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USING DEVELOPED LABORATORY PROCEDURES FOR DISCRIMINATING POTENTIAL OF SELECTED MICHIGAN-GROWN SOYBEAN VARIETIES FOR SOYMILK AND TOFU PRODUCTION

By

Dianne Thuy Trinh

A Thesis

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

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2001

ABSTRACT

USING DEVELOPED LABORATORY PROCEDURES FOR DISCRIMINATING POTENTIAL OF SELECTED MICHIGAN-GROWN SOYBEAN VARIETIES FOR SOYMILK AND TOFU PRODUCTION

By

Dianne Thuy Trinh

Seven soybean seed varieties from two Michigan locations were analyzed for physical properties including seed weight, shape, split seeds, and mixed color of seed coats, and for chemical properties such as moisture and protein contents. All soybeans exhibited significant differences in seed size, moisture content, and protein content. Soybeans from Sanilac County in general had higher seed weight, more split seeds, and were lower in moisture content than those of Allegan County. Using a developed procedure, soymilk was produced from all seven varieties from both locations. Its quality was investigated for temperature, total volume, color, pH, density, total solids, and protein content. Significant differences were detected in soymilk volume and protein content among soybean varieties. Color, pH, density, and total solids were similar among all soybean varieties studied. Soybeans from Sanilac County produced higher soymilk volume than those of Allegan County. Tofu, produced with a developed procedure from all seven varieties from both counties, was evaluated for quality. Yield, moisture and protein contents, and texture were used as parameters to discriminate soybean quality among varieties. Significant differences were exhibited for tofu yield and protein content, but not for tofu moisture and texture. Location and soybean seed size did not affect tofu yield, but there were positive correlations between soybean protein and each of the following: soymilk protein, tofu yield, and tofu protein.

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"Images in this thesis are presented in color."

1. INTRODUCTION

For more than 5,000 years, soybeans have been cultivated in the East and provided a nutritional protein source for human diets in many Asian countries. They have been utilized in the preparation of many soyfood products, which were introduced to the West less than a century ago (Chen, 1989). In recent years, soyfood products have been attracting consumers because of their functional and nutritional values (Lo, 1989). Among these various soyfood products, soymilk and tofu are non-fermented products that have attracted much attention. They are also considered good sources of protein and isoflavones (Messina, 1994). The modernization of technology and the understanding of soybean chemistry have helped improve traditional methods and increase soymilk and tofu production. These new procedures have made soyfood products more palatable and more digestible (Liu, 1999).

Although the U. S. has become the world's largest soybean producer, most of the soybeans grown are used for animal feed or oil production, and this is true of Michigan-grown soybeans as well. If more soybeans were consumed by humans, the crop value would substantially increase. The per capita consumption of soymilk and tofu has increased rapidly, and a large number of other soyfood products have entered commercial production over the past few years (Liu, 1999). Thus, evaluation of different soybean varieties for soymilk and/or tofu production potential is important, and would provide information to breeders for selecting new varieties with soymilk and/or tofu production potential. However, currently no standard procedures for the preparation of soymilk and tofu are available. Therefore, the need for developing standard procedures for preparing both soymilk and tofu is great.

II LITERATURE REVIEW

2.1 Soybeans

Soybeans are considered to be one of the oldest crops of the East and an important agricultural commodity in many Asian countries. They emerged as a domesticated crop around the eleventh century B.C. in Northern China, probably by the early Shang dynasty. The English word "soybean" can be traced back to the beginning of the Christian Era, when the word "shu" was reported to refer to soybean. With traditional processing methods and small-scale production, soybean products such as soymilk, tofu, soy paste, soy sauce, and soy sprouts as well as soybean cultivation in China were introduced to Japan, Korea, and many other countries in Asia around 1,100 years ago. The Japanese modified the traditional Chinese methods and developed a large number of new soyfood products; for example, two of those products are natto (a bacteria-fermented soyfood) and tempeh (a yeast-fermented soyfood) (Liu, 1999).

In 1712, Engelbert Kaempfer, a German botanist, first introduced the soybean to Europe. It was later given its genetical name, *Glycine max*, by a Swedish botanist, Carl Von Linne. Around the mid-eighteenth century, soybeans were introduced to the United States where they were used as a forage crop rather than as food for human consumption. This changed in the early 1900s, when the first crushing plant was built and operated in 1911 to extract soybean oil, and later in 1922, when the first soybean processing plant was developed in Illinois (APV, 1998; Liu, 1999).

In a short period of time, the U.S. became the world's largest soybean producer, responsible for almost half of the total world soybean production by 1995 (Table 2.1)

Table 2.1. World Soybean Production 1997-99

Country	1997-98 1,000 metric tons	1998-99 1,000 metric tons
Canada	2,738	2,737
United States	73,176	74,598
Argentina	19,500	19,900
Bolivia	1,071	620
Brazil	32,500	31,000
Paraguay	2,988	3,000
Italy	1,243	1,192
China	14,728	15,000
India	5,350	6,000
Indonesia	1,306	1,300
Korea, Democratic People's	420	420
Thailand	338	335
World total	158,072	158,931

USDA, 2000.

(Liu, 1999). Almost one-third of the U.S. soybean production was exported to the Netherlands, Japan, China, and other countries during 1997-1999 (Table 2.2); and of the non-exported soybean crop, the majority was pressed for oil, or used for oil products and animal feed. Only 1% of recent total soybean production was processed and used as food for human consumption in the U. S. (Liu, 2000).

Recently, the use of modern technology has improved the quality of soyfood products to suit Western tastes, resulting in subsequent increases in their consumption. Modern technology has also reduced processing costs, and improved shelf life of soyfood products. Recent medical research has delineated the health benefits of soyfoods such as reducing cholesterol level and heart diseases, inhibiting the growth of breast, colon and prostate cancer cells, and preventing osteoporosis and menopausal symptoms (Messina 1994, 1999; Riaz, 1999; Alekel and Germain, 2000). Therefore, soybeans are now considered to be functional foods (Riaz, 1999). The U.S market has classified soyfood products into six groups: soy oil, traditional soyfoods, soy protein products, new-generation soyfoods, soy-enriched foods, and functional soy ingredients/dietary supplements (Liu, 2000).

In general, there are two types of traditional soyfood products: nonfermented and fermented. Soymilk, tofu, soy sprouts, and yuba (soy film) are the most common nonfermented soyfoods, whereas soy sauce, miso, tempeh, and fermented tofu are the most common fermented soyfood products. Among the nonfermented soyfoods, traditional soymilk and tofu were developed by Lord Liu An in China around 164 B.C. (Shurtleff and Aoyagi, 1990).

Table 2.2. International Soybean Trade, 1997-1999

Principal exporting countries

Country	**1997-98** 1,000 metric tons	**1998-99** 1,000 metric tons
United States	23,761	21,813
Brazil	9,325	9,300
Argentina	3,231	3,300
Paraguay	2,390	2,400
Netherlands	560	1,100
Canada	769	850
Others	1,049	826
World total	41,085	39,589

Principle importing countries

Country	**1997-98** 1,000 metric tons	**1998-99** 1,000 metric tons
Netherlands	5,000	4,800
Japan	4,873	4,650
China	2,940	3,850
Germany	3,406	3,650
Mexico	3,479	3,600
Spain	3,044	3,050
Taiwan	2,387	2,200
Korea, Republic of	1,340	1,450
Belgium	1,279	1,200
Indonesia	810	1,150
United Kingdom	1,005	950
Italy	768	820
Thailand	600	750
Others	7,628	7,444
World Total	38,559	39,564

USDA, 2000.

2.1.1 Seed morphology

The soybean belongs to the family of leguminosae, subfamily Papilionoideae. There are three species: *Glycine ussurensis* (wild), *G. max* (cultivated), and *G. gracilis* (intermediate) (Vaidehi and Kadam, 1989). *Glycine max* is the most commonly grown and belongs to the subgenus *Soja* which has 2n = 40 chromosomes (Verma and Shoemaker, 1996).

Food legumes are classified into two types: pulses and oilseeds. Soybeans are oilseeds (Shurtleff and Aoyagi, 1990). Most legume seeds are made up of three parts: the seed coat, embryo, and food storage structures. The soybean seed, however, has only two parts: the seed coat or hull, and the food storage structures. The seed coat accounts for 8-10% of the bean's weight and half of the seed's fiber; it protects the seed from fungi and bacteria. The hilum or seed scar is linear-elliptical in shape. There is a small groove or chalaza of the hilum where the seed coat is joined to the body. Another point at the other end, called the micropyle, is the opening in the seed coat where the primary root of the young seedling comes out and through which the bean respirates. Next to the micropyle is the outline of the hypocotyl or germ, which accounts for 3% of the bean weight. This is the place where the bean sprouts or grows (Williams, 1950; Liu, 1999).

The food storage structures are comprised of two cotyledons, of which the embryo is a part. The cotyledons constitute most of the seed bulk, contain mainly protein and oil, and account for 60-89% of the seed weight. They supply food to the seedling plant during germination and initial growth (Williams, 1950; Liu, 1999).

Different soybean varieties have different shapes, sizes, color of hila and seed coats. Soybean seeds vary in shape, from almost spherical, to oval, to elongated, or flat

(Fig. 2.1). On average, the weight of a single seed is 120-180 mg, but larger seeds may weigh about 260 mg (Disoy, Magna, and Prize varieties), or even more than 500 mg (with certain genotypes in germplasm collections). There is no identified standard color for the hilum: its color is generally reported as black, imperfect black, brown, buff, gray, or yellow. The color of the seed coat appears to be yellow, green, brown, black, or a combination of these colors (Fig. 2.1). Soybeans with dark color of hilum or seed coats lend soymilk and tofu a grayish color; in contrast, soybeans with a light yellow or clear hilum and seed coats give the whitest color for the end products (Shurtleff and Aoyagi, 1990).

2.1.2 Seed composition

Although soybean seeds of different varieties have similar morphology, they may vary in chemical composition. The differences in physical and chemical compositions of soybean seeds depend not only on the variety but also on environmental influences such as climatic conditions, fertilizer level of the soil, locality where the seeds are grown, etc. (Vaidehi and Kadam, 1989).

On a dry basis, the four major chemical components of a soybean seed are proteins, carbohydrates, oil, and ash, constituting about 40%, 35%, 20%, and 5%, respectively, of the total composition (Liu, 1999). Proteins are stored in protein bodies (2-20 μm in diameter). Oil is located in small structures called spherosomes (0.2-0.5 μm) which are located in between the protein bodies (Vaidehi and Kadam, 1989). With a distinct lack of starch, the carbohydrate component consists mainly of sugars (soluble carbohydrates including sucrose, raffinose, stachyose, and verbascose) and fiber (insoluble carbohydrates including cellulose, hemicellulose, and pectin). The major minerals are

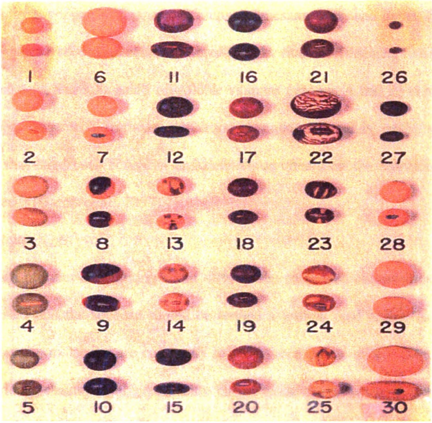

Figure 2.1. Soybean Varieties with Different Shapes and Color of Seed Coats. (William, 1950).

1. Chalky-white
2. Yellow seed coat
3. Green seed coat
4. Maternal green-cotyledon
5. Genetic green-cotyledon
6. Colorless-hilum
7. Colored-hilum
8. "Saddle"-pattern
9. "Saddle"-pattern, black eyebrown
10. Black seed coat
11. Concentric marking of black or brown
12. Flecking of brown or black
13. Noninherited molting
14. Smudging caused by black pods
15. Bloom on soybean seed
16. Black soybean
17. Brown soybean
18. Red-Brown soybean
19. Imperfect-black soybean
20. Buff soybean
21. Reddish-buff soybean
22. Netted cracking of outer layer of seed coat
23. Abnormal hilum and defective seed coat
24. Cracking of seed on buff-colored seed
25. Cracking of seed coat in pigment area
26. Seed of wild soybean
27. Small, flat seed
28. Medium-size round seed
29. Large, round seed
30. Large flat seed

potassium, iron, copper, manganese, calcium, magnesium, zinc, and cobalt (Garcia et al., 1997). Soybean seed also contains water-soluble vitamins (thiamin, riboflavin, niacin, pantothenic acid, and folic acid), oil-soluble vitamins (vitamin A and E) (Liu, 1999), and other minor components (protease inhibitors, phenolic compounds, lectin, saponins, and phytates) that have both beneficial and deleterious effects on the nutritional quality of soybean seeds (Garcia et al., 1997).

2.1.3 Proteins

With proteins constituting about 40% of the total seed composition, Liu (1999) has suggested that the soybean should be termed a protein seed rather than an oilseed. Soybean protein molecules are compactly folded in their native states. The hydrophobic amino acid side chains of polypeptides are buried inside the molecular subunits, exposing the hydrophilic amino acids on the surface. Therefore, native soybean proteins are soluble in water, and about 90% of these proteins can be extracted with water. Additionally, about 90% of soybean proteins can be precipitated at a low pH of 4.5-4.8, and these are referred to as acid-precipitable proteins and play an important role in food processing (Fukushima, 1991).

There are three types of soybean proteins: the proteins involved in metabolism, the structural proteins, and the storage proteins. Storage proteins are found in high concentration, and comprise about 80-90% of the total protein content. They can be fractionated into five groups: 2S (α-conglycinin), 7S (β- and γ-conglycinin), 9S, 11S (glycinin), and 15S globulins (Garcia et al., 1997). The main storage proteins are glycinin and β-conglycinin. Glycinin (11S) and β-conglycinin (7S) constitute about 70% of the total seed proteins (Maruyama and Sato, 1999). Upon heating, these 7S and 11S fractions each form a specific gel that is dependent on the number of free -SH groups and S-S bonds present in amino acid residues that form their respective peptide chains. The 11S globulins have 2 free -SH groups and 20 S-S bonds per molecule. These groups and bonds maintain the molecule's quaternary structure which undergoes only minor change during denaturation. On the other hand, the 7S globulins have no free -SH groups and only 2 S-S bonds per molecule. These globulins interact more upon heating and give a uniform gel with a network-like structure.

Gel formation and the stabilization of network structure also depend on the molecular forces involved during heating such as hydrogen bonding, hydrophobic interactions, ionic interactions and disulfide linkages (Utsumi, 1985). These forces are different within 11S and 7S fractions (Table 2.3). Molecules from the 11S fraction have more affinity to each other than do molecules from the 7S fraction since each fraction retains its respective number of S-S bonds during heating and 11S molecules have substantially more S-S bonds. In contrast, 7S molecules have only hydrogen bonds with which to form gel structures. As a result, gel formed from the 11S fraction is firmer than gel from the 7S fraction (Utsumi and Kinsella, 1985; Fukushima, 1991).

2.1.4 Oil

Oil constitutes about 20% of the total soybean composition. It is composed of 88.1% neutral lipids, 9.8% phospholipids, and 1.6% glycolipids (Garcia et al., 1997; Vaidehi and Kadam, 1989). The neutral lipids primarily consist of triglycerides (99%), small portions of five free fatty acids [palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2), and linolenate (18:3)], sterols, and sterol esters (Yadav, 1996). In addition to some minor phospholipid compounds, there are four major phospholipids

Table 2.3. Possible Molecular Forces Involved in Formation and Maintenance of the Structural Matrix of 11S and 7S Globulin Gels

	Possible molecular forces involved in	
Primary Globulin Component of gel	**Formation**	**Maintenance**
11S	Hydroph obic interactions	
	Electrostatic interaction	Disulfide bonds
	Disulfide bonds	Hydrogen bonds
7S	Hydrophobic interactions	
	Hydrogen bonds	Hydrogen bonds

Utsumi and Kinsella, 1985.

present: 35% phosphatidyl choline, 25% phosphatidyl ethanolamine, 15% phosphatidyl inositol, and 5-10% phosphatidic acid (Liu 1999).

