Influence of soybean variety and method of processing on tofu manufacturing, quality and consumer acceptability

by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

INTRODUCTION

The majority of Americans have lived an easy life. Many of us have never experienced hunger, except maybe an occasionally grumbling stomach. Our tables are blessed with an abundance of food, especially protein. Consequently, the United States and other developed countries set the standard on which the rest of the world measures economic success. There are undesirable repercussions caused by this. In a selfish attempt to attain western-like success, government leaders of many third-world countries isolate themselves from their people and greedily turn their countries agricultural endeavors away from filling hungry stomachs to filling their own pocket-books so that they may live in the grand western style. As a result, many people go hungry each year, especially children. It is estimated that twenty percent of the deaths in the world are caused by hunger. The seemingly endless supply of pictures of starving children disturbs many of us, especially when we compare our life-style to theirs. To ease our guilt, we are quick to point out that the United States has acted as a philanthropic provider to these starving people for years by exporting our surplus grain under various aid programs. With this in mind, many Americans have felt that they can help in their own small way by adopting a vegetarian diet. The rationale behind this is based on the fact that meat is an inefficient converter of grain to protein. It takes approximately 14 to 21 pounds of feed, such as soybeans or grain, to yield 1 pound of meat from a feed-lot steer. So the common belief has been that if we cut down on our meat consumption, we would be able to export more grain. Unfortunately, the United States aid to starving people is based not only on need, but also on the political nature of the country in question and on the economic health of the United States. Therefore in reality, the United States uses its surplus food as a tool to further United States interests. Also, numerous studies have shown that the exporting of grain to third-world countries has actually done more harm than good. Third-World countries have become over-dependent on United States aid, and in response have neglected their own agricultural welfare. As a result, developing nations are extremely vulnerable to world economic fluctuations and the agricultural productivity of grain-exporting countries.

What then is the solution to the problem of world hunger? Sterling Wortman (1976), a former vice-president of the Rockerfeller Foundation, states that, "The increased production of basic food crops on all farms everywhere has at last been accepted as the primary solution to the world food problem." How does the United States fit into this overall solution? In Chapter 1 of "World Food and Nutrition Study" prepared by the National Research Council (1977), it was concluded that the United States could contribute by "...helping to build research capabilities in the third-world countries and by orienting U.S. research activities more towards global concerns about hunger and malnutrition."

The United States is the leading producer of soybeans, contributing more than 65 percent of the total world supply. Ironically, this protein-rich crop has been neglected by the United States as a source of dietary protein for people. Americans must look into the future and realize that there may come a day when they will have to depend on other types of food as a source of protein. It is to everyone's advantage that Americans start to take soybeans seriously as a protein source. By promoting the use of soybeans, we will not only help ourselves, we will set an example for others to follow.

Soybeans have a long history as a food source in the Orient, where they have been and still are a chief source of protein, calories, and essential fatty acids. Soybeans, however, contain several undesirable characteristics such as off-flavors, extended cooking requirements (4-5 hours) and flatulent factors. Orientals have overcome these characteristics by altering the soybeans through fermentation, or by breaking the soybeans down to isolate the fractions of the soybean most suitable for human consumption. As a result, Orientals have produced a variety of soy-products that meet their food preferences. Unfortunately, Oriental food preferences may differ from American preferences, and as a result, the majority of the Oriental soy-based foods are unacceptable to Americans. However, one Oriental soyfood called tofu has caught on in the United States in the last seven years. Tofu is a bean curd made from the water-soluble proteins of the soybean. It is relatively bland, easy to prepare and can be used in many traditional dishes. It was thought that tofu could become a popular food in the United States, however, its growth in popularity has not been as rapid as was once anticipated. One reason for this may be the fact that tofu is a traditional Oriental food and Americans have not become accustomed to its texture and flavor. A second reason is that the soybeans used in the production of tofu in the United States have been bred for oil content and are not specifically for use in soyfoods as Oriental beans are. Also, many stores do not handle tofu properly, which results in a product that is often times of inferior quality due to microbial spoilage.

The purpose of this study is two-fold: 1) to determine if soybean variety and the method of processing are important factors in influencing tofu quality and 2) to determine American preferences for this traditional Oriental product.

REVIEW OF LITERATURE

History of Soybeans

Beginnings in the Orient

The soybean has a long history as a food source. In fact, the first recorded usage of soybeans dates back as far as the 11th century B.C. (Hymowitz, 1972). Smith and Circle (1972) attribute the popularity of soybeans as a foodstuff in the Orient to the Buddhist religion, which required its followers to exclude meat from their diet. Buddhist monks have been credited with the discovery of many of the traditional Oriental soybased foods such as tempeh, miso, shoyu, soysauce and tofu.

Utilization of soybeans in the United States

Soybeans have been grown in the United States since the early 1800s, however, their use was limited to forage. In the early 1900s, there was a growing interest in using soybeans as a source of oil, both industrial and food-grade, and as a result, between 1920 and 1940 there was a rapid increase in the number of soybean-oil processing plants. As a result of becoming a cash crop, soybeans became increasingly more attractive to farmers and began to replace other crops such as corn, wheat, tobacco and cotton that had previously been produced in surplus quantities.

Since 1920, there has been a 378-fold increase in soybean production in the U.S., bringing the U.S. contribution to the total world soybean production up to 75 percent (Wolf and Cowan, 1975). Smith and Circle (1972) state that "The growth of the soybean industry in the United States was influenced more by the shortage of oil and its relative high price than by the need for protein."

Industry, nevertheless, took advantage of the large amount of by-product (soybean meal) that resulted from soybean-oil processing. This protein-rich soybean meal, being inexpensive, was quickly utilized as animal feed for cattle swine and poultry. The use of soybean meal as a food-source for cattle allowed American scientists to discover that soybeans have a higher nutritional value when cooked, something that had been known in China for over a dozen centuries.

Industrial uses of soybean protein are quite varied. Soybean protein has been used as an adhesive for plywood glue and as a starting material for producing wool-like textile fibers. One of the largest industrial uses of soybean proteins is in the paper-making industry, where it is used for coating and sizing paper.

Breeding

The production of soybeans as a source of oil initially encouraged American soybean breeders to develop new varieties of soybeans with higher oil contents and better yields. As a result, U.S. varieties of soybeans are generally higher in oil content than Oriental varieties (Smith and Circle, 1972). In recent years, however, with the decrease in vegetable oil prices and the increasing appreciation of the soybean as a source of human dietary protein, soybean breeders have taken an interest in developing high-protein soybean varieties. Although it would be nice if a soybean variety could be bred for both high protein and high oil content, this is not possible because unfortunately, there exists a negative relationship between these two traits. To complicate matters, there are many other traits such as yield, seed-size, disease and lodging resistance which are all inter-related. Fehr (1978) states, however, that yield is the "...most important character in soybean breeding."

Environmental conditions can also have a great effect on soybean yield. For example, soybeans are sensitive to day length, which can affect soybean yield by altering the time of flowering and maturity of the soybean plant (Whigham and Minor, 1978). In order to produce maximum yields, breeders have divided soybean cultivars in North America into 12 maturity groups based on their response to daylight. Besides light, temperature, water, wind and a variety of pests, such as insects, may also affect yield. The effect of adverse environmental conditions on soybean production is of prime concern when considering soybeans as a foodstuff to be grown world-wide.

Nelson (1975), in his conclusions at the World Soybean Research Conference-I, stated that, "Genetics affect almost all phases of soybean growth and production". Therefore, breeders have a major responsibility in determining the successfulness of utilization of soybeans throughout the world. Productive research towards this goal will certainly depend on the success of international cooperative breeding programs.

Current and Future Usage of Soybeans and Soybean Products

Whole soybeans

Currently in this country, soybeans are being utilized both for their oil and protein. Whole soybeans, which are used in countries such as Japan, are exported for processing into meal, oil and to produce traditional Oriental soyfoods.

Soybean oil

Soybean oil was initially used primarily as an industrial oil, but, because of increasing competition from synthetic oil, it is now primarily used for producing edible oils and margarines. Very little is exported (Norman 1978).

Soybean meal

Soybean meal is gradually finding its way into the diets of Americans, although it is still being used primarily as feed for livestock, swine and poultry. The transition of soybean meal from animal feed to a human foodstuff is due mainly to the fact that in recent years the meal has been further processed into soyflour, soy concentrates and soy isolates. These different forms of soy, which are high in protein, have then been utilized in many commercial food products.

Initially, the functional properties of soy (Table 1) were exploited, however, now soy protein is being used to increase the nutritional quality of many foods. With the development of textured vegetable protein (TVP), meat analogues have now been successfully produced and marketed.

Traditional Oriental soyfoods

Besides the growing use of processed soybean meal, there has been a growing interest in producing traditional Oriental soyfoods which do not require any sophisticated technology for production. Low-technology production is one reason that these traditional soy foods are seen as a possible means of introducing soy to the third-world countries. Kellogg and Williams (1976) stated that in soy processing technology, "Governments need to be sensitive to the environments surrounding poorer, relatively isolated people if these people are to have access to soy food products for improving their nutrition". These traditional Oriental soyfoods could benefit both Americans and other people by providing a low-cost source of protein.

Tofu

In the United States, one traditional Oriental soyfood that has steadily increased in popularity is tofu. Tofu is a bean curd made from the water-soluble proteins of the soybean. Consumption of tofu and other low-technology soyfoods for 1981 was 2.13 pounds per capita. The total consumption of all products directly made from soybeans in 1981 was 8.6 pounds per capita (Leviton, 1982) compared to 2 pounds per capita in 1976 (Kinsella, 1976). Leviton (1982) expects to see

Table 1. Functional properties of soybean products in food systems (modified after Wolf, 1970)

Functional property	Protein form used[a]	Food systems
Emulsification formation	F, C, I	Frankfurters, bologna, sausages, breads, soups, whipped toppings, frozen desserts
Stabilization	F, C, I	Frankfurters, bologna, sausages, soups
Fat absorption		
Promotion	F, C, I	Frankfurters, bologna, sausages, meat patties
Prevention	F, I	Doughnuts, pancakes,
Water absorption		
Uptake	F, C	Breads, cakes, macaroni, confections
Retention	F, C	Breads, cakes
Texture		
Viscosity	F, C, I	Soups, grains, chili
Gelatin	I	Simulated ground meats
Chip and chunk formation	F	Simulated meats
Shred formation	F, I	Simulated meats
Fiber formation	I	Simulated meats
Dough formation	F, C, I	Baked goods
Film formation	I	Frankfurters, bologna
Adhesion	C, I	Sausages, lunch meats, meat patties, meat loaves, rolls, boned hams

[a]F, C, I represent flours, concentrates and isolates, respectively.

Table 1 (continued)

Functional property	Protein form used[a]	Food systems
Cohesion	F, I	Baked goods, macaroni, simulated meats
Elasticity	I	Baked goods, simulated meats
Color control		
Bleaching	F	Breads
Browning	F	Breads, pancakes, waffles
Aeration	I	Whipped toppings, chiffon mixes, confections

in the next 5 years:

1) An increase in the use of traditional Oriental soyfoods

2) An increase in the home preparation of these soyfoods.

3) The advent of convenience soyfoods (ready-to-eat entrees, dips, dressings, burgers and desserts).

4) Institutional mass feeding of soyfoods.

5) The marketing of soyfoods by large food corporations.

Although there has been a great deal of optimism expressed for the future of tofu and other soyfoods, their increased usage over the last 7 years has not been as great as was once anticipated. A major reason for this is that the flavor, texture and color of tofu are either unacceptable or unfamiliar to the majority of Americans.

Tofu, being a traditional Oriental soyfood, is made by a process which is centuries old and designed to make a product that specifically satisfies unique Oriental food-quality preferences. Therefore, Oriental food preferences may not necessarily be compatible with American preferences. The processing of soybeans to make tofu, however, involves many inter-related factors that may be manipulated to produce tofu that would suit the preferences of other peoples. Suitable parameters for making tofu that are acceptable to a particular group of consumers could be determined through research.

Justification for carrying out tofu research

Their are several reasons why tofu research would be advantageous both to the well-being of Americans and other peoples throughout the world:

1) The tofu-making process is relatively simple when compared to processes required to produce "high technology" soyfoods such as TVP. Because tofu is easy to prepare using simple equipment, it can easily be prepared in the home or could be produced in isolated third-world communities.

2) Compared to cooking soybeans, which takes 3-5 hours, very little heating is required (10 minutes) in making tofu. This makes tofu ideally suited for countries such as India which have severe fuel shortages.

3) Tofu, if prepared properly, is bland in taste and could be incorporated into domestic foods without affecting flavor.

4) Due to processing, approximately 80 percent of the flatulent-causing polysaccharides found in soybeans are absent in tofu (Shurtleff

and Aoyagi, 1979).

5) The oil in tofu is rich in polyunsaturated fatty-acids including the essential fatty acid linoleic acid (Winarno and Karyadi, 1976).

6) Tofu can be an excellent source of calcium provided that a calcium coagulant is used.

7) The soybean isoflavones which are present in tofu have been shown to have the ability to lower serum cholesterol (Siddiqui and Siddiqui, 1976; Sharma, 1979 a, b).

8) Tofu is an excellent source of high quality protein (Shurtleff and Aoyagi, 1979).

Therefore, tofu would be a valuable addition to the American diet and it has great potential for world-wide use.

Tofu Processing

Traditional tofu-making process

Although there are variations in the tofu-making process, the basic procedure (outlined in Figure 1) has not changed significantly over the years. The beans are soaked for 8-10 hours until they are completely hydrated. Any unabsorbed water remaining after soaking is drained. The hydrated beans are then combined with fresh water and ground. The resulting slurry is then cooked at 100°C for a period of time, usually 7-10 minutes. The cooked slurry is then filtered to remove the water-insoluble residue (okara). The okara is pressed, washed and then pressed again to remove any remaining soymilk. The soymilk is then coagulated either with a divalent salt of calcium or magnesium or with some sort of acid (citric, acetic etc.). To produce the finished tofu, the curds are separated from the whey, poured into a cloth-lined pressing box, pressed for a period of time and then cooled. The production of soymilk is one of the most important phases in tofu production, and for this reason a close look at soymilk and its production is warranted.

History of soymilk utilization

Soymilk is thought to have first been used in China in approximately the second century B.C. (Shurtleff and Aoyagi, 1979) and is still very popular in China and to a lesser extent in Japan. However, because of rising prices for cow's milk, soymilk has increased in popularity in Japan since 1976 (Shurtleff and Aoyagi, 1979).

Soymilk was first produced in the United States by Dr. J.H. Kellogg in 1897. Presently, it is used mainly as a milk substitute for infants who are allergic to cow's milk. Nutritional studies on infants have shown that if properly fortified with vitamins and minerals, soymilk makes an excellent replacement for cow's milk (Liener, 1972).

It has been suggested by Shurtleff and Aoyagi (1979) that the primary reason soymilk has not become popular in the United States is because Americans tend to compare the taste of soymilk with that of cow's milk. The soymilk is then rejected because it has a beany flavor. Shurtleff and Aoyagi (1979) use as an analogy the fact that Orientals compare cow's milk with soymilk and reject the cow's milk for having an "animal" taste.

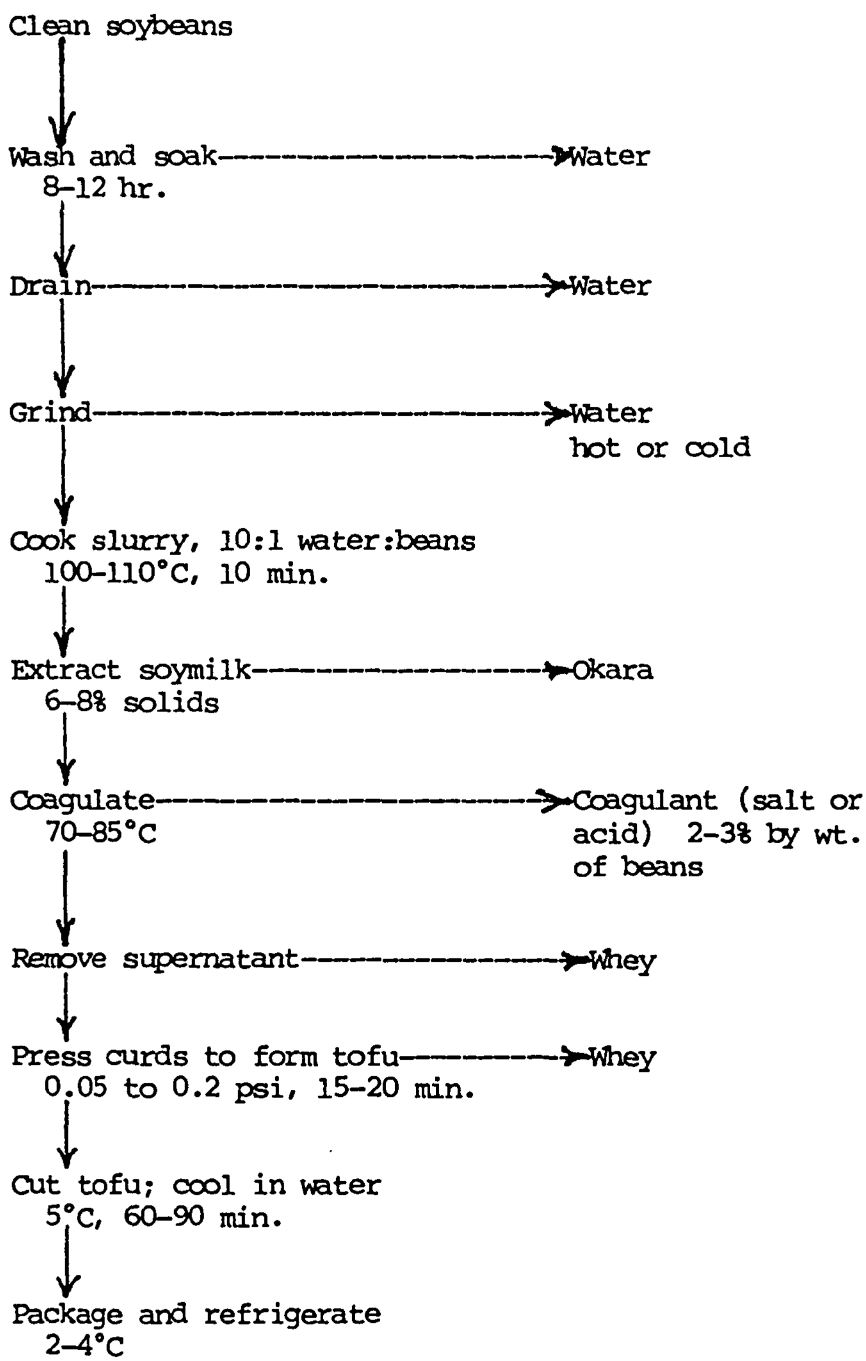


Figure 1. Flow chart for regular tofu production (Modified after Shurtleff and Aoyagi, 1979)

Soymilk processing

Purpose The processing of soybeans to yield soymilk serves two important purposes:

1) To liberate protein, oil, vitamins and minerals for efficient utilization.

