 2303					

Correlation Between Instrumental Analysis and Sensory Evaluation of Tofu

When instrumental analysis results, hardness and fracturability of tofu and L, a, b values of tofu were compared with sensory analysis results in terms of texture, flavor, and color (Table 4.11), a few correlations occurred for certain varieties. Sensory hardness correlated ($p<0.05$) with Instron hardness for A8926 tofu, cohesiveness for Pioneer 9202 and Vinton 81 tofus. For most varieties, except NK 2303, sensory evaluation color score significantly correlated ($p<0.05$) with Hunter a value. The strong correlation with color was due to the fact that the judgment of color, unlike flavor and texture, was not influenced by panelist's nationality, race and frequency of tofu consumption. It has been suggested that the frequency of tofu consumption is one of the factors which influence the flavor and texture scores in tofu sensory evaluation (Smith and Wilson, 1993). However, there were only a few statistically significant correlations, hardness of A8926 tofu, fracturability of Pioneer 9202 and Vinton 81 tofu, between the instrumental texture and sensory analysis results. The

Table 4.11: Significant ($p<0.05$) correlation coefficients between instrumental textural and color characteristics and sensory analysis results

Variety	Hardness	Fracturability	Cohesiveness	L	a	b
A8926	0.536				0.742	
LS 201					0.692	
Latham 650					0.514	
Pioneer 9202			-0.498	-0.615	0.707	
Vinton 81			-0.470		0.437	
NK 2026					0.602	
NK 2303						

lack of correlation of textural characteristics may be because the sensory analysis scores were not large enough to show any statistically significant differences whereas the instrumental textural properties changed significantly over storage time.

CHAPTER 5. CONCLUSIONS

Seven soybean varieties were different in their proximate chemical compositions, total protein, lipid, and moisture contents. They have different soy protein subunit compositions. Glycinin subunits are significantly different among these seven varieties. The textural characteristics of tofu made from these varieties showed significant differences. The soy protein subunits and distribution were correlated with texture properties of tofu among regular tofu beans. There was no correlation between soy protein subunits and texture properties in oil-use beans.

High temperature and high humidity storage condition affected the soybean quality to a different extent depending on variety. In general, NSI decreased linearly over the storage periods for most of varieties indicating that the extractability of water-soluble proteins decreased with prolonged storage. The moisture contents decreased slightly as storage time increased. Total extractable lipids decreased linearly with storage time. Total extractable protein of soybeans apparently did not change over the storage time regardless of variety. However,the relative amount of glycinin decreased linearly with time for NK 2026, Pioneer 9202 and Vinton 81. The relative amount of β-conglycinin significantly ($p<0.05$) decreased with time for A89 and LS 201. There were significant correlations between glycinin/β-conglycinin ratio and storage time for A89 LS 201. The relative amounts and distribution of subunits ex-

tracted in soybean seed changed with storage time. Vinton and LS 201 were the only two varieties with the A_4 subunit. The A_4 subunit band disappeared after 22 days storage. Statistically significant changes occurred to different subunits depending on variety. Only β% changed statistically significantly over storage time in variety A89. In NK 2303 and Pioneer 9202, α'%, β%, A_3%, and $A_{1a}A_{1b}A_2$% show significant differences over the storage time. In Vinton, α'%, A_3%,and $A_{1a}A_{1b}A_2$% were significantly different during bean storage. In Latham 650, α'% and β% were significantly difference. LS 201 showed significant difference in β%, A_3 and $A_{1a}A_{1b}A_2$% whereas NK 2026 had significant differences in the relative amounts of glycinin subunits (A_3% and $A_{1a}A_{1b}A_2$%). However, statistically significant ($p<0.05$) linear correlations between relative amounts of A_3 subunits and storage time were observed only in three varieties, NK 2303, Pioneer 9202 and Vinton. The texture characteristics, hardness, fracturability, and cohesiveness, were significantly decreased as storage time increased for all varieties except variety NK 2026. The quality of NK 2026 was not affected as much as other varieties under the storage conditions used in this study. Among Hunter L, a, and b values, only the a value showed a linearly significant change as store time increase for all seven varieties.