When the seed is cracked during storage or crushed during processing at ambient moisture and temperature, enzymes and substrates interact in the presence of oxygen and moisture resulting in rancidity. Polyunsaturated fatty acids and the enzyme lipoxygenase are accountable for off-flavors and off-aromas of soy and soyfood products during storage and processing. They are also responsible for the beany flavor. The flavor compounds produced are carbonyl compounds, phenolic acids, volatile fatty acids and amines, volatile neutral compounds, alcohols, and phosphatidyl choline (Vaidehi and Kadam, 1989). The undesired flavor limits wide acceptance and consumption of soyfood products by Westerners.

2.1.5 Nutritive value and health benefits

Soybeans are a high source of protein, dietary fiber, micronutrients (folate, iron, zinc, and calcium), and phytochemicals (isoflavones). Its low cholesterol level gives soybean unique nutritional value (Messina, 1999). Soybean protein is highly digestible (92-100%) (Riaz, 1999) and contains most essential amino acids (Table 2.4) in amounts that exceed the amino acid requirements for children and adults; therefore, soybeans are considered a good source of protein among legumes (Garcia et al., 1997). Soymilk made from soybeans also is lactose-free, thus soymilk can be used as a substitute for cow's milk for lactose intolerant people.

Many researchers have demonstrated that consumption of soybeans prevents certain heart diseases and improves kidney function for kidney disease patients (Riaz, 1999; Messina, 1999). On October 20, 1999, the Food and Drug Administration (FDA)

Table 2.4. Amino Acid Composition (g/16g Nitrogen) of Soybeans, Soymilk, and Tofu

Amino Acid	Soybeans	Soymilk	Tofu
Aspartic Acid	12.61	11.91	11.70
Threonine	4.11	4.01	4.00
Serine	5.74	5.19	5.32
Glutamic Acid	19.76	19.61	19.26
Proline	5.53	5.33	5.47
Glycine	4.46	4.16	4.14
Alanine	4.49	4.36	4.11
Valine	3.73	4.88	4.99
Cystine	0.78	0.03	trace
Methionine	1.34	1.59	1.43
Isoleucine	3.46	4.66	4.85
Leucine	7.90	7.94	8.32
Tyrosine	3.90	3.91	3.99
Phenylalanine	4.85	5.15	5.41
Lysine	6.19	6.08	6.41
Histidine	2.60	2.64	2.64
Arginine	8.64	8.65	8.52

Wand and Calvins, 1989.

approved a health claim that consuming 25g of soy protein per day, along with a diet low in saturated fat and cholesterol, will help reduce the risk of coronary heart disease (Ranhotra et al., 2000). Isoflavone compounds in soybeans (1-3 mg/g protein) are found to inhibit the growth of cancer cells, and appear to be associated with lower incidences of breast, colon, and prostate cancers. Soybean intake also lowers osteoporosis and menopausal symptoms (Messina 1994, 1999; Riaz, 1999; Alekel and Germain, 2000). Moreover, soy-based diets can help to control weight because they provide high-quality protein and high nutrient meals, yet are relatively low in calories (Riaz, 1999). Consuming soyfoods has also been reported to reduce gallstone formation, aging, Alzheimer's disease, and to strengthen the immune system, building resistance against cold, flu, and HIV (Riaz, 1999; Arditi et al., 2000); however, scientific studies are needed to confirm these claims. All the above reports promote and attract consumers and processors to soyfood and soy-enriched food products at higher rates than ever.

2.1.6 USDA soybean grades and quality evaluation

In the U. S., according to 1999 regulations, USDA Grade No. 1 soybeans must weigh a minimum of 56 pounds per bushel, not exceed 10% splits (the two cotyledons separated or more than one-quarter of the seed removed), 2% total damaged beans, 0.2% heat-damaged bean, 1% foreign material, nor exceed 1% black, brown, or bicolor beans (Table 2.5). In addition, soybean seeds must have about 13% moisture (Shurtleff and Aoyagi, 1990). Grades No. 2-4 soybeans have progressively lower minimum weight requirements per bushel and are lower in moisture content. They have higher limits for damaged beans, and the presence of foreign material, splits, and soybeans of other color. In the past, inspection was based on visual and subjective evaluation, which left room for

Table 2.5. The U. S. Grades and Grade Requirements for Soybeans

	Numerical Grades			
Grading factors	**1**	**2**	**3**	**4**
Minimum limits of Test weight (lbs/Bu)	56.0	54.0	52.0	49.0
Maximum percent limits of Damaged kernels				
Heat damaged	0.2	0.5	1.0	3.0
Total damaged	2.0	3.0	5.0	8.0
Foreign material	1.0	2.0	3.0	5.0
Splits	10.0	20.0	30.0	40.0
Soybeans of other color	1.0	2.0	5.0	10.0
Maximum count limits of other materials				
Animal filth	9	9	9	9
Castor beans	1	1	1	1
Crotalasia seeds	2	2	2	2
Glass	0	0	0	0
Stones	3	3	3	3
Unknown foreign substance	3	3	3	3
Total	10	10	10	10

Liu, 1999.

human variability and error. Recently, grain inspection has become operated with electronic imaging coupled with computer technology (Liu, 1999).

Soybean quality is determined based on moisture content using a moisture meter, protein content using the Kjeldahl method, and crude fat content using the Soxlet or Goldfish method (Moizuddin et al., 1999). The ratio of 11S to 7S protein components is calculated by quantifying these fractions using a densitometer after sodium dodecyl polyacrylamide gel electrophoresis (Iwabuchi and Yamauchi, 1987). The amount of phosphorus is measured following the method of Lim et al. (1990). Other quality parameters include assessing seed size by weighing 100 seeds, and visually determining the color of hila and seed coats. Splits and cracked seeds are counted and the level of seed cleanliness is checked.

2.2 Soymilk production

2.2.1. Introduction

Soymilk is a milk-like product, extracted from soybeans that have been soaked, followed by cold or hot grinding, filtering, and cooking. This traditional method was developed by Lord Liu An of Huai-nan around 164 B.C.. Soymilk has been used in China and some parts of Asia for a long time; but it was only in 1910 that Yu-Ying-Li, a Chinese who lived in Paris, designed a patent for soy "dairy" production. It was accepted as the first patent for soymilk in Britain. In 1923, a soymilk factory in Changsa, China, first produced soymilk in bottles. Later, in 1936, an American medical doctor, Harry Willis Miller, started the first soymilk plant in Shanghai, China. In 1940, a large-scale commercial soymilk production plant was established by K. S. Lo in Hong Kong, and in

1945, this company was renamed the Hong Kong Soya Bean Products Co. Ltd. and became the producer of the world's first soymilk drink. Now it is named Vitasoy (APV, 1998; Chen, 1989).

Over the past several decades, Chinese and other Asian immigrants have influenced and brought the art of preparing soyfoods, including soymilk, to the West. Now, soymilk is widely consumed as a nutritious drink and in soy-based infant formulas. It can be a substitute for cow's milk for lactose-intolerant people, and is similar to human milk in gross composition (Table 2.6) (Chen, 1989).

Chen (1989) described three factors contributing to the increase in consumption of soymilk: (1) technological improvement, (2) nutrition, and (3) health conscientiousness. To adapt to Western demand, modern technology has modified the traditional Chinese method, and researchers have been developing various procedures to enhance soymilk quality as well as decrease its beany flavor. There are two methods for soymilk preparation: the traditional method, wherein soybeans are soaked and ground in cold water, the slurry is filtered, and the resultant soymilk is cooked (Liu, 1999); and the modern method (hot grinding method), wherein the unsoaked beans are ground in hot water, followed by cooking the slurry and filtering out the soymilk (Wilken et al., 1967). This method reduces the beany flavor by inactivating the enzyme lipoxygenase. Today, many soymilk manufactures apply modified methods described by Schroder and Jackson (1972), Nelson et al. (1976), Johnson et al. (1981), and others, to produce soymilk. These methods involve soaking soybeans that are then blanched and ground in hot water, or even the use of defatted soy or soy flour as starting materials.

Table 2.6. Composition of Soymilk, Cow's Milk, and Human Milk

Item/100g	Soymilk	Cow's milk	Human milk
Calorie (Kcal)	44	59	62
Water (g)	90.8	88.6	88.2
Protein (g)	3.6	2.9	1.4
Fat (g)	2.0	3.3	3.1
Carbohydrates (g)	2.9	4.5	7.1
Ash (g)	0.5	0.7	0.2
Minerals (mg)			
Calcium	15	100	35
Phosphorus	49	90	25
Sodium	2	36	15
Iron	1.2	0.1	0.2
Vitamins (mg)			
Thiamine (B1)	0.03	0.04	0.02
Riboflavin (B12)	0.02	0.15	0.03
Niacin	0.50	0.20	0.20
Saturated fatty acids (%)	40-48	60-70	55.3
Unsaturated fatty acids (%)	52-60	30-40	44.7
Cholesterol (mg)	0	9.24-9.9	9.3-18.6

Chen, 1989.

2.2.2. Traditional soymilk preparation

The traditional Chinese method for preparing soymilk and tofu (Fig. 2.2) is still widely utilized by many soyfood manufacturers. Basically, whole soybeans are soaked in tap water overnight (8-12 hours), drained, washed, and ground with tap water at a water:bean ratio of around 8:1 or 10:1. The slurry is filtered through cheesecloth, the residue called okara is removed, and the filtrate is boiled and stored. Asians have accepted and enjoyed soymilk produced by this method, but Westerners do not like its beany flavor. Many soymilk producers have tried to incorporate other flavors such as chocolate, strawberry, etc., to mask the beany flavor.

2.2.3. Modern soymilk preparation

In 1967, Wilkens and colleagues developed a new method at Cornell University in the U. S.. The unsoaked, dehulled soybeans are ground in hot water, after which the slurry is heated in a steam-jacketed kettle at 80-100°C for 10 minutes to inactivate enzymes. The slurry is centrifuged to separate okara and soymilk. The soymilk is then formulated (adding sugar and flavors), bottled, and sterilized at 121°C for 12 minutes. This is known as the hot-grinding method or the Cornell method. This method has been successful in reducing the beany flavor of the final product.

Commercial soymilk producers have used sodium bicarbonate to improve soymilk flavor. They have also used defoamers, sweeteners, flavorants, vitamins, minerals, stabilizers, soy oil, lecithin, and emulsifiers to increase its creaminess, mouth feel, and to improve its quality and taste. Today, soymilk is variously treated with pasteurization, sterilization, ultra high temperature treatment, and homogenization to increase shelf life

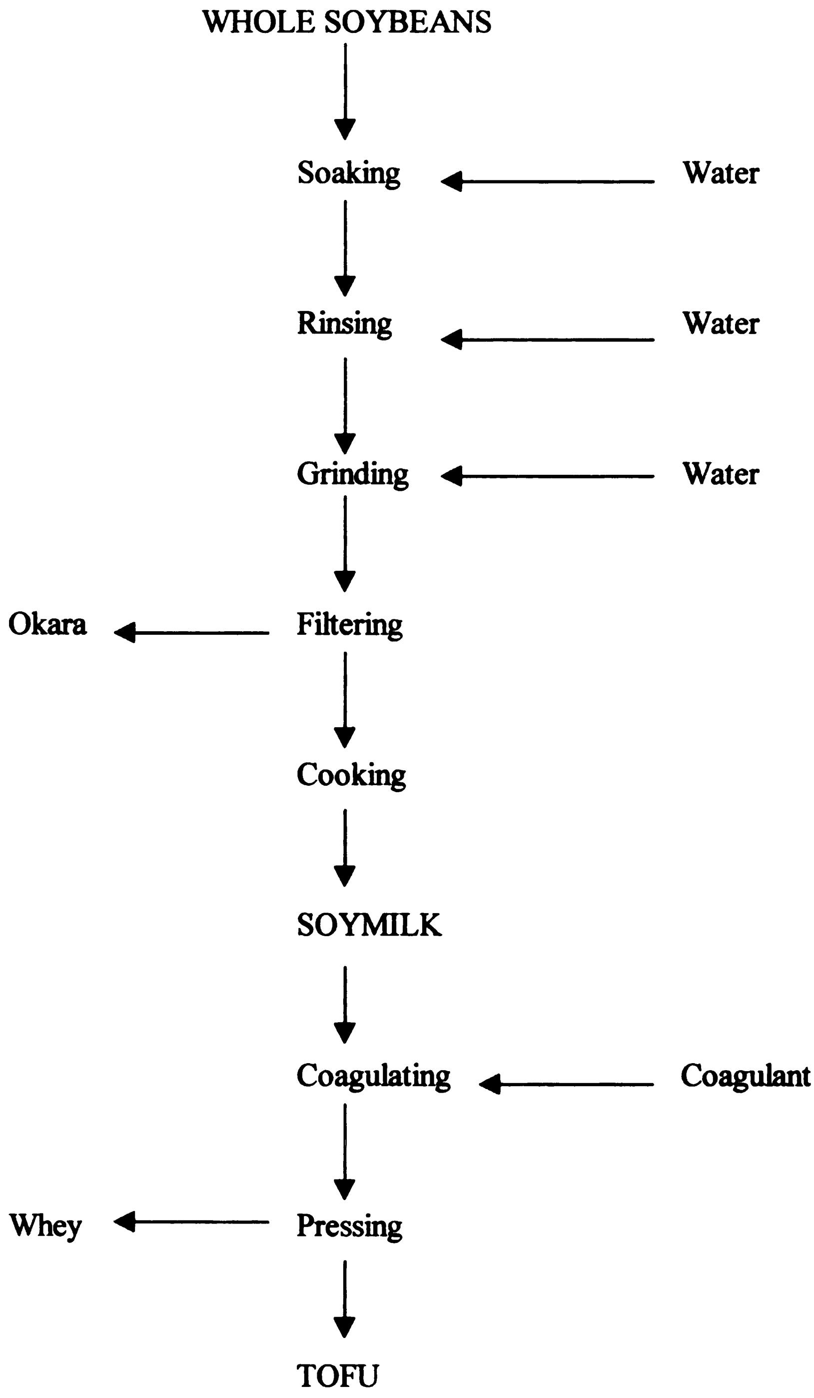

Figure 2.2. Flow Diagram for Preparation of Soymilk and Tofu. (Liu, 1999)

and reduce chalkiness of the soymilk (Chen, 1989). Soymilk is packaged and sold in aseptic cartons, refrigerated containers, or in powdered form.

2.2.4. Soymilk composition

Soymilk contains approximately 90% water, 3.5-4.0% protein, 2.0% fat (about 40-48% saturated fatty acids and 52-60% unsaturated fatty acids), 3.0% carbohydrates, 0.5% ash (calcium, phosphorus, sodium, and iron), and vitamins (thiamin, riboflavin, niacin) (Table 2.6). The total solids and protein contents of soymilk are affected by the water:bean ratio (the total weight of water added during soaking and processing per weight of dry beans) (Chen, 1989).

2.2.5 Soymilk quality

There are many factors that affect soymilk quality: soybean variety, seed size, the percentage of split seeds, the color of hilum and seed coats, total solids content (water:bean ratio), fat, protein, and carbohydrate contents, water source, soaking time, and method of soymilk preparation. The following sections briefly describe some of these factors.

2.2.5.1 Soybean variety

Not all soybean varieties are bred for the same end-use purpose; some are for animal feed and others for oil, soymilk and/or tofu production. In general, high quality varieties for soymilk and tofu have large seeds, a colorless hilum, thin clear and light yellow seed coats, yellow cotyledons, and are higher in protein content and lower in oil content (Liu, 1999). The light colored hilum beans yield the whitest soymilk. The amount of split seeds play an important role in the degree of off-flavor of the soymilk: if the seed lot has a large percentage of split and cracked seeds, natural oils inside the seeds become rancid and affect the flavor of the end products.

2.2.5.2 Beany flavor

The beany flavor of soymilk made with traditional processing has been a main drawback to Westerners. This flavor can be removed by modern processing methods, such as blanching the soybeans prior to grinding (Nelson et al. 1976), or using a hot grinding method with unsoaked soybeans (Wilkens et al. 1967). The enzyme lipoxygenase is responsible for the beany flavor. Activation of this enzyme is initiated by splitting or cracking of the seed, which releases the enzyme, but it requires the presence of oxygen and moisture. If the seeds are soaked overnight (8-12 hours), the enzymes are still inactive; the beany flavor only occurs when the tissues of the cotyledons are damaged by grinding (Liu, 1999). The undesired volatile compounds include ketones, aldehydes, and alcohols (Wilkens et al., 1967; Liu 1999).

