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The influence of storage conditions on soybean components and its relation to soymilk and tofu quality

by

Jasmine Yee Lin Kuan

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Lester A. Wilson, Major Professor
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Ames, Iowa

2005

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Graduate College
Iowa State University

This is to certify that the master's thesis of

Jasmine Yee Lin Kuan

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

To those who believed in me, even in times when I did not believe in myself, this thesis is as much yours as it is mine.

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ABSTRACT

The objective of this study was to observe the changes that occur during soybean storage and its effects on soymilk and tofu quality. Three food grade soybeans, Vinton 81, IA2032 LS, and Proto, from the 2002 and 2003 crop years were used in this study. The soybeans were stored at temperatures of 20°C, 30°C, 40°C, with two humidity conditions, 32 and 75% RH, and were evaluated every 3 months. The difference in the lengths of soybean storage was based on failure of the extracted soymilk to coagulate, which occurred at the highest temperature and humidity storage condition, at 6 months in 2002, and at 12 months in 2003. The soybeans were evaluated for moisture, protein and oil, color, total and soluble oxalates, antioxidant capacity, and soluble sugars. The soybeans were subsequently processed into soymilk and tofu using the traditional Japanese method, and were evaluated for yield, color and texture. Soybean oxalate content was not affected by different storage conditions, although there was a difference between total oxalate content and soybean cultivars. The oxalates did not make a difference in affecting tofu yield or texture. Antioxidant capacity of soybeans was found to increase when stored at a high temperature and humidity condition. Glucose was only found under high temperature and humidity storage conditions. With increased storage time, temperature and humidity, soybeans had lower Hunter *L* values and browning of the soybean seed coat was observed. This in turn carried over into soymilk and tofu produced from these soybeans, and was also observed by the gradual increase in + *a* values with increasing temperature and humidity. Soymilk solids level is a good indicator of its ensuing tofu quality. There was a very distinct color difference in different soybean cultivars at all storage conditions. Therefore, color can be used as a predictor of soybean storage conditions and its resulting product quality.

INTRODUCTION

Soybeans are considered one of the most valuable agricultural commodities since they have both economic and nutritional value with their multifaceted components. As a legume crop, the proximate composition of soybeans is about 40% protein, 20% lipid, 35% carbohydrate and 5% ash on a dry basis. In addition to these major components, there are also minor constituents such as minerals, vitamins, oxalic acid, phytic acid, isoflavones and saponins that make the soybeans a unique nutritional source.

With exports of about 14 million tons of soybeans just to Asian countries alone in 2002, U.S. soybean exports have been increasing steadily over the years, with a worldwide total export value worth $5,677 million (Soyatech 2004). The market for soy-based foods in the U.S. grew to $4 billion in 2003, even though the rate of growth has decreased, indicating a maturing stage of growth for soy foods (Soyatech 2004). However, new soy food trends and categories such as yogurts, nondairy frozen deserts, entrees and the like are beginning to show increasing growth. As such, soy food sales and demand for soybeans are predicted to be on the rise. Human consumption of soybeans and products made from them has been increasing due to its many nutritional benefits. The Food and Drug Administration (FDA) has approved a nutrition label health claim recommending 6.25g of soy protein per serving to reduce the risk of heart diseases (Henkel 2000). Soymilk is a common non-fermented beverage product made from the water-soluble extract of soybeans and is a very good nutritional alternative for people with lactose intolerance. Various methods have been employed to obtain soymilk from soybeans, but the most common method used for soymilk production is the traditional Japanese method. Tofu, a soft bean curd obtained by coagulating soymilk proteins, is another Oriental soy product that is fast gaining popularity in the

Western countries. Textural and sensory qualities are important in consumer acceptance. Oriental consumers prefer a softer tofu whereas Western consumers prefer tofu with a firmer texture.

After harvest, soybeans are stored in farm grain elevators or processing facilities, and as such, are subjected to changes during storage and transportation, before processing into soy products. Post-harvest modification of soybeans is very pronounced in the summer months, especially during storage and transporting across continents. Over prolonged storage, soybean seed quality and quality of edible products made thereof decreases.