2) To maximize the nutritional and flavor quality of the soybeans. A large portion of soymilk research has dealt with the latter purpose.

Effect of processing on soymilk nutritional quality As far back as 1917, American scientists had discovered that the nutritional quality of soy was affected by heat and moisture (Liener, 1972). One cause of this phenomenon was found to be due to a group of seven to ten proteinase inhibitors collectively termed soybean trypsin inhibitors (SBTI) which are active in raw soybeans. Although the exact mechanism by which SBTI prevents proteolysis is unknown, SBTI binds to trypsin to prevent proteolysis. Therefore, SBTI must be inactivated in order to break down soy protein for complete digestion and nutrient utilization.

Numerous studies have been carried out in order to determine the required heat treatment for SBTI inactivation (Liener and Kakadi, 1980), however, the amount of heat required can also lower the nutritive value of the soy protein by destroying certain amino acids. Consequently, studies have been conducted to determine the amount of heat necessary to obtain the optimum nutritional value of the soymilk (Van Buren et al., 1964; Hackler et al., 1965).

Soymilk flavor The characteristic flavors of soy products have probably been the limiting factor in the use of soy in the West. Green, beany, grassy, bitter, astringent and cereally are all terms that have been used to describe the flavor(s) of soy products (Kinsella and Damodaran, 1980).

Wolf (1975) did a comprehensive review of the soy flavor research conducted up to 1975. Wolf (1975) points out that the flavor problems of soy were first dealt with in 1924 by exposing the soybeans to moist heat. The actual cause of these off-flavors, however, was not elucidated until the 1960s. Interest in the poor flavor quality of soymilk was stimulated when Hand et al. (1964) studied traditionally-made soymilks produced on a pilot-plant scale and tested the flavor of these soymilks using a taste panel. It was concluded that the flavor of the soymilk would limit the acceptability. Wilkens et al. (1967) attributed the poor flavor quality of soymilk to volatile compounds which were produced by the catalyzed oxidation of polyunsaturated fats by a group of enzymes called lipoxidases or lipoxygenase.

Hot-grinding   Wilkens et al. (1967) found that to prevent the production of off-flavors due to lipid oxidation, lipoxygenase inactivation had to be accomplished simultaneously with the grinding operation. Irreversible lipoxygenase inactivation could be accomplished by grinding the beans in 100°C-water and then maintaining the slurry temperature above 93°C for 10 minutes.

Other methods have been reported for the inactivation of lipoxygenase in soybeans that do not involve high temperatures for inactivation. Several advantages have been cited for using these methods. These include:

1) The formation of 1-octen-3-ol reported by Badenhop and Wilkens (1969) is prevented.

2) A decreased loss of protein functionality when compared to heat inactivation.

3) The absence of a "cooked" flavor found in heat-treated milk.

Kon et al. (1970), using acidified water (pH 2.0), ground dry soybeans to successfully suppress off-flavor development and produce a bland soymilk by neutralizing the soymilk after heating. Protein extractability at pH 2 is approximately 80-85 percent, which is very close to maximum extractability. Unfortunately, irreversible changes have been noted in the 11S protein fraction in the pH range of two to three (Wolf and Briggs, 1958).

Badenhop and Hackler (1970) reported using a NaOH solution for producing soymilk. Several advantages were cited for soaking the beans in an alkaline solution of 0.05N NaOH:

1) NaOH increased the rate of hydration.

2) The protein content of the soymilk on a dry-weight basis increased from 46.4% to 48.0%.

3) There was a significant improvement in flavor.

The claim that the alkaline soak method gave an improved flavor over the hot-grind method of Wilkens et al. (1967) should be questioned. Badenhop and Hackler (1970) did not carry out the hot-grind method correctly. To completely and irreversibly inactivate the lipoxygenase enzyme, the beans must be ground in 100°C water and the temperature of the resulting slurry must be maintained at or above 93°C for 10 minutes

(Wilkens et al., 1967). Badenhop and Hackler (1970) failed to maintain the slurry temperature above 93°C immediately after grinding. Instead, the slurry was filtered and the milk transferred to cans before continuing to heat. It is unknown what temperature the soymilks used in the experiments were allowed to drop to before the final heating. It is possible that there may have been enough residual lipoxygenase activity to allow detectable levels of off-flavors to be produced. Therefore, it can not be concluded from the work of Badenhop and Hackler (1970) that alkaline soaking yields soymilk with improved flavor over the hot-grind method of Wilkens et al. (1967).

Ethanolic-soaking Borhan and Snyder (1979) found that a combination of ethanolic soaking and heat were effective in inactivating lipoxygenase with a minimum loss of protein functionality. Ethanol concentration, soaking temperature and soaking time could be adjusted to completely inactivate lipoxygenase. The highest protein solubility obtained (NSI=72%) was achieved by soaking the beans in a 15-percent ethanol solution at 40°C for 72 hours. It was also found that increasing the pH of the soaking solution caused an increase in the destruction of lipoxygenase. Although lipoxygenase activity was monitored, no taste panels were conducted to establish the effect of the ethanol soaking on the flavor of the soymilk.

Ashraf and Snyder (1981) did a follow-up study on the ethanolic soaking procedure to determine the effects this method had on soymilk flavor. It was found that soymilk made from beans soaked in 15-percent ethanol at 50°C for 6 hours had a painty flavor and still retained 2 percent of the original lipoxygenase activity. Based on the work of Borhan and Snyder (1979), these conditions should have been sufficient for complete lipoxygenase destruction. Ashraf and Snyder (1981), however, soaked the beans in tap water for 18 hours to remove the residual ethanol after the initial ethanolic soaking. Resoaking in tap water has been shown by Mitsuda et al. (1967) to cause the reactivation of lipoxygenase that had been previously inactivated by exposure to ethanol.

Ashraf and Snyder (1981) also looked at the effect of using the ethanolic soaking procedure in conjuction with alkaline pH to improve soymilk flavor. It was found that there was a significant difference in paintiness scores depending on which type of sodium salt was used to raise the pH of the soak solution. It was found that salt type affected the paintiness scores of the soymilks more than the residual lipoxygenase activity.

The major criticism of these non-heat methods of lipoxygenase inactivation is that they lower the protein solubility. This problem is essentially alleviated when using the Illinois process developed by Nelson et al. (1976). This process is unique in that the whole soybean is used in the milk, meaning there is no water-insoluble matter (such as insoluble protein) filtered out. Homogenization is used to adequately disperse and suspend the insoluble matter into the soymilk. The mouth-feel and colloidal stability are dependent on the tenderness of the beans. When ideal processing conditions are used, an acceptable product can be made. The most attractive feature of the process is that the final beverage contains very close to 100 percent of the original amount of soybean protein.

Soymilk in tofu production

Other than the traditional method, only the hot-grind method of Wilkens et al. (1967) for making soymilk has been used successfully to make tofu (Schroder and Jackson, 1972).

The mechanism of salt-induced coagulation involved in tofu-making has not been elucidated (Sakakibara and Noguchi, 1977) and it is unknown what effect the various methods of soymilk production would have on the coagulation of the soy proteins that make up tofu. Nutrition and flavor quality are the most important factors that soymilk producers are concerned with, however, there are other considerations that must be taken into account when the soymilk is to be used for tofu-making. Watanabe et al. (1964) as cited in Shurtleff and Aoyagi (1979) did an extensive study in which the many factors involved in soymilk production were investigated to determine their effect on the quality and yield of tofu. It was found that the maximum recovery of protein and other solids from soybeans could be achieved by using a 10:1 water to bean ratio when processing. It was also determined that the slurry cooking temperature can effect the optimum coagulant concentration, the tofu firmness and the bulk yield of tofu.

The concentration of the soymilk is also an important consideration in tofu production. Watanabe et al., 1964, as cited in Shurtleff and Aoyagi (1979) reported that the required coagulant concentration is dependent on the solids level of the soymilk and both of these factors can affect tofu firmness and bulk yield. Saio (1979) and Wang and Hesseltine (1982) also found that tofu textural quality can be affected by the soymilk temperature at coagulation, concentration, soymilk protein and phytate content. Caution should be used, however, when evaluating the findings of Wang and Hesseltine (1982). Pesek and Wilson (1983) found that the method of Wang and Hesseltine for producing tofu in a test tube gave results that did not correlate well with results obtained by producing tofu using the traditional method.

Coagulants

Traditionally, soymilk proteins have been coagulated either by lowering the pH of the soymilk to the isoelectric point of soy protein (pH 4.5) using some sort of acid (lemon juice, vinegar, etc.) or by salting out the proteins with a divalent salt of calcium or magnesium. Acid coagulation tends to produce a very soft-textured tofu which is not accepted in the United States and will not be discussed further.

Salt coagulants can be divided into two general categories, sulfate and chloride types. Sulfate coagulants, such as calcium sulfate and magnesium sulfate, are insoluble in water, which is an important factor in determining the speed of the coagulation reaction. Because of their insolubility, the sulfate coagulants react slowly with the soy proteins. As a result, the rate of coagulation is slow, allowing for the formation of large curds with a high water-holding capacity. Large curds produce a soft-textured tofu with a high bulk yield, characteristics that are highly desired by Japanese tofu consumers and producers alike. Calcium sulfate, which is the least expensive of the salt coagulants, is readily available and is currently the only coagulant generally recognized as safe (GRAS) by the Food and Drug Administration. For the above reasons, calcium sulfate is the most widely used coagulant in the world.

The chloride type coagulants are water-soluble salts. Included in this group is nigari or sea salt, the coagulant with the longest history of use in tofu production. Because of their solubility, when the chloride type coagulantsare added to soymilk, there is a rapid coagulation of soy protein. This rapid coagulation causes the formation of small curds with little water-holding capacity, which after pressing, will produce a firmer tofu than that obtained from larger curds. Sulfate coagulants are usually added quickly (i.e. dumped) into the soymilk, whereas the chloride coagulants must be added slowly in order to control the rate of the coagulation reaction. The chloride type coagulants are also noted for giving an otherwise bland tofu a subtly sweet flavor that is highly desirous. Because of the skill needed to control the coagulation reaction with chloride salts, the chloride type coagulants are used mainly in small tofu shops. Most large-scale modern tofu factories use calcium sulfate which is cheap, requires little skill to use and can be added all at once.

Effect of coagulant type and concentration on tofu quality

Research has shown that both coagulant type and concentration can affect tofu quality. Watanabe et al., 1964, as cited in Shurtleff and

Aoyagi (1979) found that as coagulant concentration increased, the bulk yield of the tofu decreased and the firmness increased. It was also found that tofu made from calcium chloride was harder than tofu made from calcium sulfate. Similar results were obtained by Saio (1979), Skurray et al. (1980), Tsai et al. (1981) and Wang and Hesseltine (1982). It is interesting to note that Lu et al. (1980) reported that tofu produced with calcium chloride was softer than tofu produced with calcium sulfate which contradicts the findings of the aforementioned workers. The method used by Lu et al. (1980) for the determination of the appropriate coagulant amount may be the reason for the difference in results. Lu et al. (1980) added coagulant until the formation of curds was noted. Because calcium chloride reacts rapidly with soy protein, localized coagulation may have occurred before the proper amount of coagulant could be added, resulting in the addition of insufficient amounts of calcium chloride and soft tofu. The opposite situation may have occurred when using calcium sulfate, which reacts slowly with the soy protein. Amounts in excess of the proper level may have been added before any coagulation was noted, resulting in uncharacteristically hard tofu.

The problem of defining the correct or optimum amount of coagulant is not confined to the research of Lu et al. (1980). Table 2 gives the coagulant concentrations reported by tofu researchers. A variety of coagulant concentrations have been used without giving any adequate explanation. Unfortunately, the problem of tofu researchers using incorrect amounts of coagulant will continue because there has been no

Table 2. Coagulant concentrations used by tofu investigators

Investigator	Coagulant Concentration
Smith et al. (1960)	_[a]
Schroder & Jackson (1972)	0.03N
Skurray et al. (1980)	_[b]
Wang & Hesseltine (1982)	_[c]
Wang et al. (1983)	0.04N

[a]Insufficient data.

[b]Coagulant added until soymilk began to coagulate.

[c]An appropriate amount of coagulant added.

general agreement among tofu researchers on how to determine correct coagulant amounts.

Factors influencing the optimum coagulant amount

There are several factors that must be taken into account when determining the optimum coagulant amount or concentration. It would therefore be inadequate for researchers to merely agree upon a certain coagulant concentrations. Soymilk volume, solids concentration, and soybean variety all play an important role in affecting the optimum coagulant amount.

Watanabe et al., 1964, as cited in Shurtleff and Aoyagi (1979) worked out a method for determining the optimum coagulant amount that took into consideration soymilk volume, concentration and varietal influences. Soymilk of a certain variety and concentration is coagulated using different coagulant concentrations. The resulting whey is measured for clearness by measuring its percent transmittance at 440 nM. The lowest concentration of coagulant to produce the clearest whey is then considered to be the optimum coagulant concentration.

The coagulation reaction

Shurtleff and Aoyagi (1979) have stated that the coagulation reaction is the most important step in the tofu-making process. It is also the most complex step since successful coagulation depends on many interrelated factors (Shurtleff and Aoyagi, 1979, list 13). Although many of these factors, such as the variety of soybeans used, are decided upon early in the tofu-making process, there are several factors that are part of the coagulation process that have an important effect on the quality of the resulting tofu. The effects of coagulant type and concentration on tofu quality have already been discussed. There are several other factors in the coagulation reaction that can exert comparable influences on tofu quality. Saio (1979) and Wang and Hesseltine (1982) have reported that the temperature of coagulation and the amount of stirring are also important factors. It was found that as both temperature and stirring increased, tofu hardness increased. It was also reported by Saio (1979) that soymilk concentration was another important factor because it was found that as the soymilk concentration was decreased, tofu firmness increased.

Effect of soybean variety on tofu quality

Soybean variety affects not only the texture, but also the flavor and color of tofu. In the late 1950s, A.K. Smith (1959) reported that the Japanese were experiencing problems using American varieties of soybeans for the production of traditional Japanese soy foods. When compared to Japanese varieties of soybeans, U.S. varieties were lower in protein, slower in absorbing water, cooked unevenly, and produced soy products that were darker in color and had less desirable flavor. These problems were reiterated by Watanabe and Shibasaki (1959).

Smith et al. (1960) compared 5 U.S. varieties and 5 Japanese varieties for their suitability in tofu-making and determined which U.S. varieties of soybeans would make a satisfactory product. The beans used in this study, however, were uncomposited samples and the author stressed that environmental factors could have had an effect on the composition of the beans used in the study. It was thought, however, that varietal effects were more important than environmental influences in determining the suitability of soybeans for tofu production. No significant difference was found between the hardness of tofus made from Japanese and American soybeans. There was a difference in the tofu color; the Japanese beans produced a gray-white tofu, whereas the U.S. beans produced a light-yellow tofu. The texture of the tofu made from U.S. beans was also deemed inferior to that of the tofu made from Japanese beans. There was no significant difference between the American and Japanese beans in overall yield of tofu.

Differences in protein composition between different soybean varieties were thought to be one cause for textural differences between varieties when Wolf et al. (1961) found a 10 to 12-percent difference in the 11S and 7S protein fractions between Japanese and American soybeans. Saio et al. (1969a) showed that the 11S and 7S protein fractions could contribute to the textural characteristics of tofu. Tofu made from crude 11S protein were much harder than tofu made from 7S protein. The crude 11S fraction contributed greatly to the springiness, chewiness and gumminess of tofu. Phytic acid greatly diminished the textural quality contributions of the 11S fraction while enhancing the springiness of tofu made from the 7S fraction. Using the electron microscope, differences were observed in the formation of protein granules of the 11S and 7S tofus. These protein granules were considered to be the fundamental structural units of the gels. In the 11S tofu, these granules were aggregated into lumps whereas in the 7S tofu the granules were dispersed. It was then thought that the differences in aggregation could have been related to the differences in the hardness between the two tofu types. Saio (1979) confirmed that the larger the protein aggregates, the harder the tofu would be if the curds are left intact and whey is not eliminated.

Saio et al. (1969b) has also shown that phytic acid content can affect the texture and bulk yield of tofu and that varietal differences in phytic acid could influence a soybeans suitability for tofu-making. It was found that the addition of phytic acid was associated with the formation of a softer tofu with increased weight. It was thought that phytic acid caused this phenomenon by retarding the coagulation reaction between soy protein and the calcium ions of the coagulant. Two Japanese varieties preferred by tofu-makers were compared to two U.S. varieties not preferred and it was found that the Japanese beans had a high phytic acid content and made a softer tofu. It was concluded, however, that a soybean variety's suitability for tofu-making could not be solely attributed to high phytic acid content.

Recent workers have questioned the significance of protein composition, phytic acid levels and other chemical factors in determining the textural qualities of tofu. Skurray et al. (1980) concluded that, although differences in protein content may lead to differences in tofu quality, these differences could be overcome by varying the amount of calcium sulfate used. These results should be questioned for 2 reasons:

1) An ideal range of coagulant concentrations was not determined and as a result, great excesses of coagulant were used. Also, intervals between the coagulant concentrations examined in the study were too wide to enable the study of texture differences due to soybean variety.

2) A taste panel was not used and therefore they failed to address the problem of "chalky" mouthfeel associated with the use of excess amounts of calcium sulfate. Therefore, the use of varying levels of calcium sulfate to overcome textural differences may be severely limited.

Skurray et al. (1980) used approximately 10 times the required amount of coagulant. At coagulant concentrations this high, it is very probable that any differences due to protein content were being masked. Watanabe et al., 1964, as cited in Shurtleff and Aoyagi (1979) showed that for a typical soymilk (ca. 6% solids) the range between an inadequate coagulant concentration and an excessive coagulant concentration is around 0.14 percent by weight of soymilk. Skurray et al. (1980) used as an interval width between coagulant concentrations 0.2 percent by weight of soymilk. This would mean that at one concentration level there could be an inadequate amount but the next highest concentration would be excessive. Again, any differences due to protein content may have been masked by the effects of low or high concentrations of coagulant.

Shurtleff and Aoyagi (1979) reported that the use of excessive amounts of coagulant (calcium sulfate) can cause tofu to have a chalky taste or mouthfeel. Subjective assessment of tofu in the work of Skurray et al. (1980) was carried out using a single person whose tolerance for a chalky flavor may have been higher than that typical of the general population. Raising calcium sulfate levels to obtain a desired texture may result in chalkiness, and therefore, coagulant concentration should not be depended upon as a sole measure for controlling the texture of tofu.