With increasing temperature of the slurry (ground beans and water) above 80°C, there is a decrease in both the number and volume of the volatile compounds produced (Liu, 1999). Wilkens et al. (1967) reported that a temperature of at least 80°C is required to prevent the development of volatile compounds. The lipoxygenase enzyme is also inactivated by altering the pH of soymilk to below pH 3 or above pH 10 (Chen, 1989), though products in these pH ranges are not suitable for human consumption.

2.2.5.3 Water:bean ratio

Soymilk is classified as "rich", "dairy-like", and "economy" depending on the amount of water added during soaking and processing (Table 2.7). When less water is added (e.g., the ratio of water:beans is only 5:1 or 6:1 (v/w)), soymilk has a higher solids

Table 2.7. Comparison of Three Basic Categories of Soymilk

Soymilk Type	Water:bean ratio (liter of water/ kg soybean)	Solids (%)	Protein (%)	Fat (%)
Rich	5:1 or 6:1	10-11.5	4.5-5.2	2.8-3.2
Dairy-like	8:1 or 8.5:1	7.4-8	3.3-3.6	2.1-2.3
Economy	10:1	6	2.7-3.3	1.2-1.6

Chen, 1989.

content (protein and fat), and is considered "rich" soymilk (Chen, 1989). This soymilk can be used to prepare silken tofu or yuba. The "dairy-like" soymilk can be used as a substitute for cow's milk because of their similarities in fat and protein contents. When more water is used during processing, the solids content is lower; the final product is referred to as "economy" soymilk and is consumed as a drink (Chen, 1989).

Water comprises a significant portion of the soymilk produced, and as a major volume contributor, can influence the flavor of the end product. To yield best-flavored soymilk, added water needs to be cold and not contain any chemical or other contaminants. Well water or spring water is preferred for soymilk and tofu production as it is cold and does not contain fluorine or chlorine (Shurtleff and Aoyagi, 1990).

2.2.5.4 Soaking time

Soybeans are soaked prior to processing to soften their cellular structures, increase protein and nutrient solubility, reduce the amount of energy required for grinding, decrease cooking time, and increase product yield (Shurtleff and Aoyagi, 1990; Liu, 1999). The soaking time depends on the temperature of the water used since water temperature affects the rate of bean hydration. The higher the temperature, the faster the rate of soybean hydration (Wilkens and Hackler, 1969). In general, soybeans are soaked in ambient temperatures for around 8-12 hours in order to achieve an increase of 2.2 times their initial weight, or an increase of around 2.4 times in volume (Shurtleff and Aoyagi, 1990). Longer soaking time will increase the loss in total solids content (Liu, 1999).

2.2.5.5 Effect of chemical composition

Soymilk has a lower fat content (2.0%) than cow's milk or human milk (3.3 and 3.1%, respectively) (Table 2.6), and is rich in polyunsaturated fatty acids and lecithin.

Lecithins are phospholipids, which are essential components of cell membranes, and essential for the growth, maturation, and functioning of all body cells. Fat in soymilk is highly digestible and has a caloric value of 16.74 cal per 200 ml of soymilk (Ang and Kwik, 1985). Higher fat content also affects the aftertaste of soymilk (sour, bitter, and astringent) resulting from the presence of higher levels of phenolic acids, oxidized phospholipids, and oxidized fatty acids (Liu, 1999).

Final protein content of soymilk (2.7-5.2%) (Table 2.7) depends mainly on the water:bean (v/w) ratio. It influences the color of soymilk; high protein soymilk usually is more opaque in appearance.

Soymilk contains only 2.9% carbohydrates which gives 2.02 cal per 200 ml of soymilk (Ang and Kwik, 1985). To increase the energy and/or the palatability of the product, sweeteners are added (in the form of sucrose) to around 9-10% (weight of sucrose/weight of soymilk) (Chen, 1989). Vitamins and minerals are also added to increase nutritional values and total solids, from around 10% (prior to additives) to 12.5-15.7% (Ang and Kwik, 1985).

2.2.6 Packaging and quality evaluation

Soymilk, with over 90% water, is an ideal medium for bacterial growth. The use of raw materials, the specific processing conditions, and the types of packaging utilized all determine the shelf life of the final product. Commercial soymilk produced using different techniques of pasteurization, sterilization, and ultra high temperature treatment keep soymilk fresh from one week to 6-8 months (Table 2.8).

Table 2.8. Heat Treatment and Packaging Considerations for Commercial Soymilk

Treatment	**Temperature (°C)**	**Time**	**Packaging**	**Shelf life**
Pasteurization	75	15 sec	Plastic bag Gable top Glass bottle	1 week refrigerated
Sterilization	121	20 min	Can Glass bottle Retort pouch	2 years nonrefrigerated
Ultra-high temperature	140	2 sec	Aseptic	6-8 mo. nonrefrigerated

Liu, 1999.

Commercial soymilk with a protein content of less than 2% is identified as soydrink (Ang and Kwik, 1985). In the West, soymilk with milk-like color, bland flavor, minimal aftertaste (sour, bitter, and astringent), and less chalkiness is considered to be high quality soymilk (Liu,1999; Chen, 1989). In general, more dilute soymilk is less preferable by consumers (Liu, 1999).

Soymilk is tested for quality by its manufacturers and in laboratories using many parameters: color with the Hunter Lab method, protein content with the Kjeldahl method, crude fat content with the Goldfish method (Moizuddin et al., 1999), bacteria with a plate count method, total solids with an oven method or following Johnson's and Wilson's method (1984), pH with a pH meter, specific gravity with a Quevenne Lactometer or hydrometer (Chen, 1989). The yield of soymilk is reported as the total volume of soymilk received after filtering. Volatile components in soymilk are determined by gas chromatography (Wilkens et al., 1967).

2.3 Tofu production

2.3.1 Introduction

Tofu (Japanese), Dau hu (Vietnamese), Teou fu or Tou fu ho (Chinese) is a cheese-like product with a mild flavor, a white or creamy color, and a bland taste. The soybean curd is precipitated from soymilk by the addition of coagulants such as calcium and magnesium salts, or an acid (Schaefer and Love, 1992).

The making of tofu is believed to have developed around the same time as that of soymilk, which was around 164 B.C., by Lord Liu An. It was made using nigari or seawater as a coagulant and this traditional method was followed for years. Then around

900 years later, tofu-making spread to Japan and other Asian countries and became popular as a meatless food. The word "tofu" started appearing in writing in the 1500s. The Japanese modified the tofu-making procedure with new coagulants (calcium sulfate, magnesium salts, etc.) and new techniques of cooking (pasteurization, steaming, etc.) (Liu, 1999). For centuries, tofu has been considered a nutritious, inexpensive, and versatile food in the East, but it was introduced to the West only less than a century ago (Liu, 1999). In the U. S., tofu started to appear on the market around 1980 and the first commercial tofu factory in the U. S. went into production around 1990 (Golbitz, 1995). Tofu manufacturers and production have kept increasing rapidly and because tofu has a bland taste, it was more easily accepted in the West than soymilk and has been consumed directly fresh, cooked, fried, or mixed with other ingredients.

Commercial tofu is classified into four types: soft, regular, firm, and extra firm (Shurtleff and Aoyagi, 1990; Liu, 2000), depending on the type of coagulant used, concentration of coagulant used, and pressure applied to the curd as well as pressing time. Tofu is sold in different packages and in several processing types: fresh refrigerated (silken or kinogoshi), pasteurized and refrigerated, aseptic, freeze-dried (kori), deep-fried (namage), etc.. In Japan, refrigerated, pasteurized, and deep-fried forms of tofu are found commonly on the market; on the other hand, only refrigerated and aseptic types of tofu are found in America and Europe (Murphy et al., 1997).

2.3.2 Tofu processing

The traditional Chinese method for preparing tofu was developed many centuries ago, but in 1964, Dr. Tokuji Watanabe and his co-workers at Tokyo's prestigious National Food Research Institute published the first scientific tofu processing method

(Shurtleff and Aoyagi, 1990). There are two main steps for preparing tofu: (1) the preparation of soymilk, and (2) the coagulation of soymilk (Fig. 2.3).

Basically, clean soybeans are first soaked overnight (8-12 hrs.), with a volume of water three times the soybean weight, to obtain double the initial weight of the soybeans. The soaked beans are drained and ground with fresh water (hot or cold). During this step, the beany flavor develops. The amount of water used for grinding is adjusted to a water:bean ratio (water is the total water uptake by the beans during soaking plus the water added for grinding) of 10:1 (Shurtleff and Aoyagi, 1990), 6.5:1 (Prosoya Inc, 1998), or 6:1 (Liu, 1999). The slurry either is filtered to collect soymilk and okara, and the soymilk then cooked; or the slurry is cooked, and then filtered to separate soymilk and okara. Next, soymilk is heated to 70-85°C, and coagulant is added to the soymilk with constant stirring for 30 sec. Soymilk is allowed to coagulate in a covered container for 5 min (Schaefer and Love, 1992), 8 min (Cai and Chang, 1999), 10 min (Ji et al., 1999; Cao and Chang, 1998, 1999), 15-20 min (Shrutleff and Aoyagi, 1990), 30 min (Liu, 1999), or 60 min (Shen et al., 1991; Escueta et al., 1986). The curd is transferred to a tofu box for molding and pressing for about 15 min (Lim et al., 1990), 30-60 min (Shurtleff and Aoyagi, 1990), or overnight (Schroder and Jackson, 1972). Tofu is covered with fresh water and stored under refrigerated conditions.

2.3.3 Tofu composition

The composition of tofu varies depending on the type: 88-90% water and 6% protein for soft tofu; 84.9% water and 7.8% protein for regular tofu; 79.3% water and 10.6% protein for firm tofu; and extra firm tofu has less than 76% water and more than 10% protein (Saio, 1979; Shurtleff and Aoyagi, 1990). Tofu, in general, also contains

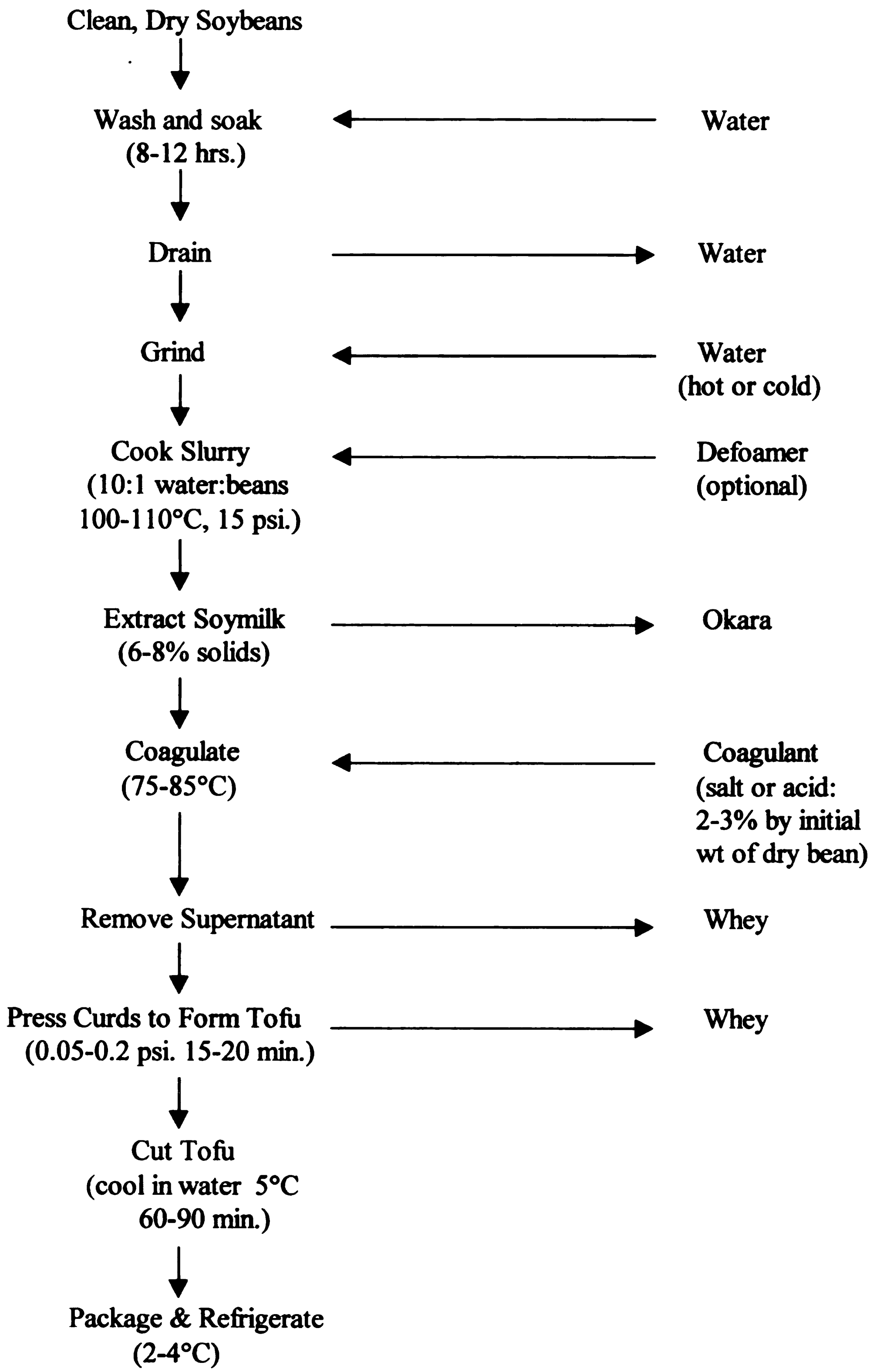

Figure 2.3. Flow Diagram for Tofu Production.

about 3.4-4.3% fat, 1.9-2.3% carbohydrates (including fiber), 0.6-0.7% ash, and vitamins (Smith et al., 1960; Shurtleff and Aoyagi 1990).

Tofu contains 17 out of 20 essential amino acids, the exceptions being asparagine, glutamine, and tryptophan. Glutamic acid and aspartic acid are found in highest concentrations of 19.53% and 11.62%, respectively. In contrast, there is very little cystine in tofu (Schroder and Jackson, 1972; Wang and Calvins, 1989).

2.3.4 Coagulants

Formation of tofu is mainly caused by the coagulation of soymilk proteins by salts, acids, or enzymes (Liu, 1999). For good tofu yield, producers need to choose an appropriate coagulant for each desired type of tofu product.

2.3.4.1 Types of coagulants

Tofu is prepared using many types of coagulants: natural nigari (salt that is extracted from sea water), sea water, magnesium chloride, calcium chloride, calcium sulfate, magnesium sulfate, glucono-delta-lactone (GDL), citrus juices, vinegar, and lactic acid (Shurtleff and Aoyagi 1990; Liu, 1999). In general, commercial tofu is mainly produced with calcium sulfate, GDL, or a mixture of calcium sulfate and GDL, since these coagulants are inexpensive and give higher tofu yield than other coagulants (Tsai et al., 1981).

In the U. S., natural nigari, magnesium chloride, and GDL are not considered GRAS (Generally Recognized as Safe), but this does not mean that they are unsafe to use (Shurtleff and Aoyagi, 1990). On the other hand, food grade calcium sulfate produces the smoothest textured tofu (Smith et al., 1960), increases nutritional value of tofu by increasing its calcium level, gives a bland taste to the soybean curd (Schroder and Jackson, 1972), and increases tofu yield (Shurtleff and Aoyagi, 1990; Metussin et al., 1992). During coagulation, calcium sulfate lowers the pH of soymilk, and calcium ions combine with phytic acid, water and protein molecules to form a larger network structure, increasing the tofu yield (Lim et al., 1990; Cai and Chang, 1998). However, calcium sulfate is very sensitive to the environment and easily absorbs moisture, which lowers its concentration and the subsequent tofu yield; therefore, it should be kept in an airtight container and measured to prepare the coagulant solution just prior to pouring into the hot soymilk (Shurtleff and Aoyagi, 1990).