Several model storage studies on soybeans have been done to determine the influence of storage conditions on their functional properties. Severe quality changes were observed in the soybeans stored under high temperature and humidity. Some physical changes include decreased lightness of the soybeans after six months, mold growth at the high humidity storage, and damaged beans.

Proximate analysis of soybeans can be measured using near infrared reflectance (NIR) spectroscopy. NIR reflectance spectroscopy is a useful, non-destructive tool for estimation of soybean components, which is not influenced by seed size or seed coat color.

Little study has been given to the effect of storage on other components that may be nutritionally valuable. Oxalic acid and its metal ion salts are widely found in plants, vegetables and nuts. Soluble and insoluble oxalates and oxalic acids are widely and naturally found in higher plants, including vegetables and legumes. Oxalic acid and soluble oxalates are capable of forming an insoluble salt with calcium (Ca) and thus interfere with Ca absorption by the body (Massey and others 2001). In addition to binding Ca in the body, oxalates have also been hypothesized to bind minerals in soy. This could be an area of

concern if oxalic acid and soluble oxalates competed with calcium-based coagulants to form a tofu curd, thus decreasing tofu yield and altering tofu texture.

Soybean, a member of the oilseed family; has tocopherols, flavonoids and phenolic acids as antioxidants. With recent interests in antioxidants and health, more studies need to be done to understand the health benefits of soy antioxidants.

Two soluble sugars of importance in soybeans are the oligosaccharides stachyose and raffinose, due to their flatulence effects. However, there have been some recent interests in soy oligosaccharides as anticarcinogenic agents and as functional food (Messina 1999).

Quality of soymilk can be measured by flavor and color whereas tofu quality can be quantified in terms of color and texture. Such attributes can influence our perception towards a food product.

THESIS ORGANIZATION

This thesis is organized into three separate papers for publication in the Journal of Food Science. There is a survey of literature regarding previous studies done on this area of study. The first manuscript will addresses the changes of oxalate levels in soybeans during storage whereas the second manuscript deals with the antioxidant potential under different storage conditions and its effect on soybean quality. The last manuscript deals with the biochemical changes of selected components in the stored soybeans and their consequences on soymilk and tofu quality.

LITERATURE REVIEW

SOYBEAN CONSUMPTION, SALES AND TRENDS

With exports of about 14 million tons of soybeans just to Asian countries alone in 2002, U.S. soybean exports have been increasing steadily over the years with a worldwide total export value worth $5,677 million (Soyatech 2004). The market for soy-based foods in the U.S. grew to $4 billion in 2003, even though the rate of growth has decreased, indicating a maturing stage of growth for soy foods (Soyatech 2004). That study indicated a slow growth in products like soymilk, tofu and soy burgers, while new soy food trends and categories, such as yogurts, nondairy frozen deserts, entrees and the like are beginning to show increasing growth. Soymilk and tofu are the most commonly used non-fermented food application processed from soybeans. As such, soy food sales and demand for soybeans will still be on the rise.

Human consumption of soybeans and products made from them has been increasing due to its many nutritional benefits. The Food and Drug Administration (FDA) has recently approved a nutrition labeling health claim recommending 6.25 g of soy protein per serving to reduce the risk of heart diseases (Henkel 2000). The health claim must have the statement, "*Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease. One serving of (name of food) provides __ grams of soy protein*".

SOYBEAN COMPOSITION

Major components

Soybeans are considered one of the most valuable agricultural commodities since they have both economic and nutritional value with their multifaceted components. As a

legume crop, the proximate composition of soybeans are about 40% protein, 20% lipid, 35% carbohydrate, and 5% ash on a dry basis.

Proteins

Soy protein is a very high quality protein and is fast gaining popularity as a substitute for animal protein (Liu 1997). Although soy protein is low in sulfur containing amino acids such as methionine, but adequate for humans, soy is nevertheless rich in the essential amino acid lysine, which is deficient in most cereal grains.