An extensive study was carried out by Wang et al. (1983) on the effect of soybean variety on the yield and quality of tofu. Five U.S. varieties and 5 Japanese varieties of soybeans were used to make tofu. Subjective assessment of the quality of the tofus was made without the use of a taste panel and therefore the results stating that all of the bean varieties produced a "satisfactory" tofu should be questioned. There were no overall differences found in the characteristics of the soybeans or the resultant tofu associated with the origin of the soybeans (U.S. vs. Japanese). Differences in the protein and oil content of the soybeans due to variety were found. These differences in turn showed up in the resulting fresh tofu. Therefore, soybean variety influenced the composition of the tofu. Also, the hardness and yield of the fresh tofu were significantly influenced by the soybean variety. Although several authors were cited that attributed tofu texture to protein composition, it was concluded that differences in the hardness of tofus were a function of water content. This conclusion was based on the work of Skurray et al. (1980) which showed that there was no significant correlation between the ratio of 7S to 11S proteins or phosphorus content and the texture of tofu. Again, as previously discussed, the work of Skurray et al. (1980) should be questioned because of their use of excess amounts of coagulant. Despite the fact that variety clearly had an influence on the composition, texture and yield of tofu, Wang et al. (1983) concluded the opposite, that there were no varietal influences. This conclusion was based on the belief that processing conditions are enough to overcome any variances contributed by soybean variety.

Summary of Tofu Research

There is disagreement amoung tofu researchers as to what role soybean variety plays in influencing the quality of tofu. Past researchers agree that soybean variety can influence tofu quality, but there is a disagreement as to the significance of this influence when compared against other influences found in processing. Reasons for this disagreement may be that a variety of methods are being used to make tofu and that processing conditions are not adequately controlled. Saio et al. (1969a, 1969b, 1971), Tsai et al. (1981) and Wang and Hesseltine (1982) used a method where tofu was made by coagulating the soymilk in test tubes. This method of producing tofu may not give a reliable representation of tofu made in the traditional manner (Pesek and Wilson, 1983). Other researchers have produced tofu in a traditional manner with minor variations at some point in the process. Wang et al. (1983) added the soymilk to a coagulant solution which is a reverse of the usual procedure. Lu et al. (1980) strained the slurry before cooking. Skurray et al. (1980) strained the slurry before heating, and did not cook the soymilk, but merely brought it to a boil before coagulation. Smith et al. (1960) excessively cooked the slurry (30 minutes under pressure). A food product is a complex system of many constituents. Interactions between the environment and the constituents and/or between the constituents themselves brings about profound changes (both good and bad) in a food product. With proper understanding of a food system we can control these interactions through processing to produce a desired product. In the case of tofu research, however, we have a simple food system in which we understand little, and are using a multitude of processes and techniques to produce undesired confusion in the research literature. The variations in the tofu-making process adopted by each researcher, although small, may be causing unnecessary and/or unknown variations in the characteristics of the resulting tofu. These variations may also influence the sensitivity of the tofu towards other factors during processing.

One additional source of variation in tofu production is in the solids content of the soymilk. Currently, most tofu researchers and producers simply mix a 10:1 ratio of water to soybeans and assume that the concentration of the soymilk will not vary. Due to the factors previously mentioned, however, the solids content can vary. Because of the importance that soymilk solids content plays in determining the textural qualities of tofu, a method is needed for quickly and accurately measuring the solids content of hot soymilk immediately before coagulation. Then, if necessary, adjustments can be made and the required coagulant amount can be accurately determined. The hand-held refractometer has been suggested as one way of measuring soymilk percent solids (Shurtleff and Aoyagi, 1979). Percent solids have also been measured by light-scattering (Johnson and Snyder, 1978). The hydrometer, a device for measuring the specific gravity of liquids, has been used in the dairy industry to measure the solids content of milk, but its ability to measure the solids content of hot soymilk has not yet been tested. The accurate measure of soymilk solids prior to coagulation would greatly add to the quality of tofu research.

There is still no agreement as to how to determine correct coagulant amounts or concentrations. As a result, there is a great variation in the coagulant concentrations used by past researchers (Table 2). Because coagulant concentrations have been shown to influence tofu textural quality, yield and flavor it is very unlikely that tofu researchers will be able to agree on the importance of other factors in influencing tofu quality until some agreement is made on what constitutes a proper level of coagulant.

Pressing pressures and duration of pressing (Table 3) have not been agreed upon. There has been a wide range of values used for both parameters among different researchers. Most of these values, unfortunately, fall outside of the range of practicality from a commercial standpoint. There are not going to be many commercial tofu producers who will be willing to press their tofu overnight when they can get the same results by pressing for 15 minutes. Also, when pressing for extended periods of time (over 1 hour), microbiological problems can develop. Tofu is usually pressed for no more than 20 minutes in commercial practices.

Table 3. Tofu pressing duration and pressures used by tofu investigators

Investigator	Duration	Pressure (psi)
Smith et al. (1960)	?	0.004
Wang (1967)	1 hr.	0.05
Schroder & Jackson (1972)	overnight	?
Skurray et al. (1980)	2 hr.	0.08
Wang et al. (1983)	1 hr.	0.14

There are an infinite number of ways that tofu could be produced, however, it would help both researchers and commercial tofu producers if a standardized method was developed for tofu production. This method should be applicable to small-scale laboratory work as well as to commercial-scale production. The usefulness of novel methods of making tofu on a laboratory-scale is questionable since many of these methods do not produce tofu like that made using traditional methods.

Finally a serious weak-point that has existed in tofu research is the insufficient use of the taste panel. Despite the fact that most of the research carried out on tofu has been done to improve its palatability, only Lu et al. (1980) has used the taste panel as a tool. The remaining researchers either used a single "expert" (Smith et al., 1960; Skurray et al., 1980) or relied on instrumental analysis to assess tofu quality (Saio et al., 1969a; Saio et al., 1969b; Tsai et al., 1981; Wang and Hesseltine, 1982; Wang et al., 1983). The use of the taste panel would help tremendously in evaluating the effects of different factors on tofu characteristics and will also be the key to producing a product that is acceptable to different groups of people with different taste and texture preferences.

Purpose of the Study

Main objectives

The objective of this study was to determine the influence of soybean variety and method of processing in tofu manufacturing and on consumer acceptability of tofu. This was accomplished in part by:

1) Comparing tofu made from three varieties of soybeans (selected on the basis of seed size and protein content and grown under identical environmental conditions) and by three processing methods (traditional, hot-grind and ethanolic soaking) to determine which variety-process combination would give the best liked tofu as judged by a taste panel.

2) Determining what coagulating and pressing conditions were required to produce a tofu with a texture liked by a taste panel.

3) Correlating instrumental analysis with results obtained by taste panels to further define what texture, flavor and color are preferred.

In order to insure that processing conditions would be identical for all tofus, preliminary work was carried out which entailed:

1) Comparing the accuracy of three methods (refractometry, spectrophotometry and hydrometry) of determining percent solids in soymilk.

2) Determining the optimum coagulant concentration for tofu-making with different soybean varieties and soymilk concentrations.

3) Designing and constructing a tofu press which allows the use of calibrated pressing pressures.

MATERIALS AND METHODS

Selection of Beans

Amsoy 71, Weber and Vinton seed-grade soybeans grown in 1979 were used for the preliminary work in this study. These soybeans were provided by Dr. Walter Fehr of the Iowa State University Agronomy Department. The beans were stored in plastic bags at 5°C.

In the main study (the influence of soybean variety and method of processing on tofu quality), Vinton 81, Weber and Prize seed-grade soybeans grown under the direction of Dr. Walter Fehr in 1982 were used. The soybean varieties were selected on the basis of protein content and seed size (Table 4). These soybeans were grown under identical environmental conditions and stored at 5°C for the duration of the study.

Table 4. Relative protein content and seed size of soybeans used in the main study

Soybean variety	Protein content	Seed size
Weber	Low	Small
Vinton 81	High	Intermediate
Prize	Intermediate	Large

Selection of Coagulant

Food-grade $CaSO_4 \cdot 2H_2O$ (U.S. Gypsum) was chosen as the soymilk coagulant because it is the most widely used coagulant, it has been shown to be suitable for large tofu operations, and is currently the only coagulant generally recognized as safe (GRAS) by the Food and Drug Administration. A 100 pound bag was purchased from The Marthens Co., Davenport, Iowa, triple-bagged (plastic) and stored at room temperature (20–25°C) in a closed cardboard barrel.

Processing Methods

Laboratory scale production of soymilk

For the preliminary study, soymilk was prepared using one of the following methods:

Traditional method Three hundred grams of soybeans were washed and soaked in distilled water 8-9 hours at room temperature (ca. 24°C). The beans were drained, combined with 2 liters of water and ground to a slurry using a Cherry-Burrell Vibroreactor. The soy slurry was then filtered through a coarse linen cloth to separate the soymilk from the water-insoluble matter. Two liters of soymilk were recovered from the slurry.

Ethanol-soak method Soybeans were processed according to the traditional method with the exception that the soybeans were soaked in a 15-percent ethanol solution at 45°C for 12 hours (Borhan and

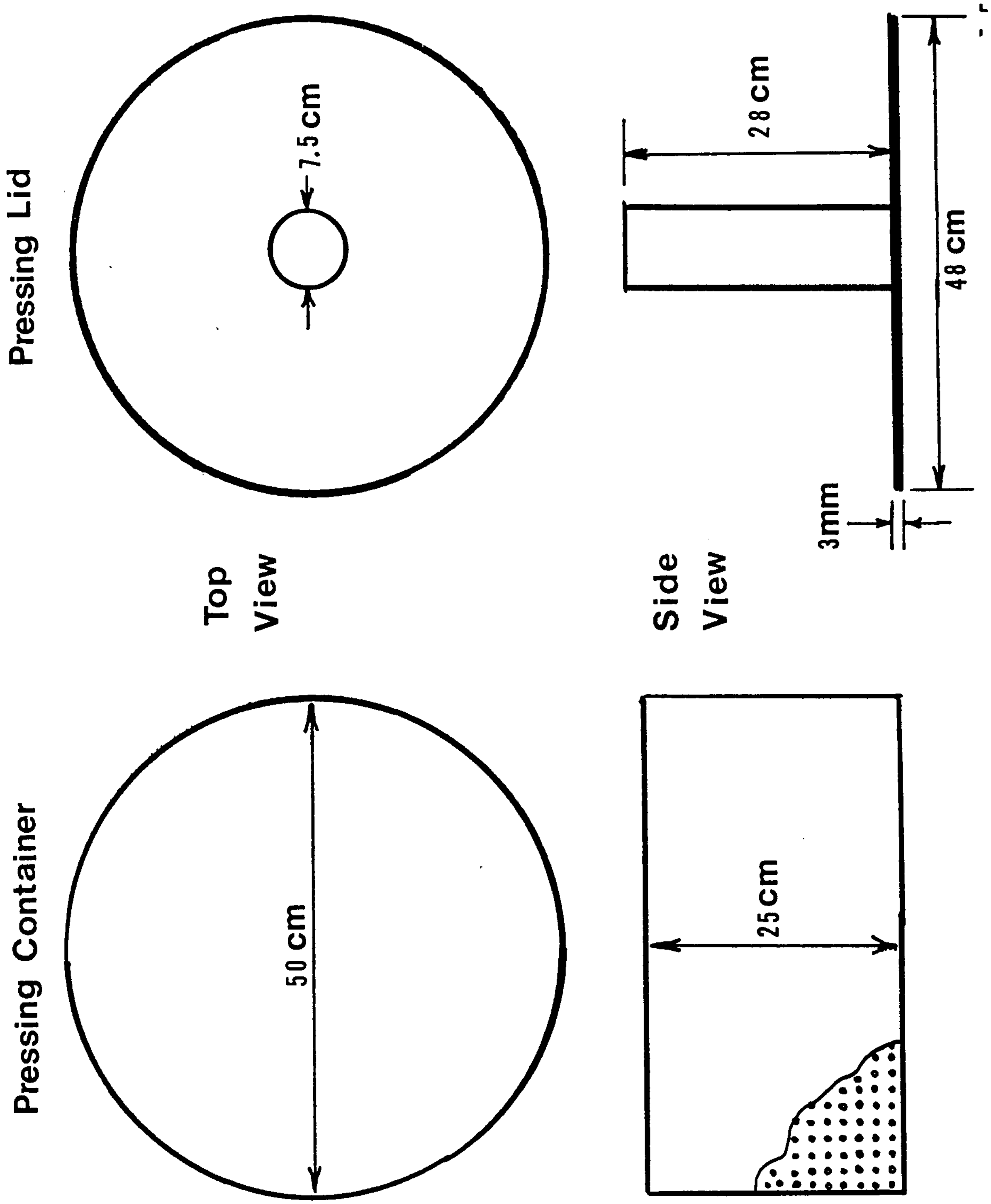
Hot-grind method Soybeans were washed, soaked and drained according to the traditional method. The beans were combined with 2 liters of boiling (100 °C) water and immediately ground using a Cherry-Burrell Vibroreactor. The resulting slurry was then rapidly heated to boiling (97-100°C) by using a steam-jacketed kettle (10 gal. cap., Lee Metals Products Co. Inc.). The temperature of the slurry was maintained above 90°C for 10 minutes to completely inactivate the lipoxygenase enzyme (Wilkens et al., 1967). The slurry was then filtered to yield 2 liters of soymilk.

Pilot-plant production of soymilk and tofu

The pilot plant production of soymilk was oriented towards supplying sufficient quantities of tofu for the consumer taste-panel (up to 60 panelists) that would be used in the main study. To insure the microbial safety of the tofu, all of the equipment used in the production of tofu was rinsed for at least 2 minutes in water containing 100 ppm available chlorine. Representative tofu samples were analyzed for total aerobic plate counts, psychrotrophic counts and coliforms. The production methods used are as follows:

Traditional method Nine hundred grams of soybeans were washed and then soaked in tap-water for 8-9 hours at room temperature (ca. 24°C). The hydrated beans were drained, combined with 6 liters of tap-water and then ground to a slurry with a Cherry-Burrell

Figure 2. Perforated, stainless-steel, flat-bottomed pressing container and stainless-steel lid (Almaco)


Pressing Lid
7.5 cm
28 cm
48 cm
3mm
Top View
Side View
Pressing Container
50 cm
25 cm

Vibroreactor. The slurry was transferred to a steam-jacketed kettle and an additional 1 liter of tap-water was added. The slurry was brought to a boil (97-100°C) and stirred constantly using a heavy-duty stirrer (Fisher brand, cat. no. 14-509-1, 1983). The temperature was maintained between 95-100°C for an additional 7 minutes. The cooked slurry was poured into a fine-mesh filtering-sack (Bean Machines Inc.) set within a perforated, stainless-steel, flat-bottomed, pressing-container (Almaco) shown in Figure 2. The mouth of the filtering-sack was then twisted closed. A stainless-steel pressing-lid (Almaco) was placed over the closed sack. A cider-press (Day Equipment Corp.) was used to provide sufficient pressure to press any remaining soymilk from the water-insoluble residue (okara). After the 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 the soymilk was then transferred back to the steam-jacketed kettle. While vigorously stirring the soymilk, a 30-ml aliquot of soymilk was removed in order to measure the solids content using the light scattering technique of Johnson and Snyder (1978). The solids content of the soymilk was then adjusted to the desired solids level using tap-water.

Ethanol-soak method Soybeans were processed according to the traditional method with the exception that the soybeans were soaked in a 15-percent ethanol solution for 12 hours at 45°C (Borhan and Snyder, 1979).

Hot-grind method Soybeans were washed, soaked and drained according to the traditional method. The beans were then combined with

6 liters of boiling (100°C) water and immediately ground by using a Cherry-Burrell Vibroreactor. The resulting slurry was then rapidly heated to boiling by using a steam-jacketed kettle. The temperature of the slurry was maintained above 90°C for 10 minutes to completely inactivate the lipoxygenase enzyme (Wilkens et al., 1967). Additional processing of the cooked slurry proceeded as in the traditional method.

Tofu production After adjusting the soymilk solids, the required coagulant amount was determined by the soymilk solids level and the final soymilk volume (as described in Determination of Optimum Coagulant Concentration on page 49). The coagulant ($CaSO_4 \cdot 2H_2O$) was then weighed out in a 800-ml beaker and 150 ml of tap-water was added to make a coagulant slurry. The soymilk was brought up to the desired temperature (80-90°C) in the steam-jacketed kettle while being stirred constantly with the heavy-duty stirrer. When the desired temperature had been reached, heating was stopped, the stirring rate was increased and the coagulant slurry was quickly added and mixed thoroughly into the milk. Stirring was stopped 10 seconds after the addition of the coagulant by stopping the stirrer and inserting a large spoon into the milk to quickly stop the swirling motion. The soymilk was allowed to stand for 2-3 minutes to allow for the formation of curds. The resulting coagulum was broken up and a colander was used to separate the curds and whey. Approximately 1.5 liters of whey was removed to prevent the formation of "watery", "mushy" tofu. The remaining curds and whey were transferred to a pressing-box (Figure 3) lined with a single layer of cheese-cloth. The curds were pressed at a