Glucono-delta-lactone is commonly used to make silken tofu. A mixture of cold soymilk and GDL is poured into sterilized packages, sealed, and heated to 70-90°C for 30-50 min to give silken tofu. There is no pressing step and whey is not separated from this tofu. The action of GDL on curd formation is different than that of other coagulants. In aqueous solution, heat dissociates the GDL to produce gluconic acid, which lowers the soymilk pH, coagulates soymilk proteins to form curd, and gives a mild-tasting final product. Tofu from this aseptic technique is protected from spoilage microorganisms and is more shelf-stable than tofu made with the regular pressing method (Shurtleff and Aoyagi, 1990; Murphy et al., 1997).

In Japan, commercial tofu is prepared with a mixture of GDL and calcium sulfate. Packaged silken tofu uses a coagulant ratio of 4:1 (GDL:calcium sulfate), and unpackaged silken tofu a ratio of 7:3 or 1:1 (GDL:calcium sulfate) (Shurtleff and Aoyagi, 1990). This mixture of coagulants gives better quality tofu and higher tofu yield (Liu, 1999).

2.3.4.2 Concentration of coagulants

Coagulant concentration also plays an important role in preparing tofu. Different concentrations will produce tofu with varying yield, protein content, and texture. According to Tsai et al. (1981), calcium sulfate concentration from 0.025-0.030M gives the best yield of tofu from soymilk and a higher protein content for the tofu. Increasing the concentration from 0.03-0.04M, will decrease the tofu yield, but increase the firmness and hardness (Metussin et al., 1992). Overall, 0.03M of calcium sulfate gives the highest yield and higher protein content of tofu (Metussin et al., 1992).

2.3.4.3 Coagulation time

Shurtleff and Aoyagi (1990) identified that good coagulation was when the soybean curd separated and moved away from the edges of the container. The color of whey is also an indicator of the completeness of coagulation (Moizuddin et al., 1999). The whey needs to be clear, without any white particles of soymilk. Recent studies have used different coagulation times to form soybean curd (see section 2.3.2), and reported tofus with different textures and yields.

2.3.5 Tofu quality

The art of making tofu from soymilk is similar to the making of cheese from cow's or goat's milk. The technique has improved with technology and the understanding of protein chemistry, but making tofu of consistent yield and quality is not easy since there are many variables affecting tofu yield and quality. These variables include the protein content of soybeans, chemical composition of soybeans and soymilk (ratio of 11S to 7S proteins), source of water, total solids content of soymilk, degree of heat-denaturation of proteins, type of coagulants, temperature of coagulant solution, coagulant concentration, stirring speed when adding coagulant to soymilk, weight for pressing tofu, and pressing time (Saio, 1979).

2.3.5.1 Sources of water

Making tofu utilizes a large amount of water, 30 pounds of water per pound of dry soybeans (washing beans 4 lbs, soaking 3 lbs, rinsing 2 lbs, grinding and cooking 8 lbs, cooling tofu 5 lbs, and cleaning equipment 8 lbs) (Shurtleff and Aoyagi, 1990). Therefore, choosing an inexpensive source of water is very important. Tap water containing fluoride and chloride may cause tofu to soften; but if well or spring water is used, minerals such as calcium or magnesium contained in the water will be the main concern. Hard water (with the presence of calcium and magnesium salts, especially calcium carbonate) is more difficult for soybeans to absorb (Jackson and Shin, 1979) and lowers the final tofu yield.

2.3.5.2 Water:bean ratio

The water:bean ratio for soymilk production has been studied as it relates to the final tofu product, with different ranges of water added from 5-10:1 (Tsai et al., 1981), 6:1 (Cai and Chang, 1998), 6.5:1 (Prosoya Inc., 1998), 7:1 (Shen et al., 1991), and 9-14:1 (Beddows and Wong, 1987). Each level of water used gave a different tofu yield and texture. Total solids relates to the protein content of both beans and soymilk. Total solids content of soymilk also relates significantly to tofu yield and is controlled by the amount of water added during grinding. The amount of water that is held in the curd relates to tofu yield. Using different water:bean ratios for tofu processing, Beddows and Wong (1987) reported that a water:bean ratio of 10:1 gave the best results for the soybean varieties they tested.

2.3.5.3 11S and 7S fractions

Upon heating, 11S and 7S polypeptides interact with coagulants and form a gel. Within the 11S fraction, disulfide bonds, and hydrophobic and electrostatic interactions form the protein networks; whereas, hydrogen bonds and hydrophobic interactions are important in the formation of gel from the 7S fraction proteins. The dissociation of proteins from both 11S and 7S fractions upon heating helps them interact with each other and form macrocomplexes that act as a matrix for holding water (German et al., 1982; Damodaran and Kinsella, 1982; Utsumi and Kinsella, 1985). In the study of Saio and Watanabe (1978) and Utsumi and Kinsella (1985), pure 11S gels were found to have a higher water-holding capacity and were harder than 7S gels. Based on these observations, it has been hypothesized that increasing the ratio of 11S to 7S globulins (1.6 to 3.2) increases tofu firmness because more covalent bonds are produced through disulfide bonding resulting in stronger overall molecular forces and a harder tofu (Ji et al., 1999).

2.3.6 Quality evaluation

To control the quality of tofu, various evaluating methods are used by manufacturers and laboratories. These include measuring protein content with the Kjeldahl method, moisture content with an oven- or vacuum-drying method, crude lipids content with the Goldfish method (Schaefer and Love, 1992; Moizuddin et al., 1999) or Soxhlet method (Metussin et al., 1992), and ash content using H_2SO_4/H_2O_2 digestion or a muffle furnace (Metussin et al., 1992). Calcium and magnesium contents are determined by the atomic absorption spectrophotometric method (Cai and Chang, 1998), or with EDTA titration (Hach Inc., 1982). Phytic acid analysis is performed with the Schaefer and Love (1992) method. The amino acid composition is analyzed using the method of

Hackler and Stillings (1967) or with a Beckman Spinco machine (Schroder and Jackson 1972).

The texture of tofu is tested based on seven parameters: hardness, brittleness, chewiness, gumminess, elasticity, cohesiveness, and adhesiveness, all of which can be evaluated with a Texturometer (Bourne et al., 1978). Many researchers have also used Instron profiling analysis to examine the rheological properties of tofu, using the Instron Universal testing machine (Lee and Rha, 1978; Saio, 1979; Lee et al., 1983; Cai and Chang, 1999) and the Rheometer (Tsai et al., 1981).

Yield of tofu is expressed by weight in grams of fresh tofu made from 100g of dry soybean (Wang, 1993; Cai and Chang, 1999), or kg of fresh tofu per kg of soybean (Lim et al., 1990). Color of tofu is determined by the Hunter Lab method (Tsai et al., 1981). Sensory evaluation is performed by panelists with visual examination, finger touching, and a grading scale method (Stone and Sidel, 1993).

Whey is also analyzed for volume, pH with a pH meter, color with Hunter Lab techniques, light transmittance with a spectrophotometer, and conductivity with a conductance meter (Moizuddin et al., 1999).

III RESEARCH OBJECTIVES

The objectives of this proposal are to test the laboratory procedures developed in the preliminary studies for producing (1) soymilk and (2) tofu for the purpose of discriminating among Michigan-grown soybeans for potential soymilk- and/or tofu-making qualities.

IV MATERIALS AND METHODS

4.1 Materials

Different Michigan-grown soybean varieties from 1999 and 2000 crops were used in this study. For developing soymilk- and tofu-making procedures in the preliminary studies, seven soybean varieties (Vinton, Vinton organic, oil feed, GMO, IA 2034, IA 3006, and NK20-20) of the year 1999 were used (Data obtained from these premilinary studies were listed in Appendices I, II, V, VI, VII, VIII, and IX). To test the procedures and discriminate among Michigan grown soybean varieties, seven soybean varieties of the year 2000 (IA 2020, IA 2034, Steyer, HP 204, Vinton 81, Novartis S20F8, and Novartis S2020) from two counties (Allegan and Sanilac) were selected.

4.2 Methods

The harvested seed samples were stored in labeled containers at 4°C in a refrigerator until use. Four phases were planned for testing the developed laboratory procedures for making soymilk and tofu.

Phase I

The physical (shape, seed weight, mixed color of seed coats, and percent of split seeds) and chemical (moisture content and protein content) characteristics of each variety from the 2000 Michigan-grown soybean crops were evaluated.

Phase II

The analyzed soybean varieties were used to prepare soymilk using the VS 40 Prosoya Soycow System (Prosoya, Ottawa, Canada). Three replicates for each variety at each location were done. Total volume, temperature, density, color, pH, total solids, and protein content of each soymilk sample were determined.

Phase III

Soymilk from each of the soybean varieties was used to prepare tofu using calcium sulfate as the coagulant. Two replicates from each variety at each location were used from which to collect quality data. Yield of tofu was determined as kilograms of fresh tofu per 1 kg of dry soybeans. Moisture and protein contents of the tofu samples were determined and texture analysis was also carried out to determine the fracturability, hardness, cohesiveness, and gumminess of each tofu sample.

Phase IV

Statistical analyses were reported as mean values with standard deviations; the coefficients of variation were calculated, and correlation relationships between parameters were carried out. The Analysis of Variance (ANOVA) was used to examine for significant differences among variables. All of the above parameters listed for Phases I, II, and III were used for comparing the quality of the soybean samples used, and of the soymilk and the tofu prepared from each of the soybean varieties.

4.2.1 Phase I: Soybean analysis

After receiving raw materials, soybeans were kept in airtight containers and stored in a refrigerator (4°C) to maintain quality. Soybean seeds were analyzed for quality based on physical and chemical characteristics (Fig. 4.1).

4.2.1.1 Physical analysis

Soybeans of each variety were tested visually for physical characteristics at room temperature. Three aliquots of one hundred seeds (or part seeds) were taken randomly from each of the soybean bags and subjected to the following analyses. Each analysis was carried out on each aliquot (i.e., three replicates), and results were recorded as percentage mean for each characteristic.

4.2.1.1.1 Seed weight

One hundred soybean seeds were transferred into a pre-weighed aluminum dish (diameter 6 cm, height 1.5 cm). Weight (g/100 seeds) of each variety was recorded and compared.

4.2.1.1.2 Shape

The same 100-soybean seed aliquots from the above analysis were visually inspected to determine whether individual seeds were spherical, oval, elongated, or flat.

4.2.1.1.3 Split seeds

When examining the same 100 soybean seeds, any split seeds (the two cotyledons separated or more than one-quarter of the seed removed) were counted individually and the results were compared with the U.S. Grading Chart (Table 2.5, P. 16).

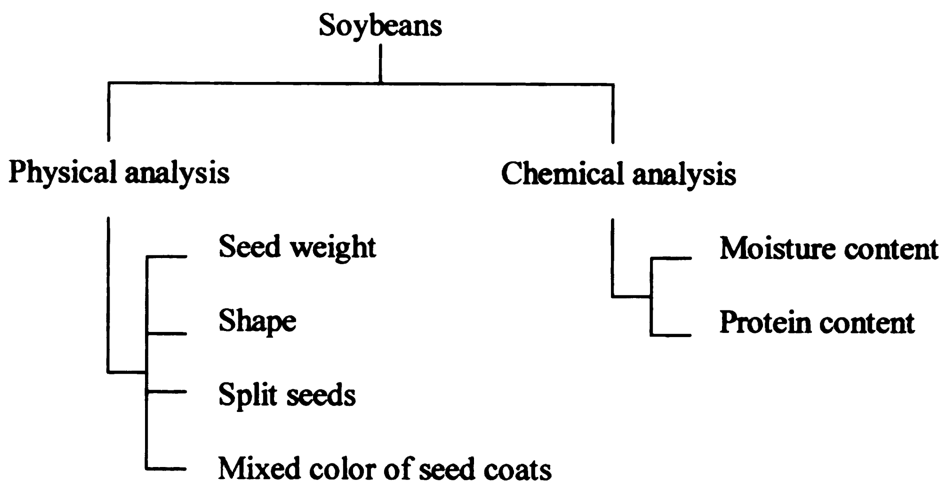

Figure 4.1. Soybean Analyses.

4.2.1.1.4 Mixed color of seed coats

The same 100-soybean seed aliquots were visually examined for mixed color of seed coats: clear, light yellow, yellow, green, brown, black, or a combination of these colors. The percentage of mixed color seed coats was reported.

4.2.1.2 Chemical analysis

Each chemical analysis was done with three replicates for each variety at each location and the results reported in percentage means. Additionally, just prior to protein content analysis, a 250-g soybean seed sample of each variety was ground with a coffee grinder (Model KSM4, Braun Inc., Woburn, MA) until very fine soy flour was obtained.

4.2.1.2.1 Moisture content

Moisture content analysis of whole soybean seeds was performed using a Motomco moisture meter (Model No. 919, Safe-Grain, Inc., Mason, Ohio) according to AACC 44-11 (AACC, 2000). Soybeans were taken out of the refrigerator, still stored in their airtight containers, and left at room temperature overnight. A 250-g sample of soybean seeds was poured into the chamber of the moisture meter after calibration and the seed temperature was recorded. Results were collected, compared with the provided soybean chart from Safe-Grain, Inc., and the moisture content was calculated based on the individual temperature of each seed sample.

The moisture content of each soy flour sample was analyzed following AACC Method 44-31 (AACC, 2000) with some modifications. A 5-g soy flour sample was weighed in a pre-weighed aluminum weighing dish, dried in an air oven (Model 737F, Fisher Scientific Co., Pittsburgh, PA) for 2 hours at 130°C, cooled in a dessicator, and the final dried weight recorded. The percent moisture was determined based on the weight loss of the sample during drying.

4.2.1.2.2 Protein content

The protein content of soybeans was determined by a micro-Kjeldahl procedure following AACC Method 46-13 (AACC, 2000) with some modifications. A 100-mg sample of soy flour was weighed on weighing paper, and then digested for 3 hours with digestion block DS-20 (Tecator, Hoganas, Sweden), followed by distillation with a 1003 Distilling Unit (Tecator, Hoganas, Sweden), and the solution was titrated with HCl for nitrogen content determination. The protein content was calculated with the nitrogen factor of 6.25.

4.2.2 Phase II: Soymilk processing and analysis

Each soybean variety was used to make soymilk with three replicates on three different days. From each batch of soymilk, an aliquot of fresh soymilk was saved and analyzed with three replicates to determine color, pH, density, total solids, and protein content.