Seed proteins in plants are of two types, metabolic and storage proteins. The majority of the soybean protein is storage protein. Two major storage proteins in soybeans are glycinin and β-conglycinin, which account for 65-80% of the soybean storage proteins (Liu 1997). The bulk of the glycinin and β-conglycinin proteins are stored in spherical protein bodies ranging from 2-20 μm in diameter, with smaller oil bodies, spherosomes, interspersed in between (Saio and Watanabe 1968, Wolf and Cowan 1975). Other proteins found in the protein bodies include lectins, trypsin inhibitors and polypeptides (Liu 1997).

When soy protein undergoes an analytical ultracentrifugation separation, β-conglycinin has a sedimentation coefficient of 7S (S = Svedberg units) and is present in the highest amount. It is organized as a trimer with a molecular weight (MW) of 180 kilo Daltons (kDa). Glycinin, occurring as a hexamer with MW of 360 kDa, is found in its purest form in the 11S fraction. As such, it is the largest single fraction of total seed protein (25-35%) and accounts for over 40% of the total seed globulin (Liu 1997).

The major storage proteins of soybeans, glycinin and β-conglycinin, differ in composition and structure, thus exhibiting distinctions in nutritional quality and functional properties. Both glycinin and β-conglycinin form gels when heated or when coagulant is

added. Heating time and temperature play a role in the hardness of gels set from the glycinin and β-conglycinin protein. The glycinin gel coagulates faster with calcium sulfate and is much harder than the β-conglycinin gels (Saio and others 1969).

Murphy and Resurreccion (1984) found that there is an impact of genetics on the glycinin and β-conglycinin content of soybeans. An earlier study (Hughes and Murphy 1983) on the genetics of glycinin in soybean was also consistent with this finding. However, the crop environment also has a greater impact on the glycinin fraction than the β-conglycinin fraction (Murphy and Resurrecion 1984).

In a study on the textural properties of soy protein gels, Kang and others (1991) demonstrated that elasticity was affected by heating temperature, hardness was affected by protein concentration, and fracturability was affected by the glycinin/ β-conglycinin ratio.

The unique tofu texture can also be attributed to both the intermolecular reactions between the free sulfhydryl (-SH) and disulfide (S-S) groups and the intermolecular hydrophobic reactions among the exposed hydrophobic amino acid residues of the major storage proteins, glycinin and β-conglycinin (Draper and Catsimpoolas 1978, Fukushima 1991).

Trypsin Inhibitors. Trypsin inhibitors (TI) are known to be antinutritional factors ubiquitous in soy. Two types of TI have been identified, the Bowman-Birk and Kunitz. There have been studies indicating that the Bowman-Birk TI are more heat stable and some evidence that it may have anticancer properties (Messina 1999). TI activities may be reduced through moist heat treatment, but soy protein solubility and essential amino acid loss can occur from extremely high heat. Consequently, optimum heating time, temperature, moisture

and pressure should be used to ensure proper inactivation (~90%) of TI without losing soy protein quality.

Lipoxygenase. Lipoxygenase (LOX) is a dioxygenase enzyme that catalyzes the hydroperoxidation of polyunsaturated fatty acids (Prigge and others 1996). Off-flavors associated with soy such as beany, grassy or astringent can be attributed to the breakdown products from hydroperoxides, such as hexanal, produced by LOX enzymes, i.e. hydroperoxidation of *cis-cis* 1,4-pentadiene-containing fatty acids by LOX (Wilson 1996).

LOX triple null soybeans are soybeans that are genetically altered through plant breeding to have the three lipoxygenase isozymes, Lox-1, Lox-2, and Lox-3, removed. The triple null soybeans are developed for use in food production since soybean cultivars that lack all three lipoxygenase isozymes contain less of the beany flavor when compared to conventional soybean cultivars. The lipoxygenase enzymes create the primary products for lipid autoxidation in soybeans, which have been known to cause off-flavor and quality in the soy products made from them. Soymilk and tofu made from LOX triple null soybeans are less astringent due to its lesser beany aroma and flavor (Wilson 1996). Weather conditions also play a role in influencing LOX activities in soybean cultivars (Marczy and others 1995).