Figure 3. Calibrated tofu press and pressing box

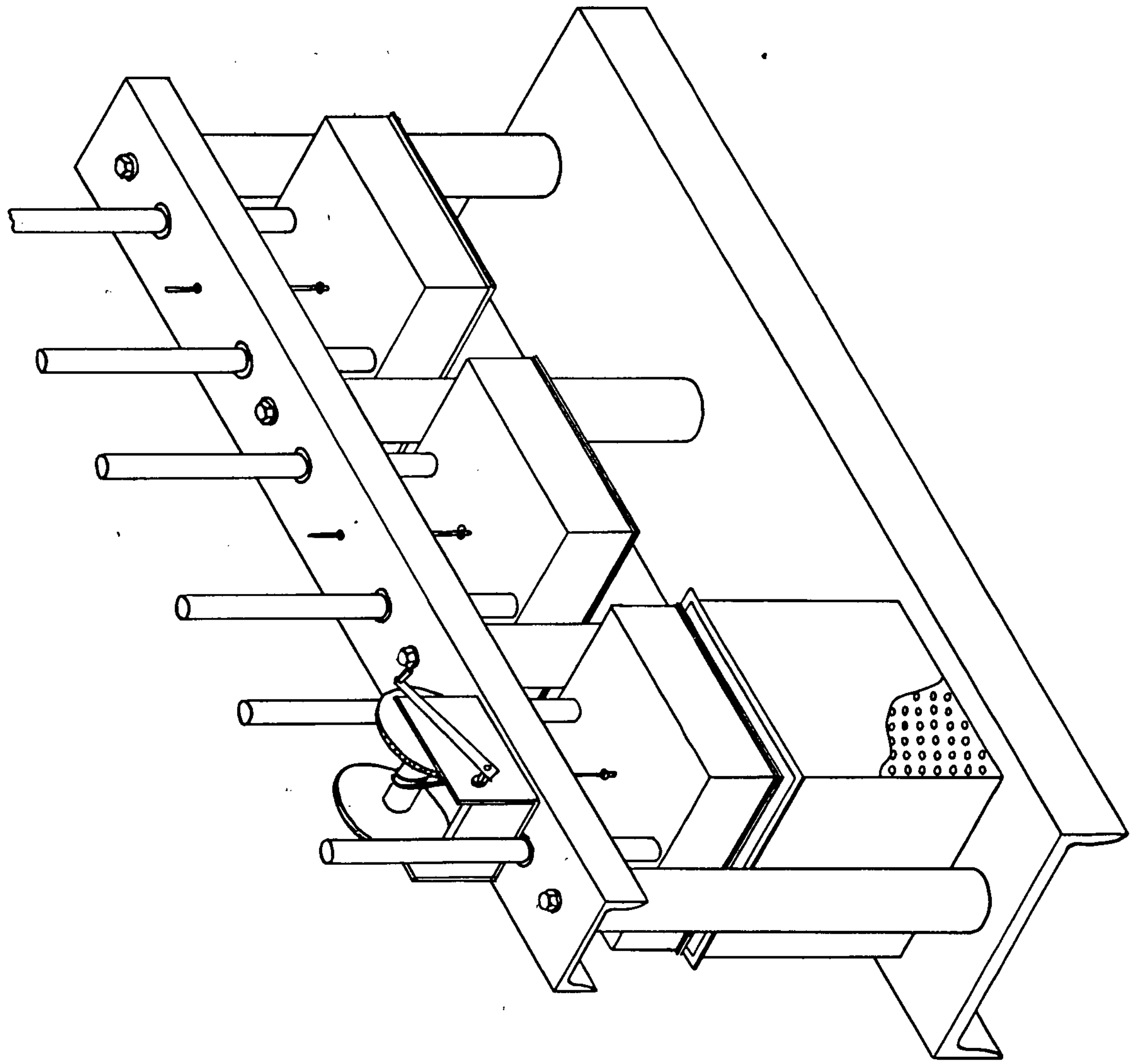
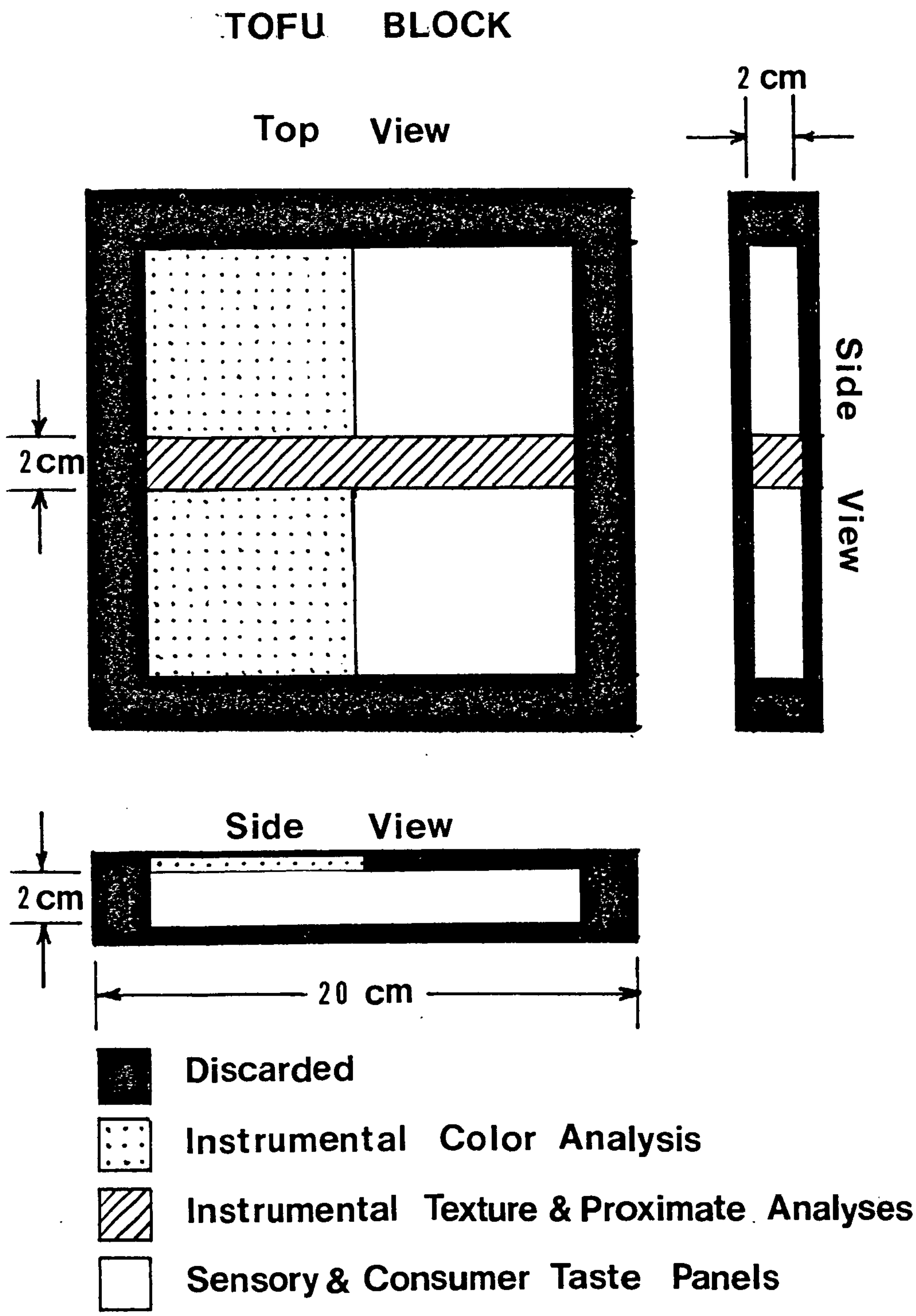


Figure 4. Schematic drawing of a tofu block illustrating the origin of the tofu samples tested in taste panels, instrumental and sensory analysis.


TOFU BLOCK
Top View
2 cm
2cm
Side View
Side View
2 cm
20 cm
Discarded
Instrumental Color Analysis
Instrumental Texture & Proximate Analyses
Sensory & Consumer Taste Panels

specific pressure between 0.6 psi and 2.2 psi for a specific duration between 10 and 30 minutes using a tofu press (Figure 3). The tofu press had been previously calibrated using a proving ring (R. C. Ames Co., Waltham, MA) provided by the Mechanical Engineering department at Iowa State University. The resulting tofu was removed from the pressing-box and weighed. Approximately 1 inch of tofu was removed from each edge of the tofu block because the edges were found to be firmer than the rest of the block. The tofu was then placed in a water-filled plastic container and stored at 5°C until tested.

Preliminary Work

Measurement of percent solids

From each batch of soymilk, the following dilutions were made: 200/0, 175/25, 150/50, 125/75, 100/100, 75/125, 50/150, 25/175 (soymilk/water). Each dilution was heated to a boil while being stirred constantly and then held at 95-100°C for 7 minutes.

The diluted soymilk was transferred to a 250-ml graduated cylinder and the temperature of the soymilk was recorded. Nine different variety-process combinations (Table 5) were used to produce soymilk.

Oven method Two 1-ml aliquots of a soymilk dilution were transferred to 2 tared aluminum weighing-boats and dried to a constant weight in a vacuum oven at 80°C at 15 inches Hg (AOAC, 1980, method 14.003).

Hydrometry Brix hydrometers ranging from 10-31° Brix (Elmer and Amend, N.Y.), specific gravity hydrometers ranging from 0.700-1.00

(Fisher Scientific Co., Pittsburgh, PA) and a milk lactometer (Quevenne) were used to measure the specific-gravity. Two samples of each diluted soymilk were measured.

Refractive index A few drops (ca. 0.1 ml) of each diluted soymilk were taken for a percent-solids reading on a Bausch and Lomb hand-held refractometer. Two samples of each diluted soymilk were measured.

Light scattering Two 1.0-ml aliquots of each dilution were diluted 1:250 with distilled deionized water. The transmittance readings were then converted to absorbance. The nine soymilk treatments were prepared again 2 weeks later for a second replication.

Table 5. Nine variety-process combinations (treatments) used to produce soymilks

Bean variety	Processing Method		
	Traditional (T)	Hot-grind (H)	Ethanol-soak (E)
Amsoy (A)	A-T	A-H	A-E
Vinton (V)	V-T	V-H	V-E
Weber (W)	W-T	W-H	W-E

Varietal influence on the optimum coagulant concentration

Two liter quantities of soymilk were prepared from Vinton, Amsoy 71 and Weber variety soybeans using the laboratory scale method of soymilk production previously described. The solids content of each type of soymilk was measured and then adjusted to 6 percent solids. Appropriate amounts of coagulant ($CaSO_4 \cdot 2H_2O$) were determined based on the coagulant concentrations chosen (Table 6) and a 400-ml volume of soymilk. To determine the coagulant amount required to make a 0.007M (0.014N) solution in 400 ml of soymilk the following calculation was used:

$$\underset{\text{Molecular weight.}}{(172.2 \text{ grams/mole})} \; \underset{\text{Coagulant concentration}}{(0.007 \text{ moles/liter})} \; \underset{\text{Soymilk volume}}{(0.4 \text{ liters})} = 0.48 \text{ grams of coagulant}$$

Table 6. Coagulation concentrations used in the determination of optimum coagulant concentrations for 4, 5, 6 and 8 percent soymilk

Soymilk % solids	Coagulant concentrations (Normality) used					
4	0.010	0.012	0.014	0.016	0.018	0.020	
5	0.012	0.014	0.016	0.018	0.020	0.023	0.026
6	0.014	0.016	0.018	0.020	0.023	0.026	0.030
8	0.023	0.026	0.030	0.032	0.035	0.037	

For each coagulant concentration tested, the calcium sulfate dihydrate was weighed out into a 50-ml beaker and 10ml of distilled deionized water was added to make a slurry. A 390-ml aliquot of hot (90-98 degree C) soymilk that had previously been cooked for 7 minutes was then transferred to an 800 ml beaker containing a magnetic stir-bar.

The soymilk was stirred using a magnetic hot-plate stirrer until the temperature of the milk had decreased to 80°C, then the stirring rate was increased to as fast a rate as possible without splattering the milk. The 10-ml coagulant-slurry was then quickly transferred to the soymilk. A wash-bottle filled with distilled-deionized water was used to wash any remaining coagulant from the 10-ml beaker (using no more than 10 ml of water for washing). The addition of coagulant was carried out in 15 seconds. After the addition of the coagulant, an additional 5 seconds was allowed in order to insure the adequate mixing of the coagulant into the milk. Stirring was immediately stopped and the magnetic stir-bar was removed for use in the next 390-ml aliquot of soymilk. This procedure was continued until all of the coagulations were finished. Each beaker of coagulated soymilk was allowed to stand for approximately 20 minutes. For each beaker of coagulated soymilk, the resulting curds and whey were transferred to a plastic 500-ml beaker with 1/8-inch holes in the sides and bottom to allow for the drainage of the whey. The beaker was lined with a double-layer of cheese-cloth in order to retain the curds. The transmittance of the whey was measured at 400 nM (Tsai et al., 1981) on a Bausch and Lomb Spectronic 20 spectrophotometer. The optimum coagulant concentration was defined as the minimum coagulant concentration needed to produce the maximum whey transmittance (Watanabe et al., 1964, as cited in Shurtleff and Aoyagi, 1979).

<u>Influence of soymilk concentration on the optimum coagulant concentration</u>

Amsoy 71 soybeans were used to make soymilk at concentrations of 4, 5 and 8-percent solids. Optimum coagulant concentrations for each of these soymilk concentrations were determined as previously described. The coagulant concentration selected for each percent-solids level is shown in Table 6.

Statistical Analysis

Statistical analysis was carried out using the 1979 edition of the Statistical Analysis System (SAS) package developed by the SAS institute Inc., Box 8000, Cary, North Carolina 27511.

Determination of Consumer Texture Preferences

Weber soybeans were processed using the traditional pilot-plant method previously described. Tofus of 2 to 3 different textures were made for each panel session. The tofu texture was controlled by varying the concentration, coagulation temperature and stirring rate of the soymilk, the amount of curd breakage, pressing duration and pressure.

<u>Instrumental analysis of tofu texture</u>

Figure 4 illustrates what section of the tofu block was used for the instrumental tofu texture measurements. The samples were obtained immediately after pressing the tofu. At least 5, 20X20X20-mm sized tofu samples were obtained for testing. A texture profile analysis descibed by Bourne (1968) was run on 3 of the tofu cubes using an Instron model 1122 Universal Testing Instrument (Instron Corporation) that was equipped with a compression anvil. The applied compression force was measured using a 500-Kg tension-compression load-cell and recorded using a chart-speed of 500mm/minute with a 10-Kg load equalling full scale. The tofu was compressed 15 mm (75 percent) with a cross-head speed of 200 mm/minute. At least 2 samples (1 penetration test per sample) were used for penetrometer measurements using a Precision Penetrometer (Precision Scientific Co.) equipped with a penetration cone.

Consumer tofu texture panel

Panelists were obtained by placing notices throughout the Iowa State University campus. There were no specific requirements for participating in the taste panel other than not being allergic to soy-protein. The taste-panels were conducted on tofu samples stored for 20 hours at 5°C after being made. The samples were cut into 20X20X20-mm cubes (Figure 4) and then heated by steaming in a covered stainless-steel pot for 3 minutes. The samples were then served to the panelists under red light to mask any color differences between samples. The instruction-score sheet used for this panel is shown in Figure 5. The tofu samples were ranked according to the panelists texture preferences, with a score of 1 indicating the most preferred sample. The significance of a ranking score for a particular sample was determined by using tables prepared by Kramer et al. (1974).

The purpose of this taste panel is to evaluate tofu texture preferences. You will be given 3 samples. Comparing only textural characteristics, please rank the samples based on your preference; 1 being the most preferred, 3 being the least preferred. Please make comments on the back of this sheet.

<u>Ranking</u>	<u>Sample #</u>
1.	
2.	
3.	

Figure 5. Instruction-score sheet for texture preference panel

Instrumental and Sensory
Analysis and Evaluation of Tofu Quality

Tofus were prepared from Weber, Vinton 81 and Prize variety soybeans using the traditional, hot-grind and ethanol-soak methods on a pilot-plant scale to make a total of 9 types of tofu (Table 5).

Consumer tofu quality preference panel

Taste panel sessions were conducted on tofus made from a single soybean variety to evaluate and compare the effects of the 3 processing methods on tofu quality. Samples for this panel were prepared as previously described for the texture panel. The samples, however, were served under white lights to enable the panelists to detect color differences. The instruction-score sheet used for this panel is shown in Figure 6.

The effects of soybean variety were evaluated by making tofus from the 3 varieties of soybeans using the processing method that the panelists had judged to make the best tofu for a particular variety. These 3 tofus were then compared under white light. The instruction-score sheet used for this panel is shown in Figure 7.

Instumental color analysis

A section of each tofu-block was taken for color analysis (Figure 4) on the same day as the taste-panel. The Hunter color system parameters L, a and b were measured on a LabScan Spectrocolorimeter (Hunter Associates Laboratory, Inc.).

The purpose of this taste panel is to evaluate Tofu preferences. You will be given 3 samples. It is important that you try to ignore textural characteristics during your evaluation of the tofu samples. You will rank each sample based on your preference; 1 being the most preferred, 3 being the least preferred. If you have no preference please write NP. The sample number is found on the bottom of each sample tray.

1. Please rank the tofu samples based on color

Sample #	Ranking
	1
	2
	3

Comments:

2. Please rank the tofu samples based on flavor

Sample #	Ranking
	1
	2
	3

Comments:

3. Ignoring texture please rank the samples for overall preference

Sample #	Ranking
	1
	2
	3

Comments:

Figure 6. Instruction-score sheet used in the consumer tofu quality preference panel (Processing affects)

The purpose of this panel is to evaluate Tofu preferences. You will be given 3 samples. You will rank each sample based on your preference; 1 being the most preferred, 3 being the least preferred. The sample number is found on the bottom of each sample tray.

1. Please rank the tofu samples based on color

Sample #	Ranking
	1
	2
	3

Comments:

2. Please rank the tofu samples based on flavor

Sample #	Ranking
	1
	2
	3

Comments:

3. Please rank the samples for overall preference

Sample #	Ranking
	1
	2
	3

Comments:

Figure 7. Instruction-score sheet used for the consumer tofu quality preference panel (varietal affects)

Instrumental flavor analysis

Immediately after being prepared, a tofu sample was removed for analysis in a later study of identifying flavor compounds using a gas chromatograph.

Sensory analysis

Tofu was made from Weber, Vinton and Prize variety soybeans using the traditional and hot-grind methods to give a total of 6 different types of tofu. These 6 types of tofu were replicated 4 times, thus a total of 24 samples were tested. These 24 samples were randomly presented to the panel, 3 samples per session. Two samples of each type of tofu were served to a panelist. One sample was served cold (5°C), the other sample was steamed for 3 minutes prior to serving.

Evaluations were based on scaling. The scale consisted of a line 15 cm in length with anchor points 1 cm from each end labeled with terms describing the extreme ends of the range of a particular attribute.

Flavor In order to confirm the results of the consumer preference panel and to be able to describe the various flavor attributes, 6 people were trained briefly (1 week) to describe the flavor attributes of the tofu samples. The following is a list of the flavor attributes that were evaluated:

1. Aroma
   a. Cooked chicken or brothy
   b. Painty
2. Flavor

a. Astringent

b. Cooked chicken or brothy

c. Painty

d. Bitter

Texture The same tofu samples were also evaluated for texture. The panelists were asked to evaluate each sample for springiness, firmness and cohesiveness. Springiness was defined as the degree to which the sample returned to its original shape after it had been compressed. Firmness was defined as the force required to compress the sample between molars. Cohesiveness was defined as the degree to which the sample could be compressed between the molars before breaking. These evaluations were based on the previously described scaling method used for the sensory flavor analysis. The resulting sensory scores were then correlated with the appropriate Instron texture profile parameters.

Composition and Yield of Tofu

Two replicates of the nine types of tofu (Table 5) were completed. For each tofu, the total weight of the resulting okara was determined and 2 samples (ca. 0.25 grams) were taken to be analyzed for percent nitrogen by a modified micro-Kjeldahl method (AOAC, 1970, method 38.012) in order to determine the protein content. A section of the resultant tofu was removed (Figure 4.) for moisture, crude lipid and nitrogen determination. Two samples, weighing ca. 3 grams each, were taken for moisture determination (AOAC, 1980, method 14.003). The resulting dry samples were then hexane-extracted to determine the crude lipid content

(AACC, 1969, method 30-20). The dried, defatted samples were then analysed for percent nitrogen using a modified micro-Kjeldahl method (AOAC, 1970, 38.012). The conversion factor of 6.25 was used to convert percent nitrogen values to percent protein.

Calculations

The crude lipid content of the tofu on an as-is basis was calculated by dividing the weight of the extracted lipid by the original weight of the tofu sample. The original weight of the tofu sample was calculated by using the following formula:

(Dry sample wgt)/(1- (% moisture of sample/100)) = Original sample wgt

The protein content of the tofu samples on an as-is basis was calculated as the weight of the protein divided by the weight of the original sample. To calculate the original weight of the dried, defatted sample, the following calculation was used:

$$\frac{\text{(dried, defatted sample wgt.)}}{1-((\%\ \text{moisture} + \%\ \text{lipid})/100)} = \text{original sample weight}$$

To ease the burden of making these simple but tedious calculations, a simple program was written for use on a Commodore Pet 2001 mini-computer.