Correlation analysis between physical-chemical properties of soybean and the texture characteristics of tofu indicated that different kinds of correlations occurred during soybean storage depending on variety. The NSI value for Vinton was significantly correlated with fracturability and cohesiveness of tofu whereas the NSI value was only significantly correlated with cohesiveness of tofu in Pioneer 9202 beans. There was not any statistically significant correlation between NSI and texture properties of tofu for other varieties. The observation, by previous researchers, that A_3

and glycinin/β-conglycinin ratio correlated with the mechanical properties of soy protein gels apparently did not apply to our results. In addition, relative amounts of β, α', α, and $A_{1a}A_{1b}A_2$ apparently correlated well with some of texture properties of tofu depending on variety. Therefore, the real food system was much more complex than isolated soy protein systems and the conclusions drawn from soy protein model systems should be carefully evaluated.

The results of correlation analysis of sensory analysis scores showed that the tofu color score, and texture, and flavor preference scores given by panelists were significantly ($p<0.05$) changed with storage time for most varieties. The changes in flavor and texture of tofu over soybean storage time were detected by most of the panelists but the evaluation scores did not large enough to show any statistically significant difference. When instrumental analysis results, (hardness and fracturability of tofu and L, a, b values) were compared with sensory analysis results in terms of texture, flavor, and color (Table 4.11), a few correlations occurred for certain varieties. Sensory hardness correlated ($p<0.05$) with Instron hardness for A8926, cohesiveness for Pioneer 9202 and Vinton 81. For most varieties, except NK 2303, sensory color score significantly correlated ($p<0.05$) with Hunter a value.

In conclusion, the composition and distribution of soy glycinin and β-conglycinin and their subunits do have certain relationships with the textural characteristics of tofu. The relationship depends on soybean variety and storage conditions. The variety has significant effect on hardness, fracturability and cohesiveness of tofu. The storage conditions used in this study caused significant decrease in the quality of soybean and significant changes in the textural characteristics and appearance of tofu.

There are many possible causes for the decrease in soy protein solubility during storage of beans. Any single hypothesis may not be able to explain all the observations made during storage. Based on this study, the variety had a significant effect on the textural characteristics of tofu. Since genetic factors seems play a very important role in the textural properties of soy products, more work needs to be done in this area. Although many researchers have reported that the soy protein composition has an effect on the textural properties of isolated soy protein gels, the recent interesting hypothesis (Wang and Damodaran, 1990) suggested that the hardness or gel strength of typical globular protein gels were related to the size and shape of the polypeptides in the gel network rather than to their chemical nature such as the amino acid composition and distribution. This hypothesis needs to be tested in a complex food system. In addition, better sensory evaluation techniques for tofu need to be developed in order to obtain more accurate and meaningful results.

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APPENDIX

Table A.1: Changes in total buffer extractable protein during storage [a] [b]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	16.7±1.3	14.3±0.3	18.4±1.2	17.6±0.2	17.8±1.0	16.0±0.6
LS 201	15.8±0.9	14.7±0.2	18.7±0.7	16.9±1.1	17.6±0.7	15.5±0.8
Latham 659	18.5±0.9	13.5±0.2	18.6±0.7	16.1±0.8	17.9±0.4	14.6±0.8
Pioneer 9202	15.8±0.8	13.1±0.4	17.3±0.8	17.9±1.1	16.8±0.1	13.7±0.9
Vinton 81	14.2±1.0	15.8±0.8	20.2±0.6	20.7±1.2	21.0±0.3	17.1±0.4
NK 2026	16.0±0.9	14.6±0.7	17.9±1.1	15.6±0.1	18.3±1.0	14.2±1.2
NK 2303	16.8±1.4	14.2±0.6	19.6±0.9	19.0±1.0	19.6±2.0	15.8±0.7

[a]Mean±SEM is based on three replications.