4.2.2.1 Soymilk preparation

A modified laboratory procedure for making soymilk was developed in preliminary studies (Fig. 4.2). Soymilk was prepared using a Prosoya Soycow System (Model VS 40, Prosoya Inc., Ottawa, Ontario, Canada). A 1-kg sample of soybeans was washed and soaked in 3 liters of distilled water at 22°C for 16-18 hours. Distilled water was chosen to help eliminate contaminants (such as chlorine and fluorine from tap water), and to control the mineral content of the water and other factors that could affect soymilk

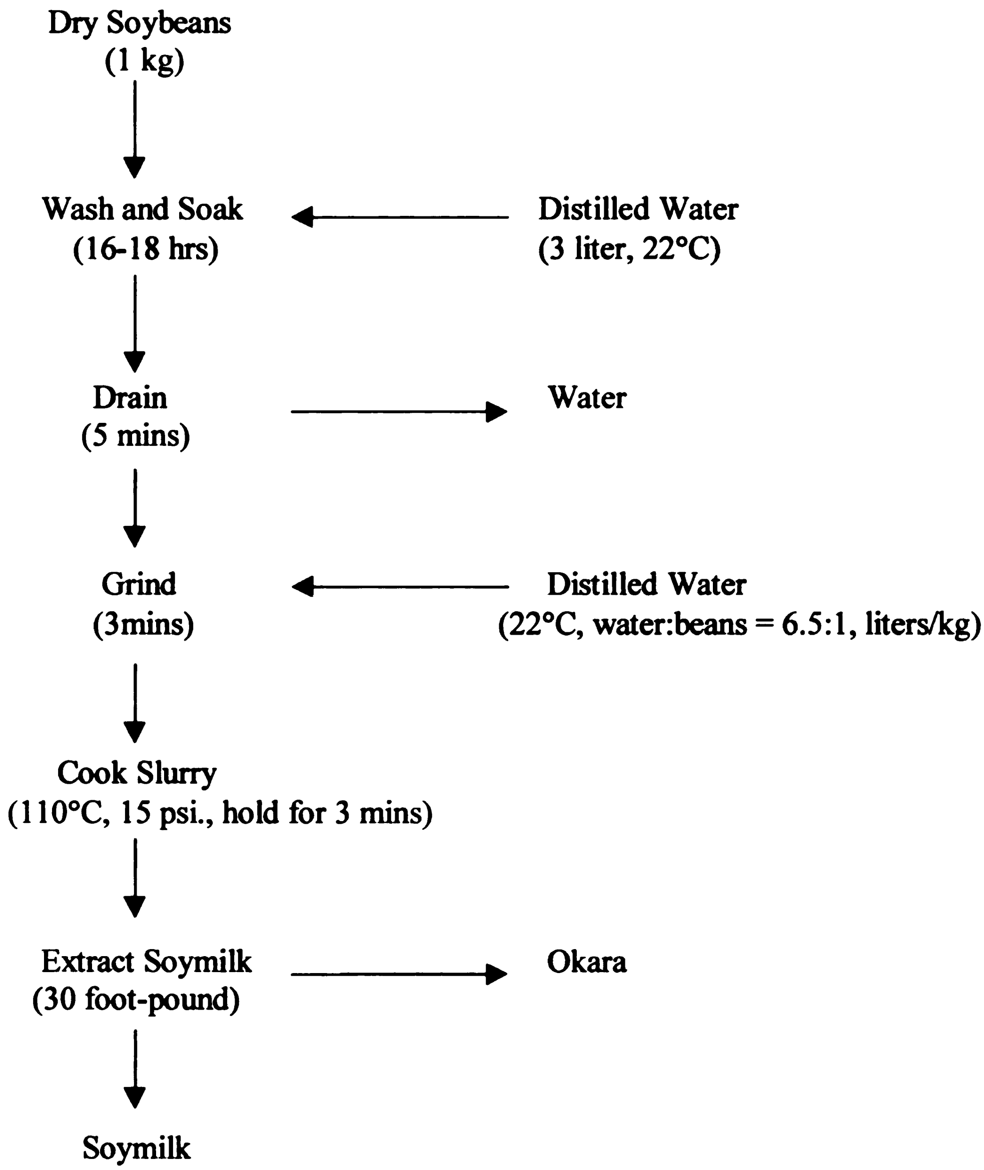

Figure 4.2. Developed Procedure for Making Soymilk.

quality. The soaked beans were drained in a colander for 5 minutes. The amount of water collected from draining was recorded. The drained beans were transferred to the Prosoya Soycow System, as outlined in the manual. The amount of water added to the grinder was adjusted to establish a final water:bean ratio of 6.5:1 (liters/kg of original dry bean weight) (Prosoya Inc., 1998). The soaked beans were then ground for 3 minutes. The slurry was cooked at 110°C until the pressure reached 15 psi; this pressure was held for 3 minutes after which the slurry was transferred to a press (Prosoya Soycow System) that was covered with a sterilized filter bag. The slurry was pressed until 30 foot-lbs of pressure was reached by a torque bar and the soymilk was collected separate from the okara.

4.2.2.2 Soymilk analysis

After filtering, 500 ml of fresh soymilk was saved for analysis (275 ml for color and pH, 200 ml for density, 20 ml for total solids, and 5 ml for protein content). All results were reported as mean values of nine replicates from three batches of soymilk.

4.2.2.2.1 Temperature

Soymilk temperature was measured directly from the flow of soymilk coming from the press. To increase the accuracy, a digital thermometer (Model 15-077-14; Fisher Scientific Co., Pittsburgh, PA) was used. It had a separate probe and results were shown within two seconds.

4.2.2.2.2 Total volume of soymilk

Total soymilk volume was reported as the total volume of milk-like product, extracted from soybeans after filtering.

4.2.2.2.3 Color of soymilk

Color of soymilk was determined with a colorimeter (Model D25-PC2Δ, Hunter Associates Laboratory, Inc., Reston, VA). An 80-ml soymilk sample at room temperature was poured into a testing dish and examined. The results were reported in L-, a-, and b- values, where L=100 indicated white, L=0 was black, negative "a" meant color was towards green, positive "a" meant color was towards red, negative "b" indicated color was towards blue, and positive "b" meant the color was towards yellow.

4.2.2.2.4 pH

Soymilk pH was measured from fresh soymilk at room temperature, the same day as soymilk processing. It was measured with a digital pH meter (Accumet portable Model AP62, Fisher Scientific Co., Pittsburgh, PA), standardized with buffer solutions of 4.0, 7.0, and 10.0.

4.2.2.2.5 Density

The density of soymilk was determined for room temperature fresh soymilk samples on the same day as processing. Soymilk was poured into a pre-weighed 50 ml volumetric flask to measure a 50-ml soymilk sample. This was then weighed and the density of the soymilk in g/ml was calculated.

4.2.2.2.6 Total solids content

The total solids content was determined according to AOAC Method 925.23 (AOAC, 1984) with some modifications. A 5-g fresh soymilk sample (the same day of soymilk processing) was weighed in a pre-weighed aluminum dish, dried in an air oven at 98-100°C for three hours, and cooled in a dessicator. The sample was weighed again and soymilk total solids was reported as percentage of initial weight.

4.2.2.2.7 Protein content

Protein content of soymilk was determined by micro-Kjeldahl Method 46-13 (AACC, 2000) with some modifications. A 1-ml sample of soymilk was pipetted into a Kjeldahl tube and digested for two hours, followed by distillation and titration, and the soymilk protein content was calculated using a Nitrogen factor of 6.25.

4.2.3 Phase III: Tofu processing and analysis

Tofu was produced by coagulating fresh soymilk. After saving an aliquot (about 500 ml) of soymilk for chemical analyses, the remaining soymilk was used for tofu production. Each soybean variety was processed from soybeans to soymilk to tofu two times to evaluate the reproducibility of the process.

4.2.3.1 Tofu processing

A modified laboratory procedure for making tofu was developed in preliminary studies (Fig. 4.3). From each batch of soymilk, two bricks of tofu were made (i.e., two replicates). Tofu was processed with calcium sulfate, following the methods of Shurtleff and Aoyagi (1990) and Prosoya Inc. (1998), with some modifications. After receiving soymilk from the Soycow, one liter of hot soymilk (85-88°C) was transferred to a plastic container and maintained at 80°C (Schroder and Jackson, 1972) in a water bath for 15 minutes (Fig. 4.3). A calcium sulfate solution (0.03M) was prepared at 65°C and the heated plastic container was removed from the water bath. Hot soymilk was stirred five times in a clockwise direction and coagulant solution was immediately added and mixed into the soymilk sample. This mixture was stirred slowly five times in an anti-clockwise direction. The container was covered and the soymilk was allowed to coagulate

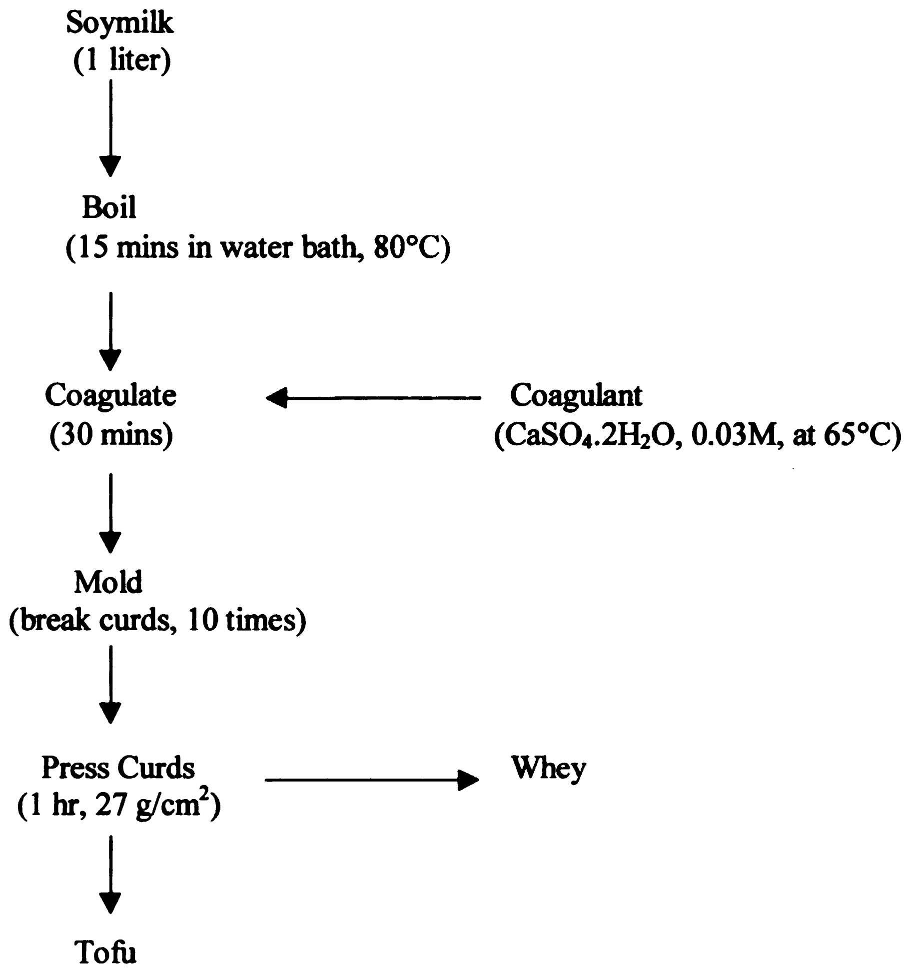

Figure 4.3. Developed Method for Tofu Processing with Calcium Sulfate.

undisturbed for 30 minutes (Liu, 1999) at about 77°C. The curd was then cut into small pieces by stirring 10 times with a regular large table fork to release whey; the curd pieces were transferred to a laboratory-designed tofu box (11.43 x 11.43 x 10.16 cm) lined with cheesecloth for molding (Fig. 4.4). Whey was separated from the curd by pressing for 1 hour with 27 g/cm^2. The tofu was unwrapped from the cheesecloth and the weight of the fresh tofu brick was recorded. The brick of tofu was placed in a labeled container, covered with tap water, and stored in the refrigerator at 4°C for texture, moisture, and protein analyses the next day.

4.2.3.2 Tofu analysis

4.2.3.2.1 Tofu yield

Tofu yield was expressed by weight in kilograms of fresh tofu produced per kilogram of dry soybeans (Wang, 1993; Cai and Chang, 1999). The weight in kilograms of each whole brick of fresh tofu sample was determined; this weight was used to calculate the tofu yield per kilogram of soybeans as follows:

$$\text{Yield} = \frac{\text{Wt of tofu (kg)}}{\text{1 liter soymilk}} \times \frac{\text{Total Volume of soymilk (liter)}}{\text{1 kg dry soybeans}}$$

4.2.3.2.2 Texture

One-day-old tofu brick samples were used for texture analysis using the TA. HDi Texture Analyzer (Texture Technologies, Scarsdale, NY) and examined with two tests: Skin test: The tofu brick was placed underneath the TA 52 probe (2 mm diameter); this test was done three times for each tofu brick sample (two tests randomly on two corners, and one test in the center of the tofu brick). The probe traveled at the speed of 10 mm/sec

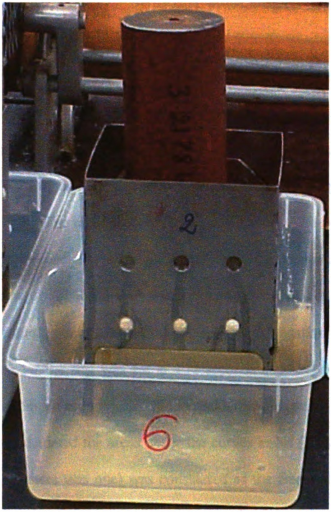

Figure 4.4. Molding and Pressing Tofu.

during the pre- and post-test. When the descending probe touched the sample, the probe speed decreased to 0.5 mm/sec for a distance of 15 mm downward and back up to constitute the test itself, after which the probe released fully back upwards. The time for the experiment was set at 60 sec. Force needed to go through the tofu skin was reported as an average of the three readings from each tofu brick in Newtons (N).

Compression test: The tofu brick was placed underneath the TA 4 probe (40 mm in diameter and 20 mm tall). The test speed was set at 0.5mm/sec. This test was done with two bite cycles: the probe touched the sample and compressed downwards to 60% of the brick height (approximately 21 mm), then released upwards (again, at 0.5 mm/sec), and then performed a second compression bite in the same location using the same parameters. The time for the test was set at 150 seconds and the test was performed randomly on two locations on each brick. All the peaks recorded were reported as forces in N and the program calculated the areas under the curves in Ns (Newton-second) (Fig. 4.5). The first peak (if there was one) was considered as the fracturability of tofu; this reflects the force in N to break the curd. The second peak of the curve was expressed as the hardness of tofu; the force (N) needed to compress the curd. The cohesiveness was determined by the ratio of Area 2/Area 1 (Fig. 4.5). The gumminess (N) was defined as the product of hardness and cohesiveness (Bourne et al., 1978).

4.2.3.2.3 Moisture content

After texture analysis, four pieces of one-day-old tofu were cut from the four corners of a tofu brick, with an area of 2 cm x 2 cm for each piece. The skins from those pieces were discarded, and the interior tofu cut and mixed together to have a uniform curd. Moisture content of the uniformly mixed tofu curd was determined following

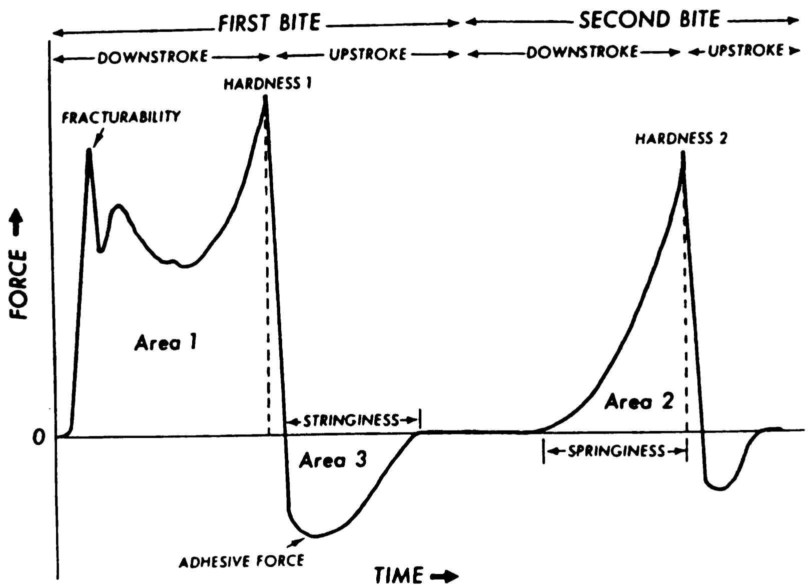

Figure 4.5. Texture Profile Analysis. (Bourne et al., 1978)

AACC Method 44-31 (AACC, 2000) with some modifications. A 5-gram sample of mixed tofu curd was weighed in a pre-weighed aluminum dish. It was further cut into small pieces to increase the surface area and dried in an air oven at 130°C for 2 hours. The sample was cooled in a dessicator and weighed again. Total solids content of each tofu brick was calculated, and the moisture content was determined and reported as a percentage.

4.2.3.2.4 Protein content

One-day-old tofu samples were used for protein determination following AACC Method 46-13 (AACC, 2000) with some modifications. A 100-mg sample of uniformly mixed tofu curd (see 4.2.3.2.3 for sample preparation) was weighed on a weighing paper and transferred to a Kjeldahl tube. The sample was digested for 2 hours, followed by distillation and titration as per the AACC Method. A Nitrogen factor of 6.25 was used to determine protein content.