Lipids

Lipids in soybeans are stored in lipid bodies, spherosomes, in the form of triacylglycerols, and the minor components including phospholipids, tocopherols, phytosterols, hydrocarbons, and free fatty acids (Saio and Watanabe 1968, Wolf and Cowan 1975, Liu 1997). Soybean oil has a fatty acid composition of 53% linoleic acid and 23% oleic acid, indicating that it is a good source of unsaturated fatty acids. Further, the sound

nutritional value of soybean oil can be attributed to its high proportion of essential fatty acids, phospholipids, phytosterols and tocopherols (White and Xing 1997).

Soybean oils are low in saturated fatty acids, and the double bonds within unsaturated fatty acids are in the *cis-* configuration. Hydrogenation is sometimes carried out to improve flavor and oxidative stability, as well as increase melting point, but some of the double bonds are isomerized from the *cis-* to *trans-* form. Recent health concerns regarding *trans-* fatty acids and higher occurrences of coronary heart diseases has prompted soybean researchers to develop new soybean cultivars with modified fatty acid composition, such as the low and ultra-low linolenic acid soybean oils, to meet nutrition labeling expectations regarding *trans-* fatty acid content (List 2004).

Carbohydrates

Carbohydrates may be grouped into soluble and insoluble carbohydrates. Total soluble sugars make up 9-12% of the soybean carbohydrate composition. Soluble sugars in soybeans include glucose, arabinose, sucrose, raffinose, stachyose, fructose and galactose (Liu 1997). The most notable soluble oligosaccharides in soybeans are raffinose and stachyose that cause flatulence in some people. The human digestive system does not possess the enzyme α-galactosidase necessary to break down the α-galactosidic linkages found in these oligosaccharides. Genetic engineering has been used to produce soybean cultivars with low oligosaccharide content. However, there has been recent interest in soy oligosaccharides as an anticarcinogenic agent and potentially leading to their classification as a functional food (Messina 1999).

A study by Wilson and others (1978) indicated that soybean starch is mostly found in the middle of the cotyledon and not at the periphery as previously thought. With less than 1%

of starch in the overall soybean composition, Wilson and others (1978) determined that the starch does not increase the viscosity of heated soymilk.

In addition to total sugars, there are also complex carbohydrates or saccharides, such as cellulose and hemicellulose, which are insoluble, and pectins, which are soluble, that contribute to dietary fiber. These carbohydrates are mainly found in the structural cell wall of the soybean.

"Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation and/or blood glucose attenuation", is the definition of dietary fiber as stated by the American Association of Cereal Chemists (AACC 2001). As a consequence, most of its oligosaccharides and its complex polysaccharides, but not raffinose and stachyose, would be categorized as dietary fiber. With increasing interests in the nutritive value of fiber, soy is beginning to gain increased popularity in most diets as a source of dietary fiber.

Proximate analyses

The composition of soybeans can be determined by proximate analysis (moisture, protein, lipid, ash, and carbohydrate, by difference). Components such as moisture, protein or lipid have been measured using Association of Official Analytical Chemist (AOAC) methods. These official AOAC methods include the vacuum oven method or Karl-Fischer titration for determining moisture and the Kjeldahl method for protein determination.

Near infrared reflectance (NIR) spectroscopy also can be used to rapidly analyze grains and soybeans for moisture, protein, oil and fiber. Soybean composition was obtained by way of reflectance from whole seed readings in a non-destructive manner. The NIR analyzer measures the potential energy of the vibrations of atoms in the molecules after excitation with near-infrared electromagnetic energy. The system was calibrated by an ideal absorbance curve obtained from analyzing a large number of seed or grain samples (Hardy and others 1996).

Minor components

In addition to these major components, there are also minor constituents such as minerals, vitamins, oxalic acid, phytic acid, isoflavones, and saponins that make the soybean a unique dietary source.