[b]Total buffer-extractable protein is determined by biuret method.

Table A.2: Changes in extractable glycinin during storage [a] [b]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	39.9±2.1	43.4±2.1	42.5±0.6	39.3±0.7	40.7±1.5	38.7±1.1
LS 201	34.7±1.9	39.9±3.4	35.4±2.5	37.9±2.5	35.4±1.2	39.9±1.0
Latham 659	46.0±1.4	46.8±2.0	38.3±1.3	37.3±1.2	41.3±2.1	34.7±1.9
Pioneer 9202	38.4±1.4	44.8±2.1	31.4±2.3	32.7±2.0	37.1±0.5	30.2±0.6
Vinton 81	53.1±1.6	52.8±5.7	40.3±2.1	34.0±1.7	35.7±0.4	43.7±0.2
NK 2026	50.2±2.2	41.1±1.2	38.2±0.6	44.0±0.8	37.1±0.2	37.9±3.4
NK 2303	34.4±0.8	40.2±0.8	35.0±2.4	28.2±1.5	38.9±1.8	40.5±0.7

[a]Mean±SEM is based on three replications.

[b]Glycinin is determined by rocket immunoelectrophoresis.

Table A.3: Changes in extractable β-conglycinin during storage [a] [b]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	21.6±0.5	23.6±1.0	20.7±0.1	16.5±1.2	14.0±0.2	20.2±2.0
LS 201	24.9±1.0	23.4±1.2	18.8±0.1	18.4±2.2	16.5±0.7	20.3±3.1
Latham 659	25.6±2.3	26.0±2.2	19.7±1.4	14.1±2.1	15.3±0.9	28.4±0.1
Pioneer 9202	26.3±0.8	19.1±0.6	16.1±0.9	14.0±2.9	15.1±0.7	23.5±7.2
Vinton 81	25.1±0.5	22.7±0.1	15.9±1.4	15.3±1.7	15.6±0.8	25.6±0.1
NK 2026	27.0±1.8	25.6±2.1	17.4±1.1	15.4±2.8	15.4±0.1	25.4±0.3
NK 2303	25.8±1.9	20.6±1.4	18.2±2.1	17.3±3.6	17.4±1.1	33.5±1.7

[a] Mean±SEM is based on three replications.

[b] β-Conclycinin is determined by rocket immunoelectrophoresis.

Table A.4: Changes in extractable α' subunit during storage [a] [b]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	34.0±5.1	32.5±1.3	29.9±0.2	33.6±3.3	30.4±1.1	37.1±2.0
LS 201	29.3±3.0	28.9±0.5	34.6±0.4	37.6±1.0	29.8±2.0	33.9±1.8
Latham 659	32.9±3.4	32.5±0.8	37.6±0.4	42.1±5.0	28.9±0.9	36.7±1.4
Pioneer 9202	34.9±0.6	41.4±1.7	36.3±0.8	32.2±0.8	33.7±1.8	37.4±1.1
Vinton 81	32.7±2.8	37.9±0.8	34.0±1.5	43.1±2.1	32.0±0.7	35.0±1.2
NK 2026	37.1±3.4	37.1±0.9	38.5±2.3	35.1±1.0	38.0±1.7	41.0±1.0
NK 2303	31.7±1.2	31.4±0.6	33.5±1.1	29.4±0.0	28.9±0.4	31.7±0.8

[a] Mean±SEM is based on three replications.

[b] Extractable α' subunit is determined by image analysis of urea SDS-PAGE gels.