RESULTS AND DISCUSSION

Preliminary Work

Solids measurements

Accurate percent solids measurements could be made with a specific gravity hydrometer (calibrated at 20°C) at room temperature for soymilks with a solids content range from 1 to 9 percent. However, when the temperature of the soymilk was raised to 90°C, the hydrometers gave a reading of 0.98 for all of the soymilks. There was no correlation between the percent solids content of hot soymilk and readings on the hydrometer scale. Due to the temperature sensitivity of the hydrometers in this temperature range (80-90°C), its use for the measuring of percent solids levels in hot soymilk was ruled out.

Generally, the refractometer gave lower percent solids readings when compared to the true value (Table 7). Also, a large deviation from the true value was obtained when measuring soymilks made from ethanol-soaked beans. Standard curves produced from these data gave correlation coefficients of 0.98 and 0.97 for the water soak and ethanol soak methods respectively. A 95-percent confidence interval was calculated for refractometer measurements of soymilk solids ranging from 1-9 percent using the following equation:

$$\frac{t(S_{y \cdot x})}{b}$$

In this equation, t is the student t value at the 95% confidence level, $S_{y \cdot x}$ is the average error and b is the slope of the standard curve. The

95% confidence intervals for these soymilks are +/-0.82 percent solids

Table 7. Mean refractometer readings (percent solids) and vacuum oven determinations of percent solids for soymilks prepared from water and ethanol soaked beans

Soaking Procedure	Percent Solids	
	Refractometer	Vacuum Oven
Water	0.0+/-0.0	1.3+/-0.0[a]
	2.0+/-0.3	2.4
	3.0+/-0.0	3.5
	3.5+/-0.5	4.8
	4.0+/-0.3	4.7
	4.5+/-0.6	5.4
	6.0+/-0.6	6.1
	6.5+/-0.5	6.5
	7.0+/-0.3	6.9
	8.0+/-0.3	8.0
	9.0+/-0.3	9.3
Ethanol	0.5+/-0.0	1.6+/-0.0[a]
	2.0+/-0.0	3.1
	2.5+/-0.3	4.1
	3.5+/-0.3	5.1
	4.0+/-0.0	5.8
	4.5+/-0.6	6.1
	5.5+/-0.3	7.1
	7.5+/-0.6	8.1

[a]Standard deviations were less than 0.01 percent solids for all determinations.

and +/-1.03 percent solids (water soaked and ethanol soaked beans respectively). Part of the error associated with this method is due to the scattering of light as it passed through the soymilk, giving a broad diffuse band instead of a sharp clear line.

For the light-scattering study, 2 replications of each of the 9 soymilk treatments (variety vs. processing) gave 18 standard curves of absorbance at 400 nM vs. percent solids. Based on statistical analysis, there was no significant difference in the standard curves within a replication. Therefore, the data from the 2 replications for each treatment were pooled to give 9 standard curves (Figure 8). The curves for Amsoy and Weber soymilks that were prepared from the same process were similar. All slopes for Vinton soymilks were lower than the slopes for Amsoy and Weber soymilks processed alike. Intercepts, slopes and correlation coefficients were calculated (Table 8). With the use of these equations, the calculated 95% confidence interval was only +/-0.3 percent solids.

The mean percent solids of the undiluted soymilks (Table 9) was significantly different at the 99% confidence level. Multiple comparisons made by using Duncan's Multiple Range Test show that the percent solids content of the ethanol-soak soymilks were significantly lower.

The percent solids in soymilks prepared from 2 different varieties of soybeans and ground with 2 different grinders at identical 10:1 water:bean ratios, were not significantly different (Table 10).

Replication of the light scattering study for each treatment were run 2 weeks apart without any significant difference; therefore, the validity of the curves is assured for at least a 2-week period. If the beans are stored in a cool (5°C), dry environment, the accuracy of the

Figure 8. Relationship between percent solids and the absorbance (400nM) of a 1:250 dilution of soymilk for different soybean varieties and processing methods

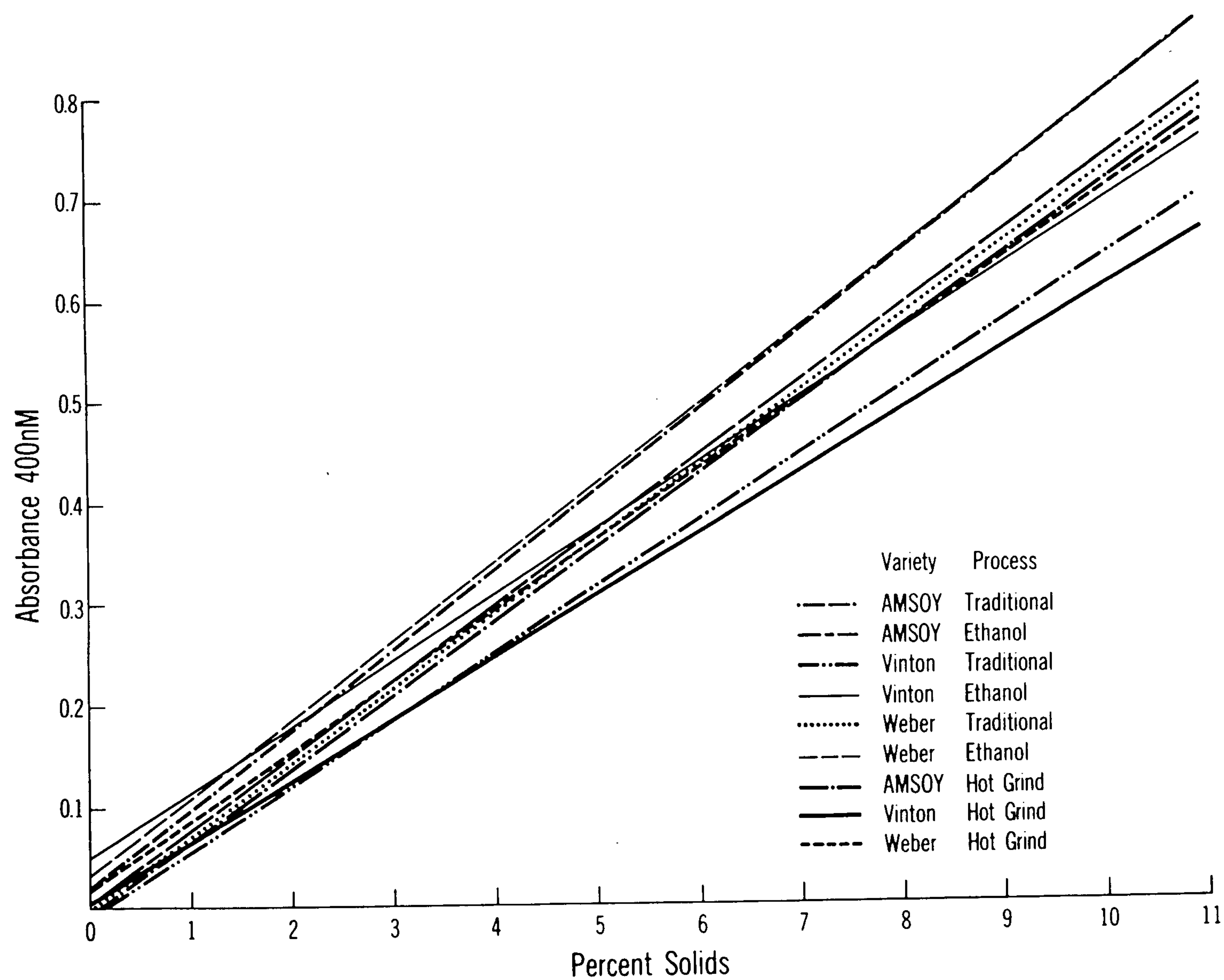

Absorbance 400nM
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
11
Percent Solids
Variety Process
AMSOY Traditional
AMSOY Ethanol
Vinton Traditional
Vinton Ethanol
Weber Traditional
Weber Ethanol
AMSOY Hot Grind
Vinton Hot Grind
Weber Hot Grind

Table 8. Parameters for Light Scattering vs. Percents Solids Standards Curves

Bean Variety	Process	Slope	Intercept
Amsoy	Traditional	0.073	0.003
Amsoy	Alcohol	0.078	0.021
Amsoy	Hot-grind	0.071	0.004
Weber	Traditional	0.073	0.003
Weber	Alcohol	0.076	0.037
Weber	Hot-grind	0.068	0.020
Vinton	Traditional	0.064	0.007
Vinton	Alcohol	0.065	0.051
Vinton	Hot-grind	0.060	0.004

Table 9. Mean percent solids[1] and standard errors of undiluted soymilks

Bean Variety	Process	Mean % Solids	Standard Error
Amsoy	Traditional	8.70 b	0.24
Amsoy	Alcohol	8.10 d	0.26
Amsoy	Hot-grind	8.80 ab	0.26
Vinton	Traditional	8.75 b	0.15
Vinton	Alcohol	8.05 d	0.19
Vinton	Hot-grind	8.85 ab	0.18
Weber	Traditional	9.10 a	0.47
Weber	Alcohol	8.10 d	0.20
Weber	Hot-grind	8.40 c	0.14

[1]Means followed by the same letter were not significantly different (p= 0.05).

Table 10. Influence of soybean variety and grinding apparatus on the mean percent solids of soymilk[a]

Grinding Apparatus	Soybean Variety	
	Weber	Vinton
Blender	6.37+/-0.16	6.54+/-0.18
Vibroreactor	6.40+/-0.10	6.40+/-0.21

[a]Average of three replications.

curves could be extended over a longer time (a standard curve prepared more than 2 months later, by using the traditional method, from soymilk made with the same lot of Weber soybeans was not significantly different from the reported standard curve). Because of the nature of commercial tofu production (small-scale operations), it is debatable whether light scattering would be a useful tool for measuring percent solids in a commercial setting. Variations in tofu due to fluctations in percent solids may be at an acceptable level, meaning that any improvement in accuracy would be unnecessary. Because commercial tofu producers work with large volumes of soybeans, it is unlikely that they would be dealing with an homogeneous bean sample, an important factor in determining the overall accuracy of the standard curves. Likewise, the cost of a spectrophotometer might be prohibitive for smaller tofu operations. For these reasons, the hand-held refractometer still seems more appropriate for percent-solids measurements at the commercial level.

For the research laboratory, the use of light scattering for measuring percent solids could be a useful tool. Certainly, if tofu texture or other characteristics affected by solids content are to be studied, some sort of accurate measure of soymilk concentration is needed.

In the nine different preparations of soymilk, identical volumes of water were added to equal amounts of beans after soaking. There were significant differences in percent solids between many of the soymilk treatments. Differences in soymilk solids can cause differences in tofu texture, yet many past researchers have studied tofu texture without reporting their measurement and adjustment of soymilk percent solids. Smith et al. (1960) reported that there were differences in the texture of tofus made from Japanese and American soybeans. There was no mention of adjusting the percent solids of the different soymilks to a standard level. Since then, there have been numerous reports (Lu et al., 1980; Skurray et al., 1980; Tsai et al., 1981; Wang and Hesseltine, 1982) that have dealt with optimum coagulant concentration or textural differences due to soybean variety but have not reported any attempts to standardize the concentration of their soymilks. Therefore, their work on tofu texture should be questioned because the texture of their tofus may have been affected by unknown variations in the solids content of the soymilk.

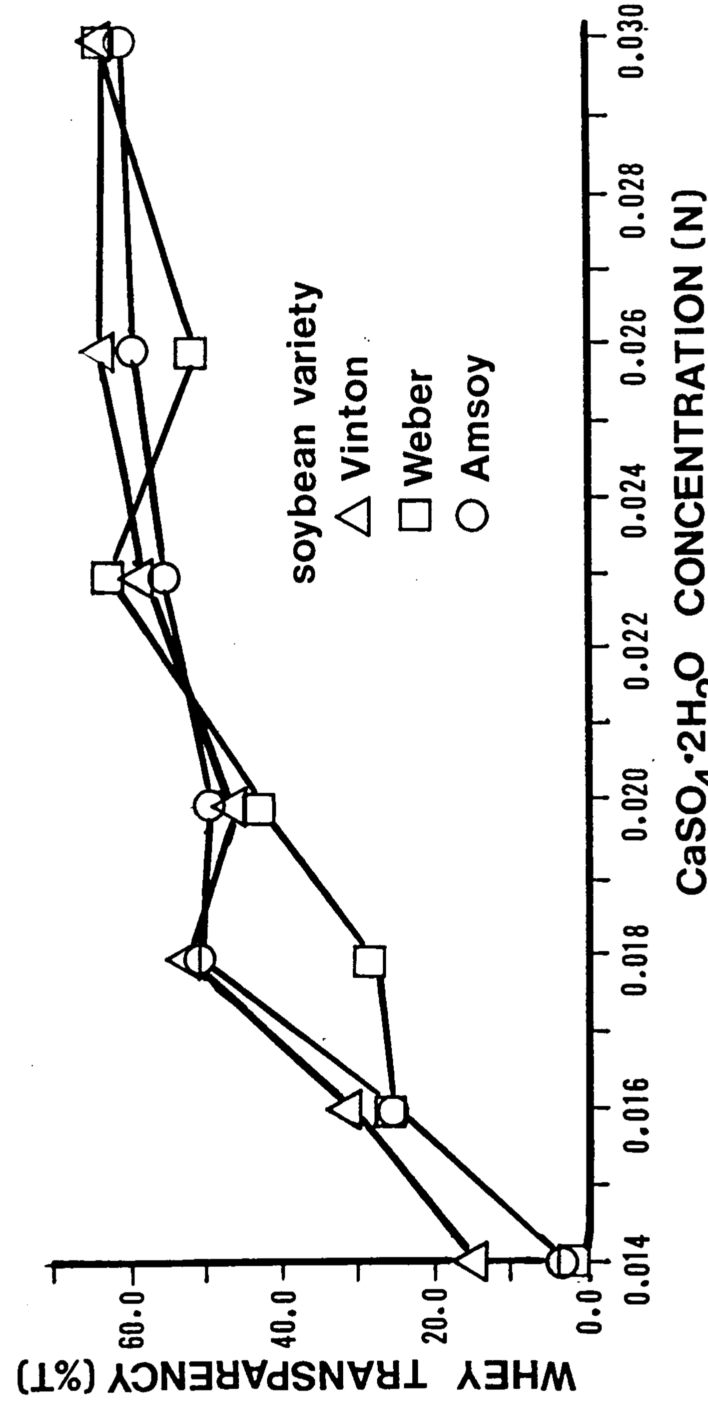
Many researchers claim that by using the same water:bean ratios, equal solids levels of the resultant soymilks will be assured. It was found in our lab that this assumption was correct when we compared the solids contents of soymilks prepared from 2 varieties of soybeans using 2 grinding procedures. This is because adjusting the water:bean ratio effectively corrects for differences in water absorption by different soybeans. However, adjusting the water:bean ratio will not correct for differences in the nitrogen solubility index (which can be significantly affected by heat, storage conditions or processing) of soybeans or the protein extraction efficiency of different grinding apparatus. Therefore, when producing soymilk for tofu research it would be best to measure the percent solids of soymilk rather than assume it is at a certain level. Also, the reporting of soymilk percent solids would aid other researchers in comparing processing methods and in replicating reported techniques.

Ideal coagulant concentration

Figure 9 shows the effect of coagulant concentration on tofu whey transparency for soymilks made from Weber, Vinton and Amsoy soybeans (referred to hereafter as Vinton, Weber or Amsoy soymilk). Maximum whey transparency for 6 percent soymilk was obtained at a concentration between 0.012M and 0.013M (0.023N) $CaSO_4 \cdot 2H_2O$ for each of the three varieties.

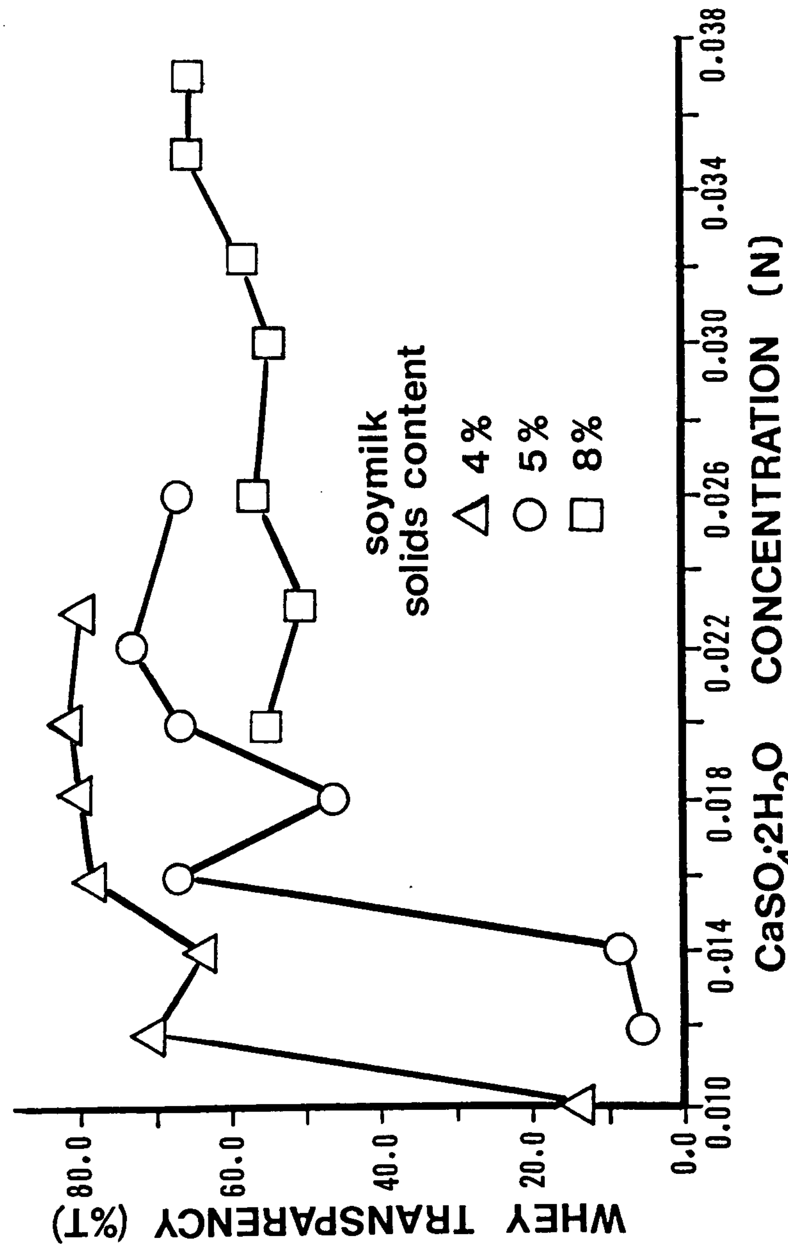
Although there was little difference noted in the required coagulant concentration for each variety, Vinton soymilk failed to coagulate at several concentrations that had previously been

Figure 9. Percent transmittance of whey vs. coagulant concentration for soymilks at 6 % solids made from Weber, Vinton and Amsoy soybeans (a concentration of 0.023N was selected as the optimum coagulant concentration)



WHEY TRANSPARENCY (%T)
0.0
20.0
40.0
60.0
0.014
0.016
0.018
0.020
0.022
0.024
0.026
0.028
0.030
$CaSO_4 \cdot 2H_2O$ CONCENTRATION (N)
soybean variety
Vinton
Weber
Amsoy

Figure 10. Percent transmittance of whey vs. coagulant concentration for Amsoy soymilk at concentrations of 4, 5 and 8 percent solids (concentrations of 0.018N, 0.019N and 0.035N respectively were selected as the optimum coagulant concentrations)


WHEY TRANSPARENCY (%T)
0.0
20.0
40.0
60.0
80.0
0.010
0.014
0.018
0.022
0.026
0.030
0.034
0.038
$CaSO_4 \cdot 2H_2O$ CONCENTRATION (N)
soymilk
solids content
4%
5%
8%

shown to be adequate for coagulation to occur. It was found that Vinton soymilk was more sensitive to inadequate stirring than the other soymilks. However, as long as the coagulant was thoroughly mixed into the soymilk, coagulation was successful.