4.2.4 Phase IV: Statistical analysis

All data were reported in mean values with standard deviations; the coefficients of variation were calculated to check reproducibility. The P-values and ANOVA parameters were calculated using the 5% significant difference level with the SAS program, version 8.01 (SAS Institute, Cary, NC). The Quattro Pro version 9 (Corel Corporation, Ottawa, Ontario, Canada, 1999) was used to assist with data computation for intercorrelation and correlation relationships between parameters of soybeans, soymilk, and tofu. The results were used to discriminate soybean varieties for soymilk and tofu production potentials.

V RESULTS AND DISCUSSION

The laboratory procedures developed in the preliminary studies were used for making soymilk and tofu, and for analyzing parameters of soybeans, soymilk, and tofu. These methods gave good results and discriminated among seven soybean varieties from two locations. Proximate analysis data for all of the soybean (physical and chemical analyses), soymilk (temperature, volume, total solids, protein content, and color) and tofu (yield, moisture content, protein content, and texture) samples among seven varieties of soybeans from both locations (Allegan and Sanilac counties) are reported in the following sections.

5.1 Soybeans

5.1.1 Physical analysis

Mean values and standard deviations of seed weight and percent of split seeds are listed in Table 5.1. The coefficients of variation of these parameters were less than 5%. Analysis of variance among seed weight data is presented in Table 5.2. Significant differences for seed weights were found among varieties from the same location as well as within the same variety grown in different locations ($p<0.05$). In each location, variety IA 2020 showed the highest weight (21.63g/100 seeds in Sanilac Co. and 18.77g/100 seeds in Allegan Co.). Overall, Allegan county soybean seeds were smaller in size than the Sanilac county seeds (except for IA 2030 and Novartis S2020). In the present study, soybean seed weight ranged from 14.69-21.63 g/100 seeds, which was relatively low compared to

Table 5.1 Mean Values of Soybean Physical Analysis Data Among Seven Soybean Varieties from Two Locations

Variety	Seed Weight (g/100 seeds)		Split Seeds (%)	
	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.
IA 2034	17.89±0.53	18.29±0.24	0.00	0.00
Steyer	16.77±0.09	15.06±0.31	0.33	0.00
HP 204	19.53±0.50	17.00±0.42	0.00	0.00
Vinton 81	19.22±0.25	16.94±0.27	0.33	0.00
IA 2020	21.63±0.05	18.77±0.16	0.00	0.00
Novartis S20F8	16.56±0.26	14.69±0.05	0.00	0.00
Novartis S2020	14.95±0.38	15.90±0.38	0.33	0.00

Table 5.2. Analysis of Variance for Soybean Seed Weight, Moisture Content, and Protein Content[a] Among Seven Soybean Varieties from Two Locations

Source of Variation	Degree of Freedom	Seed Weight	Moisture	Protein
Main Effects		F-value		
County	1	189.61[b]	14281.8[b]	40.76[b]
Variety	6	182.49[b]	242.14[b]	54.91[b]
County & Variety	6	33.16[b]	311.13[b]	5.01[b]

[a] Dry basis.
[b] Significant at $p<0.05$.

the results from Wang et al. (1983) of 15.42-35.51 g/100 seeds, DeMan et al. (1989) of 17.20-23.30 g/100 seeds, and Lim et al. (1990) of 8.71-41.27 g/100 seeds.

Soybean samples examined of all varieties grown in Allegan County contained no split seeds. But among those grown in Sanilac Co., soybean samples from Steyer, Vinton 81, and Novartis S2020 varieties had means of about 0.33% split seeds (Table 5.1). Soybeans of the seven varieties from both counties demonstrated no mixed color of seed coats: they all had clear, light yellow seed coats. Based on U.S. Standard Grades (Table 2.5), all seven varieties from both locations are Grade 1 since all seed samples contained no foreign materials, had less than 10% split seeds, and less than 1% soybeans of another seed coat color.

All dry soybean seeds were spherical in shape (Fig. 2.1 - picture 2). After soaking, they became flat in shape (Figure 2.1 – picture 30), and increased to almost three times their original size.

5.1.2 Chemical analysis

Soybean moisture content and protein content were reported in mean values and standard deviations in Table 5.3. The coefficients of variation of both parameters were less than 5%. In Sanilac County, all seven soybean varieties were shown to have a lower moisture content (range from 8.32-8.90%) than those of Allegan County (from 9.50-11.15%) which also had a wider range. Of the soybean varieties studied, Novartis S20F8 had the highest moisture content in Sanilac County and IA 2020 had the highest moisture content in Allegan County (Table 5.3). The mean values for moisture content, ranging from 8.32-11.15% in this study, were somewhat higher than those reported by Lim et al. (1990) of 6.62-11.02%.

Table 5.3. Mean Values of Soybean Chemical Analysis Data Among Seven Soybean Varieties from Two Locations

Variety	Moisture[a] (%)		Protein Content[b, c] (%)	
	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.
IA 2034	8.32±0.08	9.60±0.02	42.34±1.12	44.23±0.20
Steyer	8.36±0.07	11.06±0.08	42.19±0.62	42.54±0.14
HP 204	8.36±0.03	10.93±0.03	44.04±1.12	43.57±0.53
Vinton 81	8.35±0.06	10.73±0.04	43.50±0.28	44.18±0.49
IA 2020	8.82±0.04	11.15±0.04	42.62±0.43	43.67±0.68
Novartis S20F8	8.90±0.03	9.50±0.08	38.25±0.91	41.04±0.18
Novartis S2020	8.63±0.00	10.50±0.07	38.40±0.33	41.04±0.22

[a] n = 3.
[b] n = 6.
[c] Dry basis.

On a dry basis, varieties Novartis S20F8 and Novartis S2020 had the lowest protein contents at each of the locations (Table 5.3). Variety HP 204 from Sanilac County and variety IA 2034 from Allegan County had the highest protein contents in their respective location: 44.04% and 44.23%, respectively.

Significant differences were detected in soybean moisture content ($p<0.05$) and protein content ($p<0.05$) among all soybean varieties and between locations, they are listed in the ANOVA table of data (Table 5.2). Depending on conditions of the environment in which soybeans were grown (e.g., climate, soil, etc.), soybean varieties from different locations might have different moisture contents and protein contents. In addition to the differences in soybean moisture and protein contents, differences in the ratio of 11S/7S proteins present in soybean varieties genetical differences may also affect soymilk and tofu qualities.

5.1.3 Relationships among quality parameters and quality evaluation

The correlation coefficients among physical and chemical characteristics of soybeans are presented in Table 5.4. There was no correlation between soybean moisture content and protein content. A positive correlation between seed weight and protein content (dry basis) was found in both locations. This indicated that the larger the soybean seed, the higher its protein content. This finding is in general agreement with Smith and Circle (1978). On the other hand, DeMan et al. (1989) and Lim et al. (1990) both reported that there was no correlation between seed weight and soybean protein content, and did not observe any correlation between soybean moisture content and protein content.

Table 5.4. Correlation Coefficients for Relationships between Soybean Quality Parameters

Parameter	Sanilac Co.	Allegan Co.
Seed weight vs. Protein content[a]	0.750[b]	0.810[b]
Seed moisture vs. Protein content[a]	-0.112	0.700

[a] Dry basis.
[b] Significant at $p<0.05$.

In general, soybeans from Sanilac County had higher seed weight and lower moisture content than those from Allegan County. Among all varieties from both locations, varieties Novartis S20F8 and Novartis S2020 from Sanilac County had smaller seed size and the lowest protein contents (16.56g/100 seeds and 14.95 g/100 seeds for seed weight, and 38.25% and 38.40% for protein content on a dry basis, respectively). Soybeans from Sanilac County also were shown to have more split seeds (up to 0.33% for varieties Steyer, Vinton 81, and Novartis S2020) than those from Allegan County (0%), which would affect soymilk quality because of the off flavor produced during the soaking and grinding procedures (Fig. 5.1).

5.2 Soymilk

5.2.1 Analyses of soymilk temperature, volume, protein content and total solids

Soymilk temperature upon exiting the soycow machine was in the range of 86-90°C for all varieties. Soymilk temperature was measured to ensure its temperature was above 80°C, because this is the minimum temperature to inactivate trypsin inhibitors, denature soybean proteins, kill microbes for extending product shelf-life, increase the extraction of soymilk from the slurry, and prevent its beany flavor (Liu, 1999). Mean values and standard deviations for soymilk volume and protein content are shown in Figs. 5.1 and 5.2, respectively. The coefficients of variation for soymilk volume and protein content were less than 5%. Among seven varieties from both locations, variety Steyer grown in Allegan County gave the highest soymilk volume of 6.63 liters per kg soybeans. On the other hand, variety IA 2020 grown in the same Allegan County produced the lowest amount of soymilk: 6.27 liters per kg soybeans (Fig. 5.1).

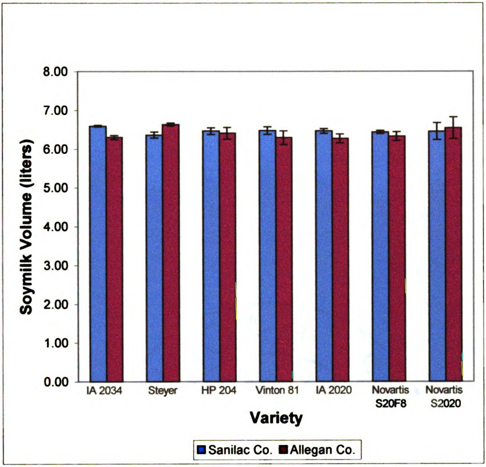

Figure 5.1. Soymilk Volumes of Seven Soybean Varieties from Two Locations.

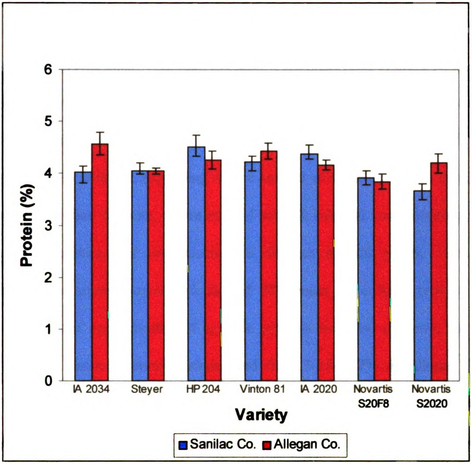

Figure 5.2. Soymilk Protein Content of Seven Soybean Varieties from Two Locations.

Significant differences were detected among soymilk volumes and protein contents ($p<0.05$) of varieties grown in the same county and within the same variety grown in both locations (Table 5.5). Although the same water:bean ratio of 6.5:1 (v/w) was used during soymilk processing, soybeans had different oil contents, and protein contents as well as containing different types of proteins, and absorbing water at a different rate during soaking. In addition, depending on the amount of soluble proteins in soybeans and their water retention capacity, the amount of soymilk released and remaining in okara differed among varieties, and thus resulted in different soymilk yields. Because of volume differences among soymilk yields and differences in soybean protein contents among varieties, the concentration of soymilk proteins was also different, ranging from 3.92-4.51% in Sanilac County varieties and from 3.84-4.57% in Allegan County varieties. Among all soybean varieties, variety IA 2034 grown in Allegan County produced soymilk with the highest protein content (Fig. 5.2). Soymilk protein contents in this study were higher (from 3.66-4.57%) than those previously reported by Chen (1989) of 3.5-4.0%.

Soymilk total solids (ranging from 8.18-8.66% in Sanilac Co. and 8.11-9.02% in Allegan Co.) were similar for all varieties in both locations (Table 5.5). Soymilk total solids is related to the water:bean ratio (Liu, 1999); therefore, in the present study, the same water:bean ratio of 6.5:1 (v/w) was used among varieties during soaking, grinding and cooking, and resulted in similar soymilk total solids in all varieties. Even though all soybean varieties produced soymilk with similar total solids, soymilk protein contents were quite different due to the differences in protein and carbohydrate contents among the soybeans samples, and the differences in extractability of soluble proteins content in

Table 5.5. Analysis of Variance for Soymilk Volume, Total Solids, Protein Content, and Color Among Seven Soybean Varieties from Two Locations

Source of Variation	Degrees of Freedom	Volume	Total Solids	Protein Content	Color		
Main Effects		F-value					
					L	a	b
County	1	12.34[a]	0.54	7.23[a]	4.50[a]	1.95	1.53
Variety	6	1.72	3.62[a]	15.82[a]	0.83	6.86[a]	6.97[a]
County & Variety	6	5.29[a]	2.16	10.00[a]	1.61	2.27	6.55

[a] Significant at $p<0.05$.

soybeans after pressing the slurry (Lim et al., 1990). In the present study, soymilk total solids were relatively lower (8.11-9.02%) compared to the total solids values of 9.09-9.90% reported by Lim et al. (1990). This was likely due to the different water:bean ratio used by Lim et al. in this study (4.7:1, v/w). A higher level of water used would result in a higher soymilk volume, but lower total solids, and lower protein content (Table 2.7).

5.2.2 Soymilk color, pH, and density

Among the varieties studied, significant differences were detected for the whiteness of soymilk (assessed by the L-value, black 0, white 100) between locations ($p<0.05$) (Table 5.5). For each soybean variety, the a-value (red +/green -) was similar for both locations (Fig. 5.3), but significant differences were detected for a-values among soybean varieties within a location. In the present study, the means of the b-value (yellow +/ blue -) were different among varieties in the same county. However, the whiteness of soymilk (the L-value) is more important than the other two factors that measure the degree of red, green, yellow or blue color present (the a- and b-values). When comparing soymilk from the varieties studied with commercially prepared soymilk, it was noted that the mean L-values (range 76.27-81.76, Appendix III) for whiteness of soymilk from all seven varieties at both locations were higher than those of soymilk from the Vitasoy Company (L = 72.4) and from the Edensoy Company (L = 66.7) (Appendix IV). Generally, commercial soymilk has higher total solids because other ingredients such as sucrose, vitamins, minerals, and gums are added; these additives also affect soymilk color. When compared with cow's milk, the soymilk produced in the present study had L-values just a little less white than 2% fat cow's milk (L = 81.2), and 3.5% fat cow's milk (L = 88.7) (Appendix IV).

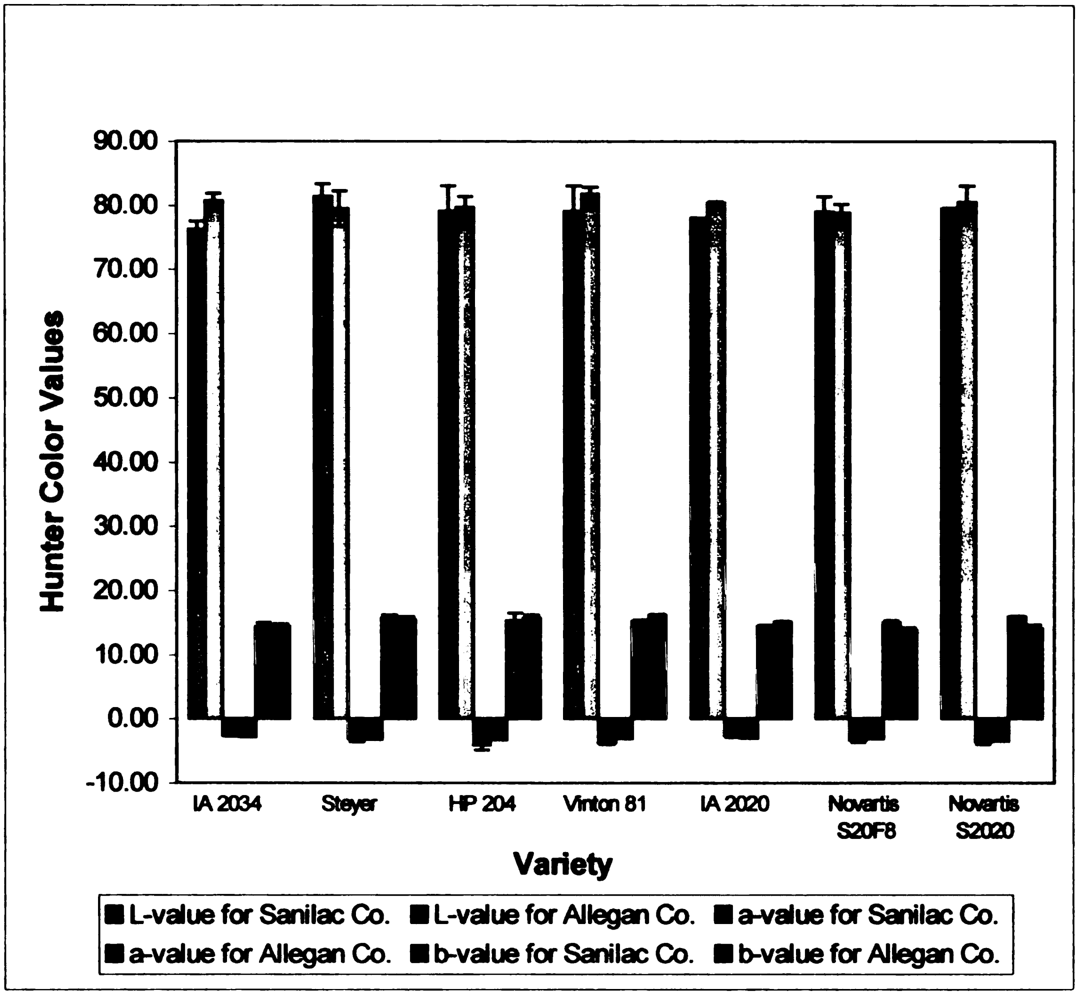

Figure 5.3. Soymilk Color of Seven Soybean Varieties from Two Locations.