Table A.5: Changes in extractable α subunit during storage [a] [b]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	37.2±3.6	40.8±2.2	40.7±0.6	40.2±0.6	40.8±0.5	41.5±1.2
LS 201	36.9±1.8	36.6±0.9	41.2±0.3	36.7±0.8	33.5±3.1	38.2±1.2
Latham 659	41.0±0.7	40.9±0.5	41.7±0.6	42.0±5.0	38.1±1.1	41.1±1.2
Pioneer 9202	42.5±1.4	40.1±1.1	42.2±1.1	41.9±0.2	39.6±0.5	43.2±0.5
Vinton 81	40.1±0.5	37.9±2.4	39.7±1.1	39.0±0.5	38.5±1.2	41.2±0.7
NK 2026	38.9±2.0	40.3±0.9	37.8±1.2	39.2±1.5	38.0±1.2	37.8±1.1
NK 2303	43.6±2.4	39.4±0.8	44.1±1.7	46.6±0.4	42.0±0.7	45.8±0.6

[a] Mean±SEM is based on three replications.

[b] Extractable α subunit is determined by image analysis of urea SDS-PAGE gels.

Table A.6: Changes in extractable β subunit during storage [a] [b]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	28.8±1.5	26.7±1.0	29.3±0.8	27.6±1.6	29.5±1.1	21.3±0.1
LS 201	29.7±4.3	34.6±0.4	13.6±9.9	25.4±1.1	36.6±1.0	27.8±1.9
Latham 659	24.3±3.0	26.6±0.9	20.8±1.7	29.4±3.5	32.9±1.3	22.2±2.1
Pioneer 9202	22.6±0.8	18.3±0.4	21.5±1.9	25.9±1.0	28.1±1.6	19.3±0.9
Vinton 81	27.2±3.3	24.2±3.3	26.3±2.7	17.9±1.6	29.5±1.9	23.7±1.9
NK 2026	24.0±1.4	25.1±1.3	23.7±1.2	25.7±1.8	24.0±0.5	24.3±3.1
NK 2303	24.7±1.6	28.5±1.0	22.7±2.0	24.0±0.4	29.0±1.0	22.5±0.5

[a] Mean±SEM is based on three replications.

[b] Extractable β subunit is determined by image analysis of urea SDS-PAGE gels.

Table A.7: Changes in extractable A_3 subunit during storage [a] [b]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	17.9±0.1	18.3±0.3	17.5±0.5	13.4±0.4	17.8±2.0	15.5±1.1
LS 201	21.3±0.8	25.1±3.1	27.2±2.7	16.0±1.6	22.5±1.9	19.9±1.1
Latham 659	21.3±0.1	19.1±3.5	20.0±0.6	22.7±3.0	18.1±2.0	15.2±0.7
Pioneer 9202	20.5±2.0	22.2±2.2	14.9±0.8	16.2±1.5	12.3±1.3	12.4±0.3
Vinton 81	21.0±0.7	20.9±0.4	21.0±1.6	19.8±1.5	10.5±1.9	17.8±1.9
NK 2026	15.6±1.0	28.4±1.4	17.6±2.4	15.8±0.2	14.2±0.3	18.0±1.6
NK 2303	23.8±2.5	27.4±0.1	22.0±1.1	24.8±0.3	14.7±0.7	15.1±0.2

[a] Mean±SEM is based on three replications.

[b] Extractable A_3 subunit is determined by image analysis of urea SDS-PAGE gels.

Table A.8: Changes in extractable $A_{1a}A_{1b}A_2$ subunit during storage [a] [b]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	82.1±0.1	81.7±0.3	82.5±0.5	86.6±0.4	82.2±2.0	84.5±1.1
LS 201	69.1±1.1	74.9±3.1	72.8±2.7	84.0±1.6	77.5±1.9	80.1±1.0
Latham 659	78.6±0.1	80.9±3.5	80.0±0.6	77.3±3.0	81.9±2.1	84.8±0.6
Pioneer 9202	79.5±2.0	77.8±2.2	85.1±0.8	83.8±1.5	87.7±1.3	87.6±0.3
Vinton 81	74.0±3.3	79.0±0.4	79.0±1.6	80.2±1.6	84.5±1.9	82.2±1.9
NK 2026	84.3±1.0	71.6±1.4	82.3±2.4	84.1±0.2	85.8±0.3	82.0±1.7
NK 2303	76.2±2.5	72.5±0.1	78.0±1.1	75.2±0.3	85.3±0.7	84.9±0.2

[a] Mean±SEM is based on three replications.