At coagulant concentrations just below the ideal level, it was noted that although there was complete coagulation with the subsequent formation of clear whey, the curds were extremely fragile and would break apart easily, clouding the whey and lowering the transparency of the whey.

Although no quantitative measurements were taken, it was noted that the hardness and degree of water retention of the curds increased with coagulant concentrations higher than the ideal concentration.

Because little difference was found in the required coagulant concentration for the three varieties, Amsoy soybeans were selected to investigate the effect of soymilk concentration on the required coagulant concentration. Figure 10 shows that there is a trend towards increasing coagulant concentrations as the soymilk concentration increased, in order to maintain clear whey. Concentrations of 0.018N, 0.019N and 0.035N $CaSO_4 \cdot 2H_2O$ were found to be ideal for soymilks at 4, 5 and 8 percent solids respectively. It was also observed that at the ideal coagulant concentrations, the volume of resultant whey decreased with increasing solids content of the soymilk.

The determination of ideal coagulant concentration via the measurement of whey transparency has been criticized because it fails to take into account tofu quality (Shurtleff and Aoyagi, 1979).

Researchers using this method in the past (Watanabe et al. cited in Shurtleff and Aoyagi, 1979; Tsai et al., 1981), however, used centrifugation to separate the curds and whey rather than the traditional method of pressing the whey out with cheese-cloth. As was previously noted, the breaking up of fragile curds (produced by the use of inadequate concentrations) caused clear whey to become cloudy only if the the curds and whey were separated with cheese-cloth. In centrifugation, the small fines that result from fragile-curd breakage tend to settle out with unbroken curds leaving a clear whey supernatant. By using centrifugation, the ideal coagulant concentration is likely to be estimated at a lower value than if cheese-cloth separation had been used. Therefore, past criticisms of this method are not unfounded. If cheese-cloth separation is used, the quality of the curds as well as the completeness of protein coagulation become important factors in determining whey transparency, which makes this method of determination more valid for tofu producers. Cheese-cloth also is considerably less expensive than a centrifuge.

It is difficult to compare coagulant concentrations used in the past by researchers because in many cases soymilk concentrations and/or coagulant concentrations were not reported. Wang et al. (1983) used 0.02M calcium sulfate to coagulate a soymilk made from a 10:1 water to bean ratio (ca. 7% solids). Although a determination of the ideal coagulant concentration for 7 percent soymilk was not completed, if one follows the trend based on 4, 5, 6 and 8 percent soymilks found in our lab, a coagulation concentration of approximately 0.014M calcium sulfate would have been sufficient for coagulation. There is a possibility that the coagulant concentration used by Wang et al. (1983) was in excess.

The higher than average number of incidents of coagulation failure exhibited by Vinton soymilks is cause for concern. It is difficult to say why the extent of stirring was more critical for successful coagulation of only the Vinton soymilk. Saio et al. (1969b) reported that phytic acid in soymilk can retard the coagulation reaction and increase the amount of coagulant needed for coagulation. The Vinton soymilk, although difficult to coagulate, did not require a higher concentration of coagulant. Because we found that adequate stirring may play an important role in successful coagulation, we found it necessary to switch from hand-stirring to mechanical stirring to assure adequate mixing of soymilk volumes of 3 liters or more. Since using an electical stirrer there have been no unsuccessful coagulations.

Adequate stirring, however, cannot make up for incorrect coagulant amounts. To be assured of a successful coagulation, required coagulant amounts, soymilk solids content and soymilk volume must be accurately determined. Most tofu makers, both commercial and those involved in research determine coagulant amounts based on total dry bean weight, water:bean ratios or by guessing. The results of our work show that the if the soymilk volume is known, the solids content of the milk can be used to successfully predict the required coagulant concentration.

Use of total dry bean weight or the water:bean ratio to determine the coagulant amount neglects the influence that protein solubility (commonly expressed as the nitrogen solubility index or NSI) plays in determining the amount of coagulant needed for coagulation. Other factors previously mentioned may also influence the amount of solids recovered and increase the uncertainty involved in the coagulation process. Because of this uncertainty, tofu producers are forced to work with relatively small amounts of soymilk to insure against losses in the event of an unsuccessful coagulation. By determining solids, total soymilk volume and correct coagulant concentration, the uncertainty of coagulation is decreased and large batches of soymilk can be successfully coagulated. This is a prerequisite for an automated tofu operation.

Coagulant concentration and soymilk solids level can also affect tofu texture. From a research standpoint, these two factors must be precisely monitored before any legitimate investigation of other factors affecting tofu texture can be made. From a commercial standpoint, by monitoring these two factors, the consistent production of high-quality tofu becomes a realistic possibility.

Microbial safety of tofu samples

Representative tofu samples had total and psychrotrophic counts of less than 70/gram and were free of coliforms.

Influence of Coagulation Conditions on Consumer Acceptability of Tofu Texture

Table 11 shows the coagulation and pressing conditions, instrumental texture analyses and the resulting texture panel scores of the tofu samples used in the study. Tofu samples with Instron hardness values ranging from 0.76Kg to 8.06Kg were made by varying the coagulation and pressing conditions. Tofu samples with a soft texture were prepared by coagulating 6 percent soymilk at 80°C. The resulting curds were gently transferred to a pressing box and pressed for 15 minutes at 0.69 psi. Extensive breakage of the curds and more rigorous pressing conditions (30 min. at 2.2 psi) failed to produce tofu with a hardness value higher than 1.5 Kg when 6 percent soymilk was coagulated at 80°C.

By diluting the soymilk and increasing the temperature of coagulation, harder tofu was produced with less severe pressing conditions. Tofu with a hardness of 8.06 Kg was produced by coagulating 4 percent soymilk at 90°C, breaking up the curds, removing the excess whey and transferring the curds to a pressing box for pressing at 1.1 psi for 20 minutes.

The Instron Universal Testing Machine proved to be a valuable tool in monitoring and evaluating tofu texture. Since many tofu-makers may be unable to afford such expensive equipment, tofu texture was also evaluated with a penetrometer, which costs considerably less than an Instron. There was a negative correlation (r=-0.92) between penetrometer readings and Instron hardness values (Figure 11). A multiple regression analysis showed that penetrometer readings correlated well with the Instron parameters brittleness, hardness, elasticity and cohesiveness (R^2=0.91).

Table 11. Comparison of tofu samples evaluated during the tofu texture taste panel and some typical commercial tofu brands using the Instron texture profile data

Panel	N	Coagulation Conditions Milk Conc/ Temp °C	Pressing Conditions Min./psi	Instron Texture Profile Analysis[a] 1	2	3	4	5	6	Penetrometer (0.1 mm)	Score[b]
1	34	6%/80	5/0.69 10/1.10	0.38	0.82	14.5	0.21	2.5	0.17	199	54
1	34	4%/90	20/1.10	2.32	4.93	24.7	0.26	30.9	1.27	77	48
2	35	6%/80	5/0.69 10/1.10	0.44	1.06	11.0	0.18	2.1	0.19	188	69
2	35	5%/90	20/0.90	1.71	4.77	21.3	0.23	23.2	1.09	132	63
2	35	4%/90	20/1.10	3.44	8.06	31.0	0.25	62.5	2.01	69	69
3	35	6%/80	15/0.69	0.41	0.76	13.0	0.22	2.2	0.17	209	82
3	35	5%/85	15/0.90	1.27	2.40	23.3	0.30	16.8	0.72	141	53[c]
3	35	4.5%/85	15/1.10	2.13	3.96	26.3	0.27	28.0	1.06	98	76
Commercial Sample #1				0.96	1.96	27.0	0.31	16.3	0.60	-	-
Commercial Sample #2				1.24	3.36	23.0	0.27	21.1	0.92	-	-

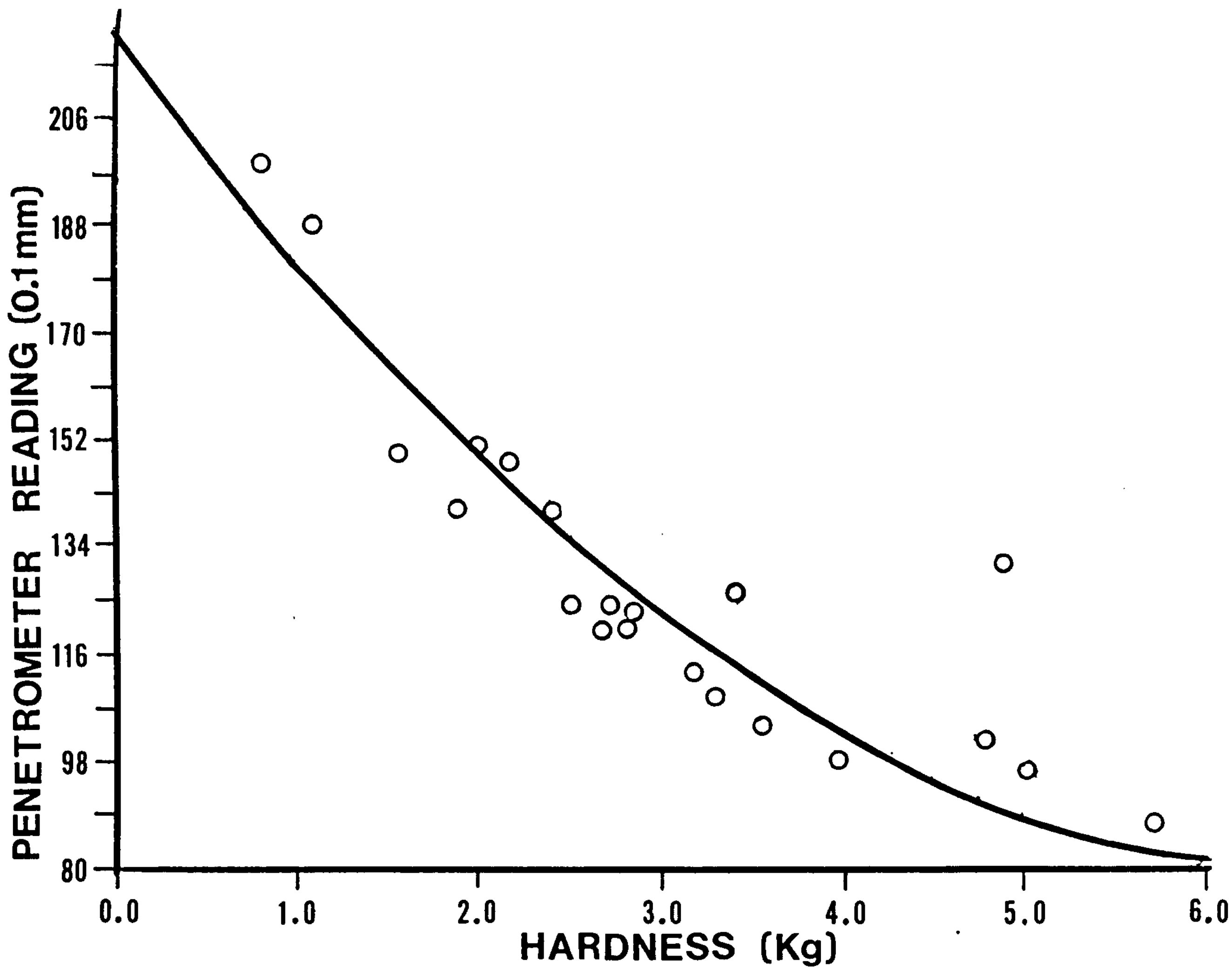
Commercial Sample #3	1.52	4.24	24.0	0.29	29.8	1.24	-	-
Commercial Sample #4	0.48	1.00	13.0	0.18	2.4	0.18	-	-

[a] 1= brittleness; 2= hardness; 3= elasticity; 4= cohesiveness; 5= chewiness; 6= gumminess.

[b] Lower score indicates a preferred sample.

[c] Preference score is statistically significant ($p=0.01$).

Figure 11. Relationship between Penetrometer readings and Instron hardness


PENETROMETER READING (0.1 mm)
206
188
170
152
134
116
98
80
0.0
1.0
2.0
3.0
4.0
5.0
6.0
HARDNESS (Kg)

In the initial taste panel, a hard tofu (4.93 Kg) was tested against a soft tofu (0.82 Kg). The scores shown in Table 11 indicate that there was no significant preference for either tofu. Judging from the comments, the panelists could be divided up into 2 groups, one group preferring the hard tofu that had a meat-like texture, and the second group preferring the softer textured tofu.

In the second taste panel, 3 samples were tested, an extremely hard tofu (8.06 Kg), a hard tofu (4.77 Kg) and a soft tofu (1.06 Kg). Again, there was no significant preference for any of the samples.

Three samples were tested in the third taste panel. One sample was produced with a hardness (2.40 Kg) that represented a compromise between the 2 opposing groups preferences of hard and soft tofu. The remaining 2 samples had hardness values of 0.76 Kg and 3.96 Kg. The taste panel significantly preferred the 2.40 Kg tofu sample. Texture profile analysis run on 4 commercial brands of tofu (Table 11) showed that only one had a texture profile similar to that of the significantly preferred tofu.

Because tofu is a relatively unfamiliar product to most consumers, it is particularly susceptible to a consumers preconceived notions about its organoleptic qualities. Being a source of protein, tofu is expected by many people to have qualities similar to meat, if not in flavor then at least in texture. Others might anticipate a cheese-like texture and flavor. Unfortunately, tofu is quite different from meat or cheese in both texture and flavor.

Because tofu is a bland product, its texture will be an important factor in determining whether it will be accepted as a desirable product. Although an avid tofu user knows that through proper preparation tofu can take on texture qualities ranging from mayonnaise to meat, the "first-time" tofu user is often times at the mercy of the tofu manufacturer when the texture qualities of a tofu dish are to be determined. Therefore, the tofu manufacturer is the most important factor in deciding the popularity of tofu with new consumers.

The results of the taste panel show that consumer texture preferences for tofu vary considerably. The significantly preferred sample merely represents a compromise between this wide range of preferences. As a result, only one panelist rated the preferred sample as least preferred, compared to an average of 14 panelists who rated the other samples presented as least preferred. Unfortunately, only one of the commercial brands of tofu sold locally was even close in texture characteristics.

The tofu industry is struggling for a share of the consumer market. In many cases, however, it is not a question of competing with other tofu producers, but a matter of local consumers accepting the product. When one compares the texture profile analysis of the preferred sample to that of the four commercial brands, one wonders what or who dictated that these textures be used? Assuming that a tofu producer has sufficient control over processing variables to make adjustments in texture, a consumer panel (which can be as unstructured as introducing coded samples to shoppers in a supermarket) seems to be a reasonably accurate means of determining what texture is best.

Past researchers (Watanabe et al., 1964, cited in Shurtleff and Aoyagi, 1979; Saio, 1979; Wang and Hesseltine, 1982) have stated that tofu texture is dependent on several processing variables such as coagulant amount, coagulant type, coagulation temperature, mixing speed and soymilk concentration. In our study, however, texture was successfully controlled by varying neither the amount nor type of coagulant. Although it is possible to control tofu texture by varying the coagulant amount as Skurray et al. (1980) has suggested, it is considered unwise since the addition of excess amounts of calcium sulfate can cause a "chalky" mouthfeel, and excess calciium chloride or nigari will impart a bitter taste to tofu (Shurtleff and Aoyagi, 1979). Insufficient amounts of either coagulant results in incomplete coagulation or fragile curds which can break apart easily.

It has been commonly known that sulfate type coagulants produce soft tofu and that chloride type coagulants produce a firm tofu. Wang and Hesseltine (1982) have stated that the anion type (SO_4^{-2} or Cl^{-1}) of the salt is important because of some type of anion-protein interaction which influences the water-holding capacity of the curds. A simpler explanation is that the anion type merely influences the solubility of the salt. The degree of solubility dictates the rate of salt dissociation and dictates the rate that calcium or magnesium ions can interact with the protein to cause coagulation. Our studies show that tofu texture differences due to coagulant type can be overcome by varying other factors such as soymilk concentration, coagulation temperature, the extent of stirring during coagulation or any factor that increases the rate of coagulation.

Effect of coagulation rate on tofu texture   Saio et al. (1969b) has linked the rate of coagulation with the water-holding capacity of curds. A fast rate of coagulation produces curds with little water-holding capacity. These curds will produce hard tofu. A slow rate of coagulation will produce curds with a high water-holding capacity and soft tofu results. Therefore, any factor that influences the rate of the coagulation reaction will also influence the tofu texture.

Effect of soymilk concentration on tofu texture   The use of soymilks with low solids content (less than 6 percent solids) to produce very firm tofu is a common practice among experienced tofu makers. Saio (1979) reported that the coagulation of soymilks with low solids content produces a precipitant consisting of small protein aggregates which have very little water holding capacity. He attributed the formation of these small aggregates to the lower concentration of soymilk causing an increase in both the rate of heat denaturation and interaction of soy protein with the coagulant.

Effect of coagulation temperature on tofu texture   Watanabe et al., cited in Shurtleff and Aoyagi (1979) has shown that tofu hardness increases with increasing coagulation temperatures. The mechanism of divalent-salt-induced coagulation of soy protein has not been completely elucidated. Hashizume and Watanabe (1979) have established, however, that heating of the soymilk causes the disruption of both the quaternary and tertiary structure of the soy protein. They also concluded that heat-induced conformational changes in the protein exposed sulfhydryl and disulfide groups. Saio et al. (1971) have shown that intermolecular bonds can form between soy protein as a result of the exposure of these groups, and that these bonds contribute to the hardness of tofu gels. When a coagulant such as $CaSO_4 \cdot 2H_2O$ is added to hot soymilk, Ca^{++} ions form electrostatic bridges between protein molecules and cause them to precipitate. The extent of cation-protein interaction is also dependent on the extent of protein unfolding and on the temperature of the soymilk. At low temperatures of below 70°C, there is little protein denaturation, little exposure of disulfide bonds for the formation of intermolecular bonds and poor cation-protein interaction which results in little or poor curd formation.