Soymilk pH and density among varieties and from both locations were similar and ranged from 6.56-6.66 for pH and from 1.01-1.02 g/ml for density (Table 5.6). The pH values were relatively higher than those reported by Lim et al. (1990) of 6.42-6.55. In the present study, the soymilk pH was in an optimum range (pH between 3 and 7) for Ca^{2+} ions from calcium sulfate coagulant to bind to soybean proteins. Liu (1999) reported that at soymilk pHs below 3 and above 7, binding between Ca^{2+} ions and soy proteins did not occur. Soymilk densities were similar among all the studied soybean varieties. This was expected since soymilk total solids had also been similar among these varieties.

5.2.3 Relationships among soymilk volume, total solids, protein content, and color

There was a negative relationship between soymilk volume and total solids (Table 5.7); this implied that adding more water during grinding would reduce soymilk total solids (Table 2.7). Soymilk protein content does not necessarily relate to soymilk yield; it depends on the types of soybean proteins present, especially soluble proteins. The presence of more soluble proteins in soybeans resulted in high soymilk volume (Liu, 1999). For soymilk volume and color (L-value for whiteness), the correlation was a negative relationship for soymilk made from soybean varieties grown in Sanilac County ($r = -0.945$) and Allegan County ($r = -0.322$).

5.2.4 Soymilk quality evaluation

Variety Steyer from Allegan County produced the highest soymilk volume (Fig. 5.1). Overall, soymilk produced from Sanilac County varieties were greater in volume than those made from Allegan County varieties. The inverse relationship between soymilk volume and total solids indicated that variety Steyer in Allegan County produced the

Table 5.6. Mean Values of Soymilk pH and Density from Seven Soybean Varieties

Variety	pH[a]		Density[b] (g/ml)	
	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.
IA 2034	6.61±0.02	6.37±0.08	1.01±0.33	1.02±0.11
Steyer	6.66±0.01	6.61±0.05	1.01±0.34	1.02±0.04
HP 204	6.61±0.01	6.64±0.03	1.02±0.22	1.02±0.10
Vinton 81	6.64±0.01	6.55±0.05	1.02±0.19	1.02±0.34
IA 2020	6.66±0.04	6.63±0.02	1.02±0.22	1.02±0.28
Novartis S20F8	6.56±0.03	6.59±0.01	1.01±0.23	1.02±0.15
Novartis S2020	6.56±0.05	6.56±0.01	1.01±0.18	1.02±0.12

[a] n = 3.
[b] n = 9.

Table 5.7. Intercorrelation Relationships between Soymilk Volume, and Total Solids, Protein Content, and Color

Parameter	Sanilac Co.	Allegan Co.
Soymilk volume vs. Total solids	-0.307	-0.688
Soymilk volume vs. Protein	0.815[a]	0.187
Soymilk volume vs. Color (L-value)	-0.945[a]	-0.322

[a] Significant at $p<0.05$.

highest soymilk volume (6.63 liters) with the lowest total solids (8.11%). In contrast, variety IA 2020 from Allegan County produced the lowest soymilk volume (6.27 liters) with the highest total solids (9.02%). With its highest soymilk protein content among the varieties studied, variety IA 2034 from Allegan County (Fig. 5.2) would be expected to produce tofu with higher yield and protein content in the following experiments of the present study.

To produce good quality soymilk, the most important factor that the producer needs to consider is the water:bean ratio (v/w). This will affect the soymilk volume produced, total solids, protein content, and the whiteness of the soymilk (Chen, 1989). Besides the main factor of water:bean ratio, a producer needs to select a proper variety of soybean that can produce high soymilk yield; choose a location for the processing plant that has a source of water without contaminants; select equipment for soybean grinding, soymilk extraction, heat treatment, and packaging; and formulate the end products (Liu, 1999). Soymilk needs to have a white appearance, be a fluid that is not too thin but not too thick, and have nutritional value. Depending on the culture and the demand of customers (for example, Western customers prefer soymilk without a beany flavor, but Eastern people favor it), the producer needs to choose the proper method of processing (hot or cold grinding, or unsoaked beans method) to produce high quality soymilk that suits consumer tastes.

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5.3 Tofu

5.3.1 Tofu yield

Mean values and standard deviations of tofu yield are presented in Table 5.8. The coefficients of variation of the tofu yield were less than 5%. In the present study, the range of 2.67-3.43 kg tofu produced per kg soybean was relatively lower than that reported by Lim et al. (1990) of 4.45-5.26 kg tofu/kg soybean. Results from analyses of variance on this data are summarized in Table 5.9. Significant differences in yield were exhibited ($p<0.05$) among varieties within each location and within the same variety from both locations. Variety IA 2020 grown in Sanilac County gave the highest tofu yields overall and variety Novartis S20F8 from both counties gave the lowest yield. Tofu yield mainly depended on the amount of water retained in the curd, and the retention probably depends on factors such as soymilk total solids, the amount of soluble proteins, the water:bean ratio, etc. (Beddows and Wong, 1987).

5.3.2 Chemical analysis

Tofu moisture and protein data are reported in Table 5.8 along with their mean values and standard deviations. The coefficients of variation were less than 5%. For protein content, on a dry basis, there were significant differences in tofu protein content ($p<0.05$) among all varieties grown in both locations (Table 5.9). Schaefer and Love (1992) reported that based on variation in soybean protein content, tofu protein content might be expected to be different among varieties. Their findings were in agreement with the present study. The mean values for protein content in the present study ranged from 48.23-58.68%, and were similar to those reported by Lim et al. (1990) of 46.03-52.50%.

Table 5.8. Mean Values of Tofu Yield, Protein Content[a], and Moisture Content of Seven Soybean Varieties from Two Locations

Variety	Yield[b] (kg tofu/kg bean)		Protein[c] (%)		Moisture[c] (%)	
	Sanillac Co.	Allegan Co.	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.
IA 2034	3.22±0.05	3.11±0.11	58.68±2.70	56.79±1.84	86.45±0.36	85.25±0.66
Steyer	3.14±0.13	3.11±0.06	51.71±1.81	53.81±2.37	86.29±0.31	86.16±0.25
HP 204	3.20±0.05	3.10±0.04	53.90±2.96	55.72±2.87	86.42±0.47	85.53±0.52
Vinton 81	2.93±0.08	2.96±0.01	53.90±2.13	56.72±2.38	85.56±0.31	85.82±0.15
IA 2020	3.43±0.16	2.99±0.10	52.41±2.70	53.79±1.84	86.12±0.45	86.47±0.62
Novartis S20F8	2.69±0.01	2.67±0.03	48.23±1.90	51.42±1.14	84.87±0.73	84.82±0.22
Novartis S2020	2.90±0.11	3.04±0.02	50.11±2.96	50.98±2.87	86.37±0.29	86.17±0.67

[a] Dry basis.
[b] n = 4.
[c] n = 8.

Table 5.9. Analysis of Variance for Tofu Yield and Protein Content[a] Among Seven Soybean Varieties from Two Locations

Source of Variation	Degrees of Freedom	Yield	Protein
Main Effects		F-value	
County	1	9.12[b]	20.56[b]
Variety	6	30.22[b]	29.35[b]
County & Variety	6	8.89[b]	3.76[b]

[a] Dry basis.
[b] Significant at $p<0.05$.

Tofu moisture contents obtained in the present study (84.82-86.47%) were similar to those observed by Lim et al. (1990) (86.58-88-58%). Water retained in the tofu was similar among all varieties and resulted in tofu samples with almost the same moisture contents. There is no standard moisture content for commercial tofu. Hard tofu contains 75.00-79.00% moisture and soft tofu ranges from 84.70-85.90% (Gebre and Summer, 1983), or 84.07% moisture for hard tofu and 87.08% for soft tofu (Tsai et al., 1981). Therefore, based on moisture content, tofu from the present study could be classified as soft tofu. Variety Novartis S20F8 produced tofu with the lowest moisture and protein contents (dry basis). Variety IA 2034 from both locations gave tofu with the highest protein contents (Table 5.8). This was due to the fact that IA 2034 produced soymilk with a high protein content, from which the tofu was made.

5.3.3 Tofu texture analysis

Mean values and standard deviations of tofu texture are presented in Fig. 5.4. The texture parameters included fracturability, hardness, and gumminess, for which values ranged from 6.46-11.19 N, 7.84-11.67 N, and 3.01-5.17 N, respectively. The cohesiveness values ranged from 0.38 to 0.50. Results of the four parameters of texture of fresh tofu samples from the seven varieties in the same location were significantly different ($p<0.05$) (Table 5.10). Similar findings were reported by Wang (1993). However, within the same variety, results from the texture evaluations were similar ($p>0.05$) between locations; except for cohesiveness, tofu samples from the seven varieties were similar in both locations.

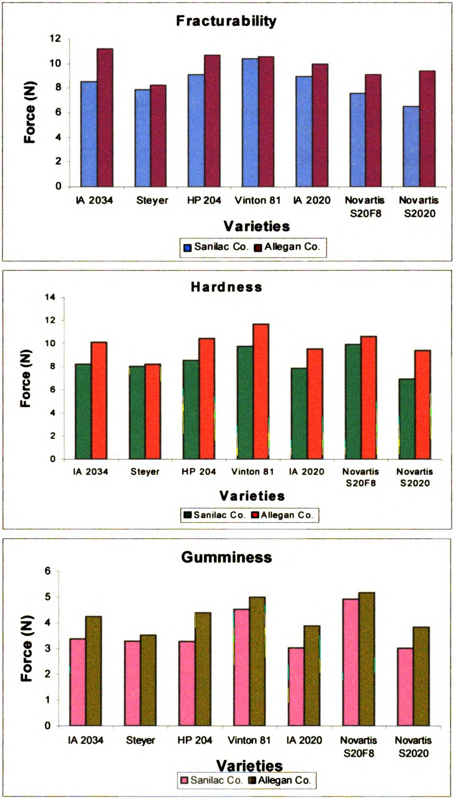

Figure 5.4. Tofu Texture Analysis Among Seven Soybean Varieties from Two Locations.

Table 5.10. Analysis of Variance for Tofu Texture Among Seven Soybean Varieties from Two Locations

Source of Variation	Degrees of Freedom	Fracturability	Hardness	Cohesiveness	Gumminess
Main Effects		F-Value			
County	1	29.26[a]	20.14[a]	0.35	14.43[a]
Variety	6	8.04[a]	4.58[a]	6.75[a]	6.50[a]
County & Variety	6	2.26	0.77	0.57	0.45

[a] Significant at $p<0.05$.

In the present study, the coefficients of variation were less than 20% for all texture parameters. These results were similar to those reported by Wang (1993), but less than the coefficients of variation of 33% reported by Schaefer and Love (1992) for tofu hardness. Variety Vinton 81 from Allegan County produced the hardest tofu. Tofu made from variety Novartis S20F8 from both locations had high values of hardness, cohesiveness and gumminess (Fig. 5.4). As an example, Fig. 5.5 shows a computer readout and the calculation of fracturability, peaks of hardness, and areas 1 and 2 for the tofu texture analysis of Vinton 81 grown in Allegan County.

Texture is an important factor that affects consumer acceptance of tofu and tofu products. This property plays a main role in the selection of type of coagulant and methods for making tofu. Improvements in technology, especially the development of the Texture Profile Analysis and computerized instruments (e.g., Texture Analyzer TA-DHi) have been found very helpful in tofu quality evaluation and assurance.

5.3.4 Relationships among tofu yield, moisture content, protein content, and texture

No correlation was obtained between tofu yield and protein content, and tofu yield and moisture content (Table 5.11) at $p>0.05$, but a negative correlation was found between tofu yield and cohesiveness ($p<0.05$), and tofu yield and gumminess (significantly for Sanilac Co. at $p<0.05$, but not for Allegan Co.). A negative correlation was also found between tofu moisture and hardness for Sanilac county. This indicates that high water retention in tofu is associated with an increase in product moisture content, and with a product that is softer. On the other hand, increasing tofu yield is associated with less cohesiveness and less gumminess of the final product.

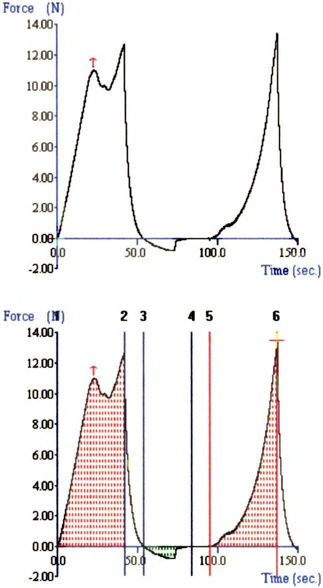

Figure 5.5. Tofu Texture Curve from Variety Vinton 81 Grown in Allegan County.

Table 5.11. Intercorrelation Relationships Among Tofu Yield, Moisture Content, Protein Content[a], Hardness, Cohesiveness, and Gumminess

Parameter	Sanilac Co.	Allegan Co.
Tofu yield vs. Protein[a]	0.618	0.613
Tofu yield vs. Moisture	0.684	0.532
Tofu yield vs. Hardness	-0.483	-0.418
Tofu yield vs. Cohesiveness	-0.931[b]	-0.745[b]
Tofu yield vs. Gumminess	-0.738[b]	-0.611
Tofu hardness vs. Moisture	-0.875[b]	-0.501

[a] Dry basis.
[b] Significant at $p<0.05$.

5.3.5 Tofu quality evaluation

Among all seven soybean varieties from both locations, variety Novartis S20F8 produced tofu with the lowest yield, moisture content, and protein content, but which was high in all the texture parameters studied (hardness, cohesiveness, and gumminess). Based on results from the present study, soybeans from variety Novartis S20F8 would be a good choice for a producer wishing to make hard tofu, or from variety Novartis S2020 harvested in Sanilac County to produce soft tofu. In general, varieties IA 2034, Steyer, and HP 204 gave high tofu yield, protein content, and medium hardness. These properties are considered good for the production of regular tofu (section 2.3.1). Based on fracturability results, variety IA 2034 from Allegan County produced tofu with the highest regained force to break the curd. The results from the tofu skin test (Table 5.12), with coefficients of variation less than 20%, confirmed that tofu produced from variety IA 2034 of Allegan County (the hardest to fracture) also had the hardest skin. These tofu characteristics exhibited by IA 2034 of Allegan County are suitable for the production of certain tofu products (e.g., fried tofu, tofu pouches, etc.).