[b] Extractable $A_{1a}A_{1b}A_2$ subunit is determined by image analysis of urea SDS-PAGE gels.

Table A.9: Changes in tofu color during storage [a]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	7.7	6.1	8.0	5.3	9.9	8.7
LS 201	4.4	5.0	4.4	3.9	8.5	7.6
Latham 650	5.5	5.6	4.6	5.0	10.1	8.9
Pioneer 9202	3.1	4.8	7.1	6.3	9.4	9.6
Vinton 81	5.8	5.7	6.9	6.0	7.8	6.6
NK 2026	3.4	5.4	5.9	6.2	9.3	8.7
NK 2303	7.8	8.3	5.3	7.6	8.9	6.5

[a]Data unit is centimeter on line scale rating (see sensory evaluation sheet Figure 3.1).

Table A.10: Changes in tofu sensory beany flavor during storage [a]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	5.8	5.6	5.9	5.4	6.8	7.7
LS 201	5.7	5.4	6.7	5.6	8.2	7.6
Latham 650	5.3	7.0	4.8	6.0	7.4	8.2
Pioneer 9202	6.7	6.8	5.5	7.8	8.5	8.3
Vinton 81	6.1	6.0	6.3	5.6	6.9	6.1
NK 2026	6.1	6.3	6.5	6.2	9.3	7.4
NK 2303	6.6	5.7	5.7	5.7	7.4	6.9

[a]Data unit is centimeter on line scale rating (see sensory evaluation sheet Figure 3.1).

Table A.11: Changes in tofu sensory texture during storage [a]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	7.1	6.3	4.0	6.0	3.2	5.7
LS 201	7.0	7.4	4.6	4.7	4.5	4.0
Latham 650	7.2	6.0	3.6	1.8	3.3	4.8
Pioneer 9202	5.8	7.5	5.9	4.4	6.2	4.7
Vinton 81	6.5	8.6	8.6	5.7	7.2	8.7
NK 2026	3.6	5.0	8.3	4.5	5.2	4.6
NK 2303	6.0	6.9	4.2	6.8	3.1	5.0

[a]Data unit is centimeter on line scale rating (see sensory evaluation sheet Figure 3.1).

Table A.12: Changes in tofu sensory flavor preference during storage [a]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	5.8	5.6	4.3	5.6	3.9	4.6
LS 201	5.2	6.6	6.3	6.6	4.3	4.0
Latham 650	5.8	5.8	5.5	3.0	3.2	4.1
Pioneer 9202	5.8	6.1	6.2	5.0	4.4	4.2
Vinton 81	8.0	6.2	6.8	5.7	4.9	6.2
NK 2026	6.0	6.4	6.5	5.0	4.0	3.7
NK 2303	7.0	5.9	6.0	5.7	3.1	5.0

[a]Data unit is centimeter on line scale rating (see sensory evaluation sheet Figure 3.1).

Table A.13: Changes in tofu sensory texture preference during storage [a]

Variety	0 Day	22 Days	44 Days	66 Days	88 Days	110 Days
A8926	6.3	6.5	4.9	6.8	2.8	4.8
LS 201	16.8	6.1	6.1	5.4	4.1	4.1
Latham 650	6.1	6.2	3.8	2.0	2.7	3.3
Pioneer 9202	6.1	7.8	5.5	5.3	6.0	4.1
Vinton 81	7.1	6.5	7.0	5.6	5.6	6.9
NK 2026	6.1	6.0	7.8	5.0	3.8	3.8
NK 2303	6.4	6.4	4.1	6.4	2.9	5.1

[a]Data unit is centimeter on line scale rating (see sensory evaluation sheet Figure 3.1).