Influence of Soybean Variety and Method of Processing on Consumer Acceptability of Tofu

Table 12 shows the panelists scores for the tofu samples presented. The ethanol-soak process consistently produced tofus (henceforth referred to as ethanol-soak tofu) that were rated inferior in color, flavor and overall quality when using Prize, Vinton or Weber soybeans. The panelists comments on the ethanol-soak tofus included complaints of off-flavors, darkened color and coarse texture.

No significant preference was found between tofus produced using the traditional and hot-grind methods (henceforth referred to as traditional and hot-grind tofu respectively) when Prize or Vinton soybeans were used. When Weber variety soybeans were used, the hot-grind tofu was rated superior in color, flavor and overall quality when compared to traditional tofu.

In the fourth taste panel, Prize-traditional, Vinton-traditional and Weber-hot-grind tofus were compared. The Weber-hot-grind tofus were ranked significantly inferior in color and overall quality when compared with the Vinton and Prize tofus. There was no significant preference in the flavor of tofu tested in the forth panel.

Two conclusions may be drawn from the above results. One, soybean variety can cause differences in the color, flavor and overall quality of tofu and these differences can be perceived by an untrained group of panelists. Two, the hot-grind method may significantly improve the flavor quality of tofu depending on what variety of soybeans is used. This would imply that some discretion should be used by tofu manufacturers when selecting a particular variety of beans for processing.

The results of the taste panels indicate that Vinton and Prize soybeans may be processed using either the traditional or hot-grind method to yield tofus of equal quality. Weber processed using the hot-grind method only will produce a tofu with a flavor quality equal to that of Vinton or Prize soybeans, however, both the color and overall quality will be inferior. For this reason, Weber would not be ideally suited for tofu-making. The ethanol-soak method should not be used in tofu-making because it yields a product that is inferior in color, flavor and overall quality when compared with tofus made using the traditional and hot-grind methods.

It was noted that the Weber produced tofus that were darker than Vinton or Prize. This dark color may be due to anthocyanins which are the principal pigments making up the dark hilum

Table 12. Influence of soybean variety and processing method on tofu preference scores[1]

	Soybean Variety	Processing Method	Color	Flavor	Overall
Panel 1	Vinton	Traditional	31[a]	47	46[b]
	Vinton	Hot-grind	36	41[a]	46[b]
N=30	Vinton	Ethanol-soak	81[d]	81[d]	78[d]
Panel 2	Prize	Traditional	53[a]	61[b]	59[b]
	Prize	Hot-grind	61	61[b]	54[a]
N=36	Prize	Ethanol-soak	90[d]	96[d]	91[d]
Panel 3	Weber	Traditional	57	67	63
	Weber	Hot-grind	42[a]	50[a]	49[a]
N=33	Weber	Ethanol-soak	81[d]	81[d]	78[d]
Panel 4	Prize	Traditional	49	55	72
	Vinton	Traditional	45[a]	53	60
N=34	Weber	Hot-grind	74[d]	66	75[c]

[1]Letter designations are valid only when comparing scores within a panel.

[a]Significant preference (p= 0.01).

[b]Significant preference (p= 0.05).

[c]Significant non-preference (p= 0.05).

[d]Significant non-preference (p= 0.01).

of certain varieties of soybeans (Howell and Caldwell, 1972). The anthocyanins are water-soluble pigments and may interact with the soy-protein (Smiley and Smith, 1946). Therefore, it is possible that they are co-extracted during the grinding process and carried along with the proteins after coagulation. For this reason, soybean varieties possessing a dark hilum (such as Weber soybeans) should be considered unsuitable for making high-quality tofu.

The flavor characteristics of tofu were also shown to be influenced by the bean variety used. Some bean varieties may produce a tofu with a high-quality flavor with or without using the hot-grind method. However, as in the case of the Weber soybeans, there are some varieties in which the hot-grind method should be used in order to obtain a tofu with a high quality flavor. It is important to realize, however, that although there may be no difference in flavor quality between traditional and hot-grind tofus for some varieties, the actual flavors may be perceivably different.

The results of our consumer panel are in disagreement with those obtained by Wang et al. (1983) where it was concluded that "Soybean variety does not appear to play an important role in tofu processing." The results of our panel show however, that soybean variety should be an important factor in both the selection of the beans and in the way they are processed.

Influence of Soybean Variety and Method of Processing on Tofu Flavor

Because of the short duration of the training period (1 week), many of the panelists could not discern between the flavor attributes astringency and bitterness which resulted in inconclusive data. Therefore these two flavor attributes will not be discussed.

Tables 13 through 16 show the mean flavor scores and tables 17 through 20 the mean aroma scores given to the tofu samples. Serving temperature proved an important factor in detecting differences in the intensity of chicken flavor and aroma. No significant differences could be detected in cold tofu for these two attributes. However, when the tofu samples were served hot, chicken flavor and aroma scores for hot-grind tofus were found to be significantly higher than scores for traditional tofus. Also, Vinton tofus had a significantly higher chicken flavor score than Prize or Weber tofus and a significantly higher chicken aroma score than Weber tofu.

Serving temperature did not affect the panelists ability to detect differences in painty flavor or aroma. Painty flavor and aroma scores for traditional tofus were significantly higher than scores for hot-grind tofus. There were no significant varietal differences in the tofu painty aroma or flavor scores.

The hot-grind method was effective in reducing the painty flavor associated with traditional tofus. Lipoxygenase activity, besides being associated with painty flavors (Snyder, 1973), has also been associated with green-beany (Mattick and Hand, 1969) and rancid flavors (Wilkens et al., 1967). Paintiness was chosen in our study as a suitable descriptive term because it has been strongly associated with lipoxygenase activity (Ashraf, 1979).

Table 13. Effect of soybean variety and method of processing on cold tofu chicken aroma scores[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-Soak	
Prize	10	20	-	15 x
Vinton	20	18	-	19 x
Weber	13	7	-	10 x
Process Means[3]	14 a	15 a		

[1]Mean Square Error = 102 for a variety-process mean (n=4).

[2]Variety and process differences are not statistically significant.

[3]Means followed by the same letter were not significantly different (p= 0.05) Duncan's multiple range test.

Table 14. Effect of soybean variety and method of processing on hot tofu chicken aroma scores[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	26	56	-	41 xy
Vinton	41	74	-	54 x
Weber	28	51	-	39 y
Process Means[4]	32 b	59 a		

[1]Mean Square Error = 156 for a variety-process mean (n=4).

[2]Process differences are statistically significant (p= 0.0001).

[3]Varietal differences are statistically significant (p= 0.0167).

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 15. Effect of soybean variety and method of processing on cold tofu painty aroma scores[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	53	34	-	43 x
Vinton	59	20	-	39 x
Weber	43	33	-	38 x
Process Means[3]	52 a	29 b		

[1]Mean Square Error = 172 for a variety-process mean (n=4).

[2]Process differences are statistically significant (p= 0.0005).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 16. Effect of soybean variety and method of processing on hot tofu painty aroma scores[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	26	14	-	20 x
Vinton	28	8	-	21 x
Weber	32	17	-	25 x
Process Means[3]	29 a	14 b		

[1]Mean Square Error = 107 for a variety process mean (n=4).

[2]Process differences are statistically significant (p= 0.0023).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 17. Effect of soybean variety and method of processing on cold tofu chicken flavor scores[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	14	13	-	14 x
Vinton	21	19	-	20 x
Weber	11	14	-	13 x
Process Means[3]	15 a	16 a		

[1]Mean Square Error = 90 for a variety-process mean (n=4).

[2]Variety and process differences are not statistically significant.

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 18. Effect of soybean variety and method of processing on hot tofu chicken flavor scores[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	15	31	-	23 y
Vinton	21	54	-	33 x
Weber	11	29	-	20 y
Process Means[4]	16 b	37 a		

[1]Mean Square Error = 86 for a variety-process mean (n=4).

[2]Process differences are statistically significant (p= 0.0001).

[3]Varietal differences are statistically significant (p= 0.003).

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 19. Effect of soybean variety and method of processing on cold tofu painty flavor scores[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	56	35	-	46 x
Vinton	40	30	-	35 x
Weber	55	24	-	39 x
Process Means[3]	50 a	29 b		

[1]Mean Square Error = 236 for a variety-process mean (n=4).

[2]Process differences are statistically significant (p= 0.0037).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 20. Effect of soybean variety and method of processing on hot tofu painty flavor scores[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	54	40	-	47 x
Vinton	53	26	-	43 x
Weber	57	36	-	46 x
Process Means[3]	55 a	34 b		

[1]Mean Square Error = 160 for a variety-process mean (n=4).

[2]Process differences are not statistically significant (p= 0.0012).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

The compounds associated with off-flavors in soy products have been identified as aldehydes, alcohols and ketones (Wolf, 1975). These compounds are volatile and may be removed by steaming (Wolf, 1975). This may explain the decrease in painty aroma scores for the tofu served hot. That the painty flavor scores did not decrease with heating may be due to the inside of the tofu not reaching a high enough temperature to release the volatile flavor compounds. These flavor compounds can be bound to the soy protein (Kinsella and Damodaran, 1980; Aspelund and Wilson, 1983) possibly preventing their release until mastication.

Although the hot-grind method is effective in inactivating lipoxygenase and reducing painty flavor, the hot-grind process seems to be giving tofu a chicken-like flavor. The presence of this flavor may be due to a passive phenomena where the off-flavor compounds associated with lipoxygenase activity are not present to mask the chicken flavors. It may also be an active process where the hot-grind method is promoting the production of different flavors. Ashraf (1979) has suggested that the appearance of new flavors with the use of the hot-grind method is merely a masking phenomena. Kinsella and Damadaran (1980) have indicated, however, that the heat inactivation of lipoxygenase can also cause protein denaturation and generate a cooked or toasted flavor. The generation of "cooked" flavors is a common problem in cow's milk heated during the Pasteurization process (Ferretti, 1978). The origin of the chicken flavor detected by the panelists may be due to one or both of the above explanations. No literature was found on possible flavor compounds that could be associated with chicken-like flavors. Isolation and identification of the compound(s) associated with this flavor would help in elucidating its origin.

Influence of Soybean Variety and Method of Processing on Tofu Texture

Results of the Instron texture profile analysis are shown in Tables 21 to 26. Cohesiveness (Table 21) was the only texture parameter that was found not to be significantly influenced by the method of processing

Table 21. Effect of soybean variety and method of processing on tofu cohesiveness as measured by the Instron texture profile analysis[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	0.285	0.291	-	0.287 x
Vinton	0.314	0.277	-	0.296 x
Weber	0.293	0.264	-	0.281 x
Process Means3	0.296 a	0.276 a		

[1]Mean Square Error = 4.4×10^{-4} for a variety-process mean (n=3).

[2]Variety and process differences are not statistically significant.

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 22. Effect of soybean variety and method of processing on tofu brittleness as measured by the Instron texture profile analysis[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	1.808	1.327	-	1.648 x
Vinton	1.929	1.560	-	1.744 x
Weber	1.327	1.022	-	1.196 y
Process Means[4]	1.666 a	1.300 b		

[1]Mean Square Error = 0.052 for a variety-process mean (n=3).

[2]Process differences are statistically significant (p= 0.003).

[3]Varietal differences are statistically significant (p= 0.002).

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 23. Effect of soybean variety and method of processing on tofu elasticity as measured by the Instron texture profile analysis[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	24.9	18.2	-	22.7 xy
Vinton	30.2	22.2	-	26.2 x
Weber	21.8	12.9	-	18.0 y
Process Means[4]	25.2 a	17.7 b		

[1]Mean Square Error = 25.14 for a variety-process mean (n=3).

[2]Process differences are statistically significant (p= 0.025).

[3]Varietal differences are statistically significant (p= 0.005).

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 24. Effect of soybean variety and method of processing on tofu gumminess as measured by the Instron texture profile analysis[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	0.93	0.74	-	0.87 xy
Vinton	1.17	0.89	-	1.03 x
Weber	0.82	0.62	-	0.74 y
Process Means[4]	0.95 a	0.75 b		

[1]Mean Square Error = 0.023 for a variety process mean (n=3).

[2]Process differences are statistically significant (p= 0.008).

[3]Varietal differences are statistically significant (p= 0.011).

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 25. Effect of soybean variety and method of processing on tofu chewiness as measured by the Instron texture profile analysis[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	26.7	24.5	-	25.9 x
Vinton	25.9	24.8	-	25.3 x
Weber	26.3	20.1	-	23.7 x
Process Means[3]	26.3 a	23.0 b		

[1]Mean Square Error = 5.95 for a variety-process mean (n=3).

[2]Process differences are statistically significant (p= 0.016).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 26. Effect of soybean variety and method of processing on tofu hardness as measured by the Instron texture profile analysis[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	3.28	2.55	-	3.03 xy
Vinton	3.72	3.28	-	3.50 x
Weber	2.81	2.34	-	2.61 y
Process Means3	3.23 a	2.75 a		

[1]Mean Square Error = 0.364 for a variety-process mean (n=3).

[2]Varietal differences are statistically significant (p= 0.049).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 27. Effect of soybean variety and method of processing on tofu penetrometer readings[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	110	120	-	115 y
Vinton	104	112	-	108 y
Weber	134	152	-	143 x
Process Means[4]	116 a	127 a		

[1]Penetrometer readings are in units of 0.1 mM.

[2]Mean Square Error = 193 for a variety-process mean (n=3).

[3]Varietal differences are statistically significant (p= 0.0002). (p= 0.05) Duncan's multiple range test.

Table 28. Effect of soybean variety and method of processing on cold tofu firmness scores[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	67	44	-	55 x
Vinton	69	51	-	60 x
Weber	33	20	-	27 y
Process Means[4]	56 a	38 b		

[1]Mean Square Error = 201 for a variety-process mean (n=4).

[2]Process differences are statistically significant (p= 0.0062).

[3]Varietal differences are statistically significant (p= 0.0003)

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 29. Effect of soybean variety and method of processing on hot tofu firmness scores[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	73	62	-	67 x
Vinton	71	72	-	72 x
Weber	43	33	-	38 y
Process Means[3]	61 a	52 a		

[1]Mean Square Error = 243 for a variety-process mean (n=4).

[2]Varietal differences are statistically significant (p= 0.0005).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

and/or soybean variety. Brittleness or fracturability (Table 22), elasticity or springiness (Table 23) and gumminess (Table 24) were all found to be influenced both by soybean variety and method of processing. Differences in chewiness (Table 25) were found to be due to the method of processing. Tofu hardness (Table 26) was influenced by varietal differences.

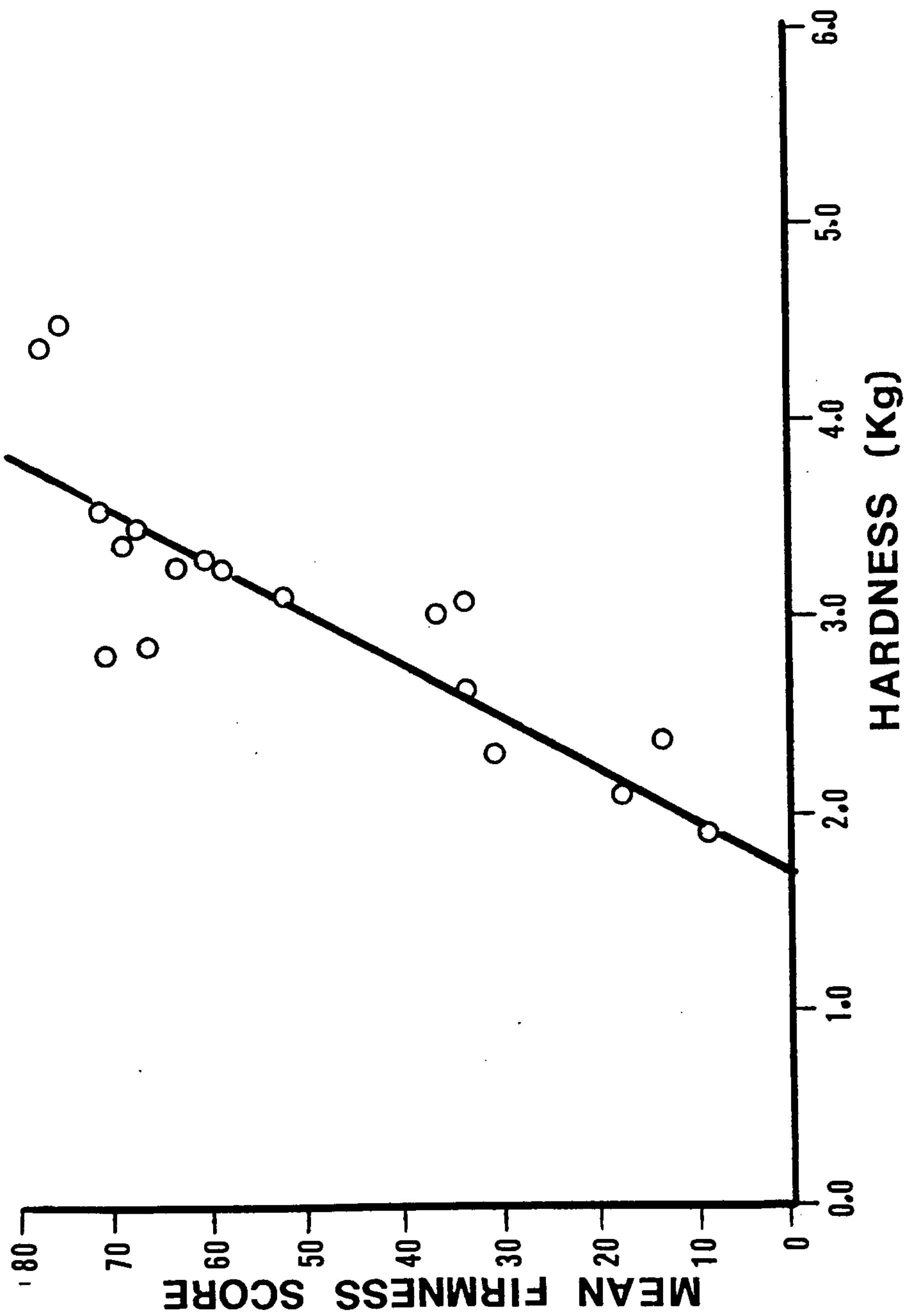
Taking a closer look at the varietal effects on texture shows that Vinton soybeans produced tofu with increased hardness, elasticity and gumminess. Both Vinton and Prize soybeans produced tofu with increased brittleness over tofu made from Weber soybeans. Using the penetrometer, it was found that Vinton and Prize soybeans produced harder tofu than Weber soybeans (Table 27). Sensory panel scores for firmness, which had a correlation of 0.83 with Instron hardness (Figure 12), also showed that Prize and Vinton soybeans produced firmer tofu (Tables 28 and 29).