5.4 Correlation relationships among quality parameters of soybeans, soymilk, and tofu

Positive correlation relationships were detected between soybean protein content and soymilk total solids among varieties from Sanilac county ($p<0.05$) (Table 5.13). Positive relationships were also found between soybean protein and soymilk protein contents ($p<0.05$), and between soymilk protein and tofu protein contents, especially for varieties from Allegan County. Therefore, soybean protein content and soymilk protein

Table 5.12. Mean Values for Tofu Skin Tests (N) of Tofu Samples from Seven Soybean Varieties Grown in Two Counties

Variety	Sanilac Co.	Allegan Co.
IA 2034	0.19±0.03	0.29±0.04
Steyer	0.16±0.02	0.19±0.01
HP 204	0.20±0.04	0.24±0.03
Vinton 81	0.27±0.04	0.22±0.04
IA 2020	0.19±0.03	0.21±0.02
Novartis S20F8	0.19±0.03	0.19±0.03
Novartis S2020	0.15±0.02	0.19±0.04

Table 5.13. Correlation Relationships Among Evaluative Parameters of Soybeans, Soymilk, and Tofu from Seven Soybean Varieties Grown in Two Locations

	Sanilac Co.	Allegan Co.
Soybean and Soymilk Correlation		
Soybean protein[a] vs. Soymilk total solids	0.847[b]	0.426
Soybean protein[a] vs. Soymilk protein	0.852[b]	0.777[b]
Soymilk and Tofu Correlation		
Soymilk total solids vs. Tofu yield	0.541	-0.094
Soymilk total solids vs. Tofu protein[a]	0.185	0.217
Soymilk total solids vs. Tofu hardness	0.213	0.444
Soymilk volume vs. Tofu protein[a]	0.768[b]	-0.403
Soymilk protein vs. Tofu yield	0.618	0.613
Soymilk protein vs. Tofu protein[a]	0.349	0.803[b]
Soybean and Tofu Correlation		
Soybean protein[a] vs. Tofu protein[a]	0.634	0.947[b]
Soybean protein[a] vs. Tofu yield	0.688	0.505
Soybean protein[a] vs. Tofu hardness	0.105	0.317
Soybean seed weight vs. Tofu yield	0.693	0.494
Soybean seed weight vs. Tofu hardness	0.183	0.105

[a] Content on a dry basis.
[b] Significant at $p<0.05$.

content played important roles in producing tofu with high protein content. These findings are similar to those reported by Wang et al. (1983), DeMan et al. (1989), Lim et al. (1990), and Schaefer and Love (1992). In the present study, the correlation between soybean protein and soymik protein ($r = 0.852$ for Sanilac County and 0.777 for Allegan County) was lower than that reported by Schaefer and Love (1992) of $r = 0.96$, but higher than the result reported by Liu (1999) of $r = 0.593$. No relationship was found between soymilk total solids and tofu yield ($p>0.05$). This finding was similar to that reported by Johnson and Wilson (1984). This lack of relationship is likely due mainly to the differences in soybean protein content among varieties, and the different types of protein as well as differences in the protein fractions present in soybean varieties, causing proteins to coagulate in different ways during tofu making. There was no correlation between soybean protein and tofu yield ($p>0.05$), in contrast to the report from Wang et al. (1983), but similar to the results of Lim et al. (1990) and Wang (1993).

No relationship between soybean seed weight and tofu yield ($p>0.05$) was found in the present study (Table 5.13). This indicated that larger soybean seed weight would not be expected to give higher tofu yield. Thus, in comparison to soybean protein characteristics, soybean seed weight probably is not a key factor for selecting soybean variety to produce tofu (Wang, 1993).

VI. SUMMARY AND CONCLUSIONS

In the present study, the laboratory procedures developed in the preliminary studies for making soymilk and tofu were used to discriminate among seven varieties of soybeans from two locations. All replicated data were similar with acceptable coefficients of variation. This indicates that the developed procedures could be used, in a reproducible way, to evaluate soybean varieties for soymilk- and tofu-making potentials.

The two main factors influencing the differences in soybean protein content among and within varieties are: (1) the location where the seeds were grown and affected by soil and other environmental conditions, and (2) the genetics of each of the varieties planted (Smith and Circle, 1978). To produce good soymilk and a high quality tofu products, Smith and Circle (1978) suggested selecting larger soybean seeds with higher protein content, lower oil content, and those that had reached maturity.

For soymilk processing in the current study, soybean varieties from Sanilac County gave the highest soymilk volume--especially variety Steyer. Soybean varieties that yield high soymilk volumes can be more profitable to producers. For making tofu, soybeans from variety IA 2034 from Allegan County had a high protein content and produced tofu with a high protein content. Greater force was required to break the curd, and tofu from this variety had a harder skin, hence, variety IA 2034 grown in Allegan County would be preferred for fried tofu products. Varieties Novartis S20F8 grown in both counties had low seed weights, low protein contents, and produced hard curd which might be selected to make hard tofu. With medium tofu hardness and high yield, soybeans from IA 2034, Steyer, and HP 204 grown in both counties might be selected by manufacturers to produce regular tofu, another marketable form of tofu. And variety Novartis S2020 grown in Sanilac County would be a good choice for making soft tofu.

VII. SUGGESTIONS FOR FURTHER RESEARCH

In the present study, all seven varieties of soybeans grown in two locations from one crop year (2000) showed significant differences in soybean physical and chemical properties. To further improve and understand soymilk and tofu qualities, the following suggested research could be conducted:

- More crop years (e.g., two or three) are needed with the same varieties grown in the same locations, to compare all properties among the varieties and evaluate for possible differences among harvest years.
- The same harvest year comparisons could be applied with the making of soymilk and tofu with the new crops of soybean varieties.
- The ratio of 11S/7S proteins in the soybean varieties studied needs to be analyzed and manipulated to achieve high tofu yield and desirable tofu texture.
- All soymilk samples among varieties studied need to be analyzed for their beany flavor via sensory analyses.
- The influence of processing conditions, including the technique of adding coagulant and the stirring rate of all soymilk during coagulation, should be investigated further.

APPENDICES

APPENDIX I

Comparison between Hot and Cold Grinding Procedures for Making Soymilk Using 1999 Vinton Soybeans

Method	Cold Grinding	Hot Grinding
Soybean (kg)	1	1
Soaking Time (hrs)	15.5	15.5
Waer:Bean Ratio	6.8:1	6.8:1
Soymilk Volume (l)	6.12	6.00
Soymilk pH	6.7	6.7
Soymilk Total Solids (%)	8.5	5.8

Comparison Between Hot and Cold Grinding Procedures for Making Tofu Using 1999 Vinton Soybeans

Method	Cold Grinding	Hot Grinding
Soybean (kg)	2	2
Soaking Time (hrs)	15.5	15.5
Water:Bean Ratio	4.6:1	4.6:1
Soymilk Temperature (°C)	65	55
Soymilk used (l)	3	3
Coagulant[a] Concentration (M)	0.03	0.03
Average Tofu Weight (g tofu/liter soymilk)	122.20	very few and small curds; could not press for tofu

[a] Calcium sulfate.

APPENDIX II

Data for Different Soybean Grinding Times to Produce Soymilk Using 1999 Vinton Soybeans

Grinding Time (min)	3	5
Dry Soybean (kg)	1	1
Water:Bean Ratio	6.8:1	6.8:1
Soymilk Volume (l)	7	7.4
Soymilk Temperature (°C)	79.2	78.0
Soymilk Total Solids (%)	9.5	9.5
Soymilk Color		
L	80.5	81.7
a	-3.0	-2.8
b	15.5	15.6
Coagulant[a] Concentration (M)	0.03	0.03
Coagulant[a] Temperature (°C)	65	65
Soymilk Volume to Make Tofu (l)	1	1
Soymilk Temperature (°C)	80	80
Curd Forming Time (min)	5	5
Pressing Weight (kg)	3.4	3.4
Pressing Time (hr)	1	1
Average Tofu Weight (g tofu/liter soymilk)	367.1	443.6

[a] Calcium sulfate.

APPENDIX III

Mean Values of Soymilk Volume, Total Solids, Protein Content, and Color of Seven Soybean Varieties (2000) from Two Locations

Variety	Volume[a] (liter)		Total Solids[c] (%)		Protein[b] (%)		Color[c]					
							L		a		b	
	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.
IA 2034	6.59 ±0.02	6.30 ±0.06	8.34 ±0.22	8.41 ±0.41	4.03 ±0.11	4.57 ±0.22	76.27 ±1.20	80.62 ±1.23	-2.59 ±0.17	-2.80 ±0.32	14.39 ±0.59	14.42 ±0.32
Steyer	6.36 ±0.08	6.63 ±0.04	8.66 ±0.22	8.11 ±0.08	4.05 ±0.14	4.04 ±0.06	81.36 ±1.96	79.47 ±2.74	-3.16 ±0.50	-3.19 ±0.37	15.66 ±0.48	15.36 ±0.48
HP 204	6.46 ±0.09	6.40 ±0.15	8.62 ±0.09	8.76 ±0.39	4.51 ±0.22	4.26 ±0.17	79.07 ±3.94	79.64 ±1.74	-4.06 ±0.83	-3.30 ±0.38	15.26 ±1.22	15.68 ±0.43
Vinton 81	6.47 ±0.10	6.29 ±0.18	8.64 ±0.15	8.66 ±0.24	4.21 ±0.12	4.43 ±0.16	79.07 ±3.94	81.76 ±1.09	-3.81 ±0.19	-3.14 ±0.28	15.21 ±0.13	16.13 ±0.09
IA 2020	6.46 ±0.06	6.27 ±0.12	8.61 ±0.14	9.02 ±0.18	4.37 ±0.18	4.16 ±0.09	77.96 ±0.06	80.40 ±0.17	-2.59 ±0.31	-3.00 ±0.16	14.41 ±0.19	15.11 ±0.06
Novartis S20F8	6.43 ±0.04	6.32 ±0.12	8.29 ±0.10	8.45 ±0.04	3.92 ±0.13	3.84 ±0.14	78.96 ±2.24	78.76 ±1.39	-3.29 ±0.37	-3.16 ±0.35	15.00 ±0.26	13.80 ±0.42
Novartis S2020	6.45 ±0.22	6.54 ±0.28	8.18 ±0.33	8.43 ±0.35	3.66 ±0.13	4.19 ±0.18	79.43 0.15	80.50 ±2.54	-3.62 ±0.40	-3.47 ±0.31	15.84 ±0.09	13.94 ±0.59

[a] n = 3.
[b] n = 6.
[c] n = 9.

APPENDIX IV

Mean Values of Color Analysis from Commercial Soymilk and Cow's Milk

Sample	Cow's Milk		Commercial Soymilk	
	Low Fat (2%)	Whole Milk	Vitasoy	Edensoy
L	81.2	88.7	72.4	66.7
a	-0.9	-4.2	-2.7	0.2
b	3.7	7.7	5.8	19.2

APPENDIX V

Tofu-Making[a] Formulae Used for Different Soymilk Volumes, and Resultant Tofu Yield Using 1999 Vinton Soybeans

Soymilk Volume (l)	Soybean used (kg)	Water: Bean Ratio	Soymilk Temperature (°C)	Coagulant[b] Concentration (M)	Coagulant[b] Temperature (M)	Average Tofu Weight (g)
2	2	6.8:1	75	0.38	65	421.20
1.5	2	6.8:1	73	0.30	65	318.65
1	2	6.8:1	73	0.38	65	211.65

[a] Using cold grinding method.
[b] Citric acid.

APPENDIX VI

Select Tofu Parameters for Tofu Made with Different Coagulant (Citric Acid) Concentrations Using 1999 Vinton Soybeans

Concentration (%)	Average Tofu Weight (g tofu/liter soymilk)	Moisture (%)	Texture (N)
0.18	209.53	79.10	4.25
0.19	178.83	77.75	12.17
0.38	187.23	84.03	8.27

APPENDIX VII

Comparison between Food Grade Calcium Sulfate and Pure Chemical Calcium Sulfate as Coagulants for Making Tofu

	Variety			
Formula and Resultant Data	**Mixed Soybeans**		**Vinton (1999)**	
Weight of Soybean (kg)	1		1	
Water:Bean Ratio	6.8:1		6.8:1	
Soymilk Volume (l)	6.5		7	
Soymilk Total Solids (%)	8.5		10	
Calcium Sulfate	Food grade	Chemical	Food grade	Chemical
Coagulant Concentration (M)	0.03	0.03	0.03	0.03
Coagulant Temperature (°C)	65	65	65	65
Soymilk Volume (l)	1.5	1.5	1.5	1.5
Soymilk Temperature for Making Tofu (°C)	73.8	74.0	70.0	70.0
Average Tofu Weight (g tofu/liter soymilk)	373.8	404.3	439.2	487.1

APPENDIX VIII

Effect of Curd-Forming Time on Tofu Yield Using 1999 Vinton Soybeans

	Curd-Forming Time (min)				
Formula Used and Measured Parameters Data	5		15	30	60
Dry Soybean (kg)	1	1	1	1	1
Water:Bean Ratio	6.8:1	6.6:1	6.6:1	6.6:1	6.8:1
Soymilk Volume (l)	7	7	7	7	7
Soymilk Temperature (°C)	79.2	86	86	86	79.2
Soymilk Total Solids (%)	9.5	6.3	6.3	6.3	9.5
Coagulant[a] Concentration (M)	0.03	0.03	0.03	0.03	0.03
Coagulant[a] Temperature (°C)	65	65	65	65	65
Soymilk Volume to Make Tofu (liter)	1	1	1	1	1
Soymilk Temperature (°C)	80	80	80	80	80
Pressing Weight (kg)	3.4	3.4	3.4	3.4	3.4
Pressing Time (hr)	1	1	1	1	1
Average Tofu Weight (g tofu/liter soymilk)	367.1	313.4	313.4	315.7	409.2
	Curds were stuck in cheese cloth, very soft, and separated apart		Curds were easy to take out of cheese cloth	Tofu was firmer and had good shape	Tofu had higher yield because soymilk had higher total solids

[a] Calcium sulfate.

APPENDIX IX

Effect of Curd-Forming Time on Tofu Yield Using 1999 Vinton Organic Soybeans

	Curd-Forming Time (min)		
Formula Used and Measured Parameters Data	**5**	**15**	**30**
Dry Soybean (kg)	2	1	1
Water:Bean Ratio	6.5:1	6.5:1	6.5:1
Soymilk Volume (l)	13	13	13
Soymilk Temperature (°C)	88	87	88
Soymilk Total Solids (%)	13.5	13.6	14.5
Coagulant[a] Concentration (M)	0.03	0.03	0.03
Coagulant[a] Temperature (°C)	65	65	65
Soymilk Volume to Make Tofu (l)	1	1	1
Soymilk Temperature (°C)	80	80	80
Pressing Weight (kg)	3.4	3.4	3.4
Pressing Time (hr)	1	1	1
Mean Tofu Weight (g tofu/liter soymilk)	489.65±27.02	502.65±54.06	493.53±41.06
	Curds were stuck in cheese cloth, very soft, and separated apart	Curds were easy to take out of cheese cloth	Tofu was firmer and good shape

[a] Calcium sulfate

APPENDIX X

Mean Values of Tofu Texture Analysis of Seven Soybean Varieties (2000) in Two Locations

Variety	Fracturability (N)		Hardness (N)		Cohesiveness		Gumminess	
	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.	Sanilac Co.	Allegan Co.
IA 2034	8.50±0.43	11.19±0.55	8.19±0.93	10.10±0.89	0.41±0.06	0.42±0.04	3.36±0.75	4.23±0.56
Steyer	7.90±0.51	8.24±0.72	8.01±0.72	8.19±0.91	0.41±0.06	0.43±0.05	3.28±0.48	3.52±0.68
HP 204	9.14±0.93	10.70±0.85	8.53±1.13	10.42±1.54	0.38±0.03	0.42±0.05	3.27±0.47	4.39±1.10
Vinton 81	10.44±0.96	10.58±0.95	9.76±0.96	11.67±1.32	0.46±0.09	0.43±0.02	4.52±1.19	4.99±0.76
IA 2020	8.95±1.17	9.94±1.67	7.84±1.57	9.52±1.39	0.39±0.03	0.40±0.04	3.02±0.55	3.88±0.74
Novartis S20F8	7.59±0.97	9.12±0.61	9.91±0.98	10.59±0.76	0.50±0.10	0.49±0.06	4.91±1.12	5.17±0.82
Novartis S2020	6.48±0.79	9.39±1.28	6.93±0.67	9.39±1.14	0.43±0.07	0.41±0.04	3.01±0.51	3.83±0.49

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