The hot-grind method caused a significant decrease in tofu Instron brittleness, elasticity, gumminess and chewiness. A sensory panel confirmed these results, rating the hot-grind produced tofu as being significantly less firm than the traditionally made tofu.

Comments were made during the consumer panel about the "mushy" or "watery" texture of tofus made from Weber soybeans. Both the instrumental and sensory panel data confirm these comments. Therefore, it can be concluded that soybean variety is an important factor in determining the texture of the resulting tofu.

Both Wang et al. (1983) and Skurray et al. (1980) have acknowledged the influence of soybean variety on tofu texture; however, they have

Figure 12. Relationship between mean sensory firmness scores and Instron hardness


MEAN FIRMNESS SCORE
0
10
20
30
40
50
60
70
80
HARDNESS (Kg)
0.0
1.0
2.0
3.0
4.0
5.0
6.0

reduced its importance by claiming that varietal influences may be overcome by other factors such as coagulant amount and other processing variables. As was previously stated, relying on coagulant amount to control the texture of tofu is unwise and should not be considered as a primary means for texture control. Although other processing variables (percent solids, temperature and stirring) may exert considerable influence on tofu texture, if these variables are kept under adequate control, as was maintained in our experiment, varietal effects will play an important role in the texture quality of tofu.

For the small-tofu-shop craftsman, who treats each individual batch of tofu with meticulous care, precise control of all processing variables is really not necessary. An experienced tofu-maker can work with the resulting curds during the coagulation process to compensate for any fluctuations in tofu quality due to fluctuations in processing variables. This would not be practical for a factory-scale operation where tofu is mass produced using an abundance of automated equipment and a minimum of human intervention. In an ideal factory-scale operation, there can be precise control of all processing variables. It would be in this setting that tofu textural differences due to variety could show up. As the use of automation increases in the tofu industry and as consumer requirements for a consistent product become more demanding, the role of soybean variety in influencing texture will become more important.

Protein composition

Murphy and Resurreccion (1983) determined the 11S and 7S content of the Weber, Vinton and Prize soybeans used in this study (Table 30). They found a high correlation (r= 0.9) between the glycinin content of these soybeans and the hardness, brittleness, elasticity and gumminess of the resultant tofu. These results are in agreement with Saio et al.

Table 30. Storage protein composition of Weber, Vinton and Prize soybeans used in this study (modified after Murphy and Resurreccion, 1983)[1,2]

Soybean Variety	% Total Protein		11S/7S Ratio
	11S	7S	
Prize	42.6 b	19.7 a	2.16
Vinton	45.6 a	19.0 ab	2.41
Weber	38.2 c	17.9 b	2.13

[1]Sample size n=4.

[2]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

(1969a) where 11S was shown to contribute to the hardness, gumminess and chewiness of tofu. Although there is considerable evidence pointing towards protein as being an important factor in tofu textural quality, other factors such as phytic acid content and the protein content of the soymilk need to be explored.

Processing method

Because of the use of boiling water and the extreme shearing forces that the soybeans are subjected to during hot-grinding, it is reasonable to expect some denaturation of the soy protein. During denaturation disulfide bonds can be broken and the conformation of the protein altered. Saio et al. (1969b) has shown that the hardness of tofu decreases as the rate of coagulation decreases. It is possible that certain conformational changes caused by hot-grinding interfere with calcium-protein interaction thus lowering the rate of coagulation and producing soft tofu.

Influence of Soybean Variety and Method of Processing on the Protein and Oil Content of Tofu

The percent moisture content of tofu shown in Table 31 was significantly reduced by the ethanol soak method. There were no significant differences in the percent moisture of tofu due to soybean variety or the traditional and hot-grind methods.

The percent lipid content of tofu shown in Tables 32 and 33 was affected by the method of processing and soybean variety. There was no significant difference in the lipid content of traditional and hot-grind tofus, however, the lipid content of ethanol-soak tofu was significantly lowered by 30 percent.

Table 34 shows the proximate analysis of the 3 varieties of soybeans used in the study. Comparing the lipid contents of the three bean varieties gives an indication of the relative lipid contents of the resultant tofu. This is illustrated by the fact that Vinton soybeans had the lowest lipid content as did the resultant tofu.

Tofu protein content shown in Tables 35 and 36 was affected by the variety of soybean used. Because only 3 soybean varieties were investigated, no relationship could be established between the protein content of the soybean and that of the resultant tofu. The method of processing had no significant effect on the protein content of the tofu.

Although processing did not have a significant effect on the protein content of the tofu, the actual protein yield (Table 37), that is, the percentage of protein in the soybean that was recovered in the tofu, was reduced significantly when the ethanol-soak method was used. The tofu protein yield was not affected by the variety of soybeans used.

The protein/oil ratio of tofu (Table 38) was influenced by the variety and process and was found to be closely related to the protein content of the beans used.

In any food-processing operation one of the most important factors that must be considered is recovery and yield. It is essential for these two parameters to be maximized if maximum profits are to be attained. For the tofu manufacturer specifically, it is important to obtain the maximum of protein from a given quantity of beans.

Two important factors in determining the amount of protein recovered from the beans is the protein content and the solubility of the protein which is usually measured by the nitrogen solubility index (NSI). Soybeans with a high protein content and a high NSI value should yield more protein than soybeans that have low values for one or both factors. Although protein content is considered a varietal trait, it can be affected by environmental factors such as soil conditions and

Table 31. Effect of soybean variety and method of processing on the percent moisture content of tofu[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	75.3	74.2	74.4	74.6 x
Vinton	76.3	77.2	73.4	75.6 x
Weber	75.9	77.0	74.2	75.7 x
Process Means[3]	75.8 a	76.1 a	74.0 b	

[1]Mean square error = 0.93 for a variety-process mean (n=2).

[2]Process differences are statistically significant (p=0.008).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 32. Effect of soybean variety and method of processing on the percent lipid content of tofu on an as-is basis[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	6.51	6.76	4.81	6.03 x
Vinton	6.28	5.53	2.77	4.86 y
Weber	7.37	6.68	5.63	6.56 x
Process Means[4]	6.72 a	6.32 a	4.40 b	

[1]Mean Square Error = 0.234 for a variety-process mean (n=2).

[2]Process differences are statistically significant (p= 0.0001).

[3]Varietal differences are statistically significant (p= 0.0005).

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 33. Effect of soybean variety and method of processing on the percent lipid content of tofu on a dry-weight basis[1,2,3]

Soybean Variety	Processing Method Traditional	Hot-grind	EtOH-soak	Varietal Means[4]
Prize	26.35	26.32	18.76	23.81 y
Vinton	26.45	24.22	10.41	20.36 z
Weber	30.55	28.99	21.81	27.11 x
Process Means[4]	27.78 a	26.51 a	16.99 b	

[1]Mean Square Error = 3.52 for a variety-process mean (n=2).

[2]Variety differences are statistically significant (p= 0.0005)

[3]Process differences are statistically significant (p= 0.0001).

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 34. Proximate analysis of soybeans used in this study[a]

Soybean Variety	% Moisture	%Lipid	%Protein	Protein/Lipid Ratio
Prize	4.84+/-0.45	21.38+/-1.0	39.52+/-0.16	1.85
Vinton	5.72+/-0.11	17.07+/-0.19	40.81+/-0.43	2.39
Weber	3.83+/-0.17	23.02+/-0.38	37.11+/-0.59	1.61

[a]Sample number n=3.

Table 35. Effect of soybean variety and method of processing on the percent protein content of tofu on an as-is basis[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	14.90	15.33	15.31	15.18 x
Vinton	14.19	13.76	15.67	14.54 xy
Weber	13.56	12.76	14.40	13.57 y
Process Means3	14.22 a	13.95 a	15.13 a	

[1]Mean Square Error = 0.754 for a variety-process mean (n=2).

[2]Varietal differences are statistically significant (p= 0.031).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 36. Effect of soybean variety and method of processing on the percent protein content of tofu on a dry-weight basis[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	60.3	59.4	59.7	59.8 x
Vinton	59.9	60.2	58.9	59.7 x
Weber	56.3	55.4	55.9	55.8 y
Process Means[3]	58.8 a	58.3 a	58.2 a	

[1]Mean Square Error = 5.81 for a variety-process mean (n=2).

[2]Variety differences are statistically significant (p= 03).

[3]Means followed by the same number are not significantly different (p= 0.05) Duncan's multiple range test.

Table 37. Effect of soybean variety and method of processing on the percent recovery of soybean protein in tofu[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	62.0	57.4	41.7	53.7 x
Vinton	59.2	59.8	41.2	53.4 x
Weber	54.6	55.1	43.0	50.9 x
Process Means[3]	58.6 a	57.4 a	42.0 b	

[1]Mean Square Error = 7.98 for a variety-process mean (n=2).

[2]Process differences are statistically significant (p= 0.0001).

[3]Means followed by the same number are not significantly different (p= 0.05) Duncan's multiple range test.

Table 38. Effect of soybean variety and method of processing on the protein-oil ratio in tofu[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	2.29	2.32	3.19	2.60 y
Vinton	2.29	2.50	5.71	3.50 x
Weber	1.85	1.91	2.62	2.13 z
Process Means[3]	2.15 b	2.25 b	3.84 a	

[1]Mean Square Error = 0.071 for a variety-process mean (n=2).

[2]Variety and process differences are statistically significant (p= 0.0001).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

climate. The NSI can be affected by storage conditions, heat and solvents.

Based on our protein recovery data, a tofu producer using the traditional method would spend an extra $7,000 a year to obtain the same amount of protein from Weber soybeans as from Vinton soybeans (based on the daily processing of 1,000 pounds of beans at $6.50 per bushel of beans). A large factory operation processing 6,000 pounds of beans daily would lose $42,000 a year. Therefore, it is in the tofu producers best interest to select those bean varieties that are both high in protein and have a high NSI value.

Unfortunately, protein content and NSI value are not used as criteria for bean quality. Rather, test weight, moisture content and percentages of splits, damaged kernals and foreign material are presently used as the criteria. This puts the responsibility of finding the best beans for tofu production on the tofu producer.

The processing method used by the producer can also affect the NSI of the beans causing a reduction in the protein yield. Borhan and Snyder (1979) showed that the combination of heat and soaking in ethanol solutions used in the ethanol soak process lowered the NSI of soybeans. Therefore, it is the responsibility of the tofu producer to pick a method of processing that will not markedly decrease the NSI of the beans once they are obtained.

The protein content of the tofu produced by the ethanol-soak process was not significantly lower; however, these results may be misleading because the actual yield of protein was significantly lower.

Influence of Soybean Variety and Method of Processing on Tofu Color

All three parameters of the Hunter Lab color system were significantly influenced by the soybean variety and method of processing used. The lightness dimension (Hunter L) for Weber tofu shown in Table 39 was significantly lower than for Prize or Vinton tofus. The Hunter L values for the hot-grind tofus were found to be significantly higher than for the traditional tofus. The green to red color dimension (Hunter a) shown in Table 40 was highest for tofus made from Weber soybeans and/or by the traditional method. The blue to yellow color dimension (Hunter b) shown in Table 41 was highest in tofus made from Vinton soybeans and/or by the hot-grind method

There was very litte color variation within a replicate of tofu. Also, the use of a standard color reference for the standardization of the HunterLab color difference meter aided in improving the overall precision of measurement. Together these two factors resulted in a low mean square error. As a result, measured color differences, although small, were statistically significant. For example, there was just over a 1 unit difference in the Hunter L dimension between traditional and hot-grind tofus. Although this was a significant difference statistically, it may have been an insignificant difference on a sensory basis.

The inferior rated color quality of the Weber tofus may be related to either the low Hunter L value, the high Hunter a value or the low Hunter b value. However, it is most likely due to a combination of

Table 39. Effect of soybean variety and method of processing on tofu color (Hunter L) as measured by the Hunter Lab color system[1,2,3]

Soybean Variety	Processing Method			Varietal Means[4]
	Traditional	Hot-grind	EtOH-soak	
Prize	87.61	87.80	-	87.70 x
Vinton	87.06	87.14	-	87.09 x
Weber	82.45	84.42	-	83.44 y
Process Means[4]	85.70 b	86.45 a		

[1]Mean Square Error = 0.701 for a variety-process mean (n=4).

[2]Process differences are statistically significant (p= 0.042).

[3]Varietal differences are statistically significant (p= 0.0001).

[4]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 40. Effect of soybean variety and method of processing on tofu color (Hunter a) as measured by the Hunter Lab color system[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	0.938	0.613	-	0.775 y
Vinton	0.900	0.470	-	0.685 z
Weber	1.463	0.660	-	1.061 x
Process Means[3]	1.100 a	0.581 b		

[1]Means Square Error = 0.0055 for a variety-process mean (n=4).

[2]Process and Varietal differences are statistically significant (p= 0.0001).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

Table 41. Effect of soybean variety and method of processing on tofu color (Hunter b) as measured by the Hunter Lab color system[1,2]

Soybean Variety	Processing Method			Varietal Means[3]
	Traditional	Hot-grind	EtOH-soak	
Prize	13.41	14.15	-	13.78 y
Vinton	14.51	15.55	-	15.03 x
Weber	11.83	13.45	-	12.64 z
Process Means[3]	13.25 b	14.38 a		

[1]Mean Square Error = 0.189 for a variety-process mean (n=4).

[2]Process and Varietal differences are statistically significant (p= 0.0001).

[3]Means followed by the same letter are not significantly different (p= 0.05) Duncan's multiple range test.

these color attributes. Also, during the consumer preference panel, when Vinton or Prize soybeans were used, a significant preference was shown for the color of the traditionally made tofu over the hot-grind tofu. There were significant differences found in all three color dimensions between traditional and hot-grind tofus. The most likely cause for the preference, however, is the Hunter a dimension which differed by approximately 35 percent between the traditional and hot-grind tofus. While the Hunter L and b dimensions differed only by 0.1 percent and 6 percent respectively.

Varietal influences on tofu color are probably due to genetically determined levels of plant pigments such as anthocyanins, which are responsible for the dark colored hilum of some soybean varieties, or carotenoids and flavonoids, which contribute to the yellow color of soybeans. Plant phenols have also been named as a contributor of off-colors associated with oilseed-proteins (Blouin et al., 1981).

CONCLUSIONS AND RECOMMENDATIONS

To accurately determine varietal and processing method effects, it is necessary to control other variables, such as the growing conditions of the soybeans, soymilk solids content, soymilk volume, coagulant amount, coagulation temperature, stirring rate, volume of whey removed prior to pressing, pressing duration and pressure. Preliminary work was carried out to insure that some of these variables could be controlled or monitored. From this preliminary work, it was found that the hand-held refractometer may be sufficiently accurate for the measurement of soluble solids in hot soymilk. The light-scattering method of Johnson and Snyder (1978) is recommended for use in the research laboratory to monitor the soymilk solids level with the accuracy needed for scientific research.

For the determination of the optimum coagulant amount, the method of Watanabe et al., 1964, cited in Shurtleff and Aoyagi (1979) proved reliable in determining the correct coagulant amount for successful and complete coagulation of soymilk. Using this method, it was found that the optimum coagulant concentration is affected by the soymilk solids level.

Soymilk solids concentration, coagulation conditions (coagulation temperature, stirring rate and duration) and volume of whey removed (prior to pressing) are important factors affecting the texture and consumer preference of tofu. Although coagulant type and amount are used to vary the textural characteristics of tofu, this study showed that controlled variation of soymilk solids concentration, coagulation conditions and volume of whey removed are equally effective and can be exploited without the risk of producing chalky or bitter tofu (which results from the use of excess amounts of coagulant) or causing incomplete coagulation of the soymilk (caused by insufficient amounts of coagulant.

Soybean variety and method of processing are important factors that must be considered in successful tofu manufacturing. These two factors also can influence the quality and consumer acceptability of tofu. Soybean variety can cause differences in the color, flavor and overall quality of tofu and these differences can be perceived by an untrained group of consumers.

The hot-grind method may significantly improve the flavor quality of tofu when compared to traditionally made tofu; however, this is dependent on what variety of soybeans are used and an individuals flavor preferences. Ethanol soaking produces tofu that is significantly not preferred in color, flavor and overall quality by consumers.

Results from a trained sensory panel indicate that both soybean variety and method of processing can affect the intensity of chicken-like flavors and aromas in tofu. Tofu painty flavor is reduced by the hot-grind method but was not affected by the soybean varieties used in this study.

For evaluating tofu texture, the Instron Universal Testing Instrument, penetrometer and a trained sensory panel were used. Both the penetrometer readings and sensory panel firmness scores correlated well with Instron hardness determinations. It was found that soybean variety and method of processing can affect the texture of tofu. Other factors in processing may to some extent overcome tofu texture differences due to variety, however, further study needs to be done to demonstrate this.

The lipid content of tofu is affected by the soybean variety and method of processing. The protein content, however, was only affected by the soybean variety. The recovery of soybean protein in tofu was significantly lowered using the ethanol-soak method.

Although both the soybean variety and processing method caused significant differences in the color of tofu (as measured by the Hunter color difference meter), many of these differences may be small enough in magnitude as to be undetected by consumers.

To produce a tofu that is acceptable to American consumers, it is recommended that yellow soybeans lacking a dark hilum be used. Whether the beans are processed using the traditional or hot-grind method will, depend on the variety of beans. The resulting soymilk should be adjusted to a 5 percent solids content and coagulated at 85°C. The amount of coagulant needed should be determined using the method of Watanabe et al., 1964, cited in Shurtleff and Aoyagi (1979). An electric stirring device should be used to insure the adequate dispersal of the coagulant. A volume of whey equalling 20 percent of the total soymilk volume should be removed prior to transferring the curds to a pressing-box. The curds should be pressed for 15 minutes at 0.9 psi to produce a tofu with an Instron hardness of 2.4 Kg or a penetrometer reading of 141 (0.1 mm).

The question of the origin of tofu texture differences due to variety (such as variations in the 11S and 7S composition of soy proteins) needs to be investigated. Before this can be effectively investigated, it is recommended that a mechanical curding and pressing device be designed so that human intervention can be removed from the coagulation process. From the time of coagulant addition to the finished product, there are an infinite number of undefined variables that may be introduced into this process if human intervention is allowed. These unnecessary variables would make it impossible to measure any significant variations due to such factors as the 11S or 7S composition of the protein. Meaningful data will only be generated by the most careful researcher.

The use of a consumer taste panel is the most important link between the tofu producer and the consumer and is highly recommended as a means of determining consumer preferences. Taste panels could be highly effective in screening new varieties of soybeans or new processing methods for their suitability in tofu making. Persons unfamiliar with the correct procedures for setting up a taste panel (including the specific wording of score sheets) are strongly advised to seek out help to prevent the unintentional influencing of panel members.

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