Phytic acid

Phytic acid, the main source of phosphorus in soybean seeds, amount to about 1-1.5% and is located almost exclusively in the protein bodies (Liu 1997). Phytic acid has the ability to chelate metal ions, particularly iron, calcium, magnesium and potassium. Determination of phytic acid in soybeans is important in terms of bioavailability of minerals and coagulation of tofu during production (Schaefer and Love 1992). A higher phytic acid content in soymilk would result in a lower coagulation rate between soy protein and added calcium, which is of importance in tofu curd formation (Saio and others 1969).

Phytate occurs as the calcium-magnesium-potassium salt of inositol hexaphosphoric acid (Liu 1997). Interestingly, even though phytate in soybeans have been negatively implicated due to its mineral absorption ability, there is interest in phytic acid as anticarcinogenic (Messina and Barnes 1991) and an antioxidant (Graf and Eaton 1990).

Oxalic acid

Oxalic acid (Figure 1) and its metal ion salts naturally occur in plants, vegetables and nuts. Oxalate exists in soluble or insoluble forms of oxalic acid. The soluble forms include the potassium and sodium (K^+ and Na^+) salts of oxalic acid, whereas the insoluble form is present as calcium oxalate (Table 1).

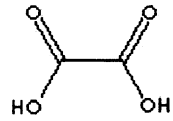

Figure 1: Chemical structure of oxalic acid

Calcium oxalate, being the least soluble form of oxalic acid (Table 1), occurs principally as a monohydrate or dihydrate, although the monohydrate is more stable (Hodgkinson 1977). Since calcium oxalate constitutes the largest part of the insoluble oxalate fraction, it is worthwhile to know the concentrations of both the soluble and insoluble oxalates (Hodgkinson 1977).

Table 1: Solubility of some oxalic acid salts
Source: Properties of oxalic acid (Stephen and Stephen 1963)

Metal	Temperature (°C)	Solubility (g/l)
Ca^{2+}	18	0.0060
Ca^{2+}	20	0.0066
Ca^{2+}	25	0.0086
Ca^{2+}	37	0.0071
Ca^{2+}	45	0.0090
Ca^{2+}	55	0.0100
Ca^{2+}	65	0.0120
Ca^{2+}	95	0.0145
K^+	20	266.8
K^+	30	285.0
K^+	50	326.0
Na^+	10	30.1
Na^+	20	33.9
Na^+	30	37.6
Na^+	50	43.4

Oxalic acid and soluble (Na^+ or K^+) oxalates are capable of forming an insoluble salt with calcium; and thus, interfere with mineral absorption by humans and other animals (Massey and others 2001). Since the soluble oxalates are absorbed more readily than the insoluble salts, therefore, it is worth identifying the relative amounts of these fractions in food products.

Despite the soy nutrient content, the nutritional consequences of oxalate content of soybeans and its binding with calcium have been overlooked due to the lack of extensive scientific research on the oxalate content of soybeans. Presently, there is very little information regarding the oxalate content in mature soybean seeds as well as information on the effects of oxalic acid on tofu coagulation. Some commonly consumed soy foods have been found to contain 0.11-2.0 mg of oxalate per g of soy food (Massey and others 2002). Soy foods are considered high-oxalate foods, since foods containing more than 0.08 mg of oxalate per g of food are considered high-oxalate foods for patients with CaOx kidney stones (The Chicago Dietetic Association and others 2000).

Table 2 lists the oxalic acid content of selected vegetables in the USDA nutrient data laboratory. The published values are limited to selected food items and data for soybean is not available in the database.

Table 2: Oxalic acid content of selected vegetables
Source: USDA-ARS Nutrient Data Laboratory (Available from www.nal.usda.gov/fnic/foodcomp/Data/Other/oxalic.html)

Vegetable	*Oxalic acid (g/100g)*
Amaranth	1.09
Asparagus	0.13
Beans, snap	0.36
Beet leaves	0.61
Broccoli	0.19
Brussels sprouts	0.36
Cabbage	0.10
Carrot	0.50
Cassava	1.26
Cauliflower	0.15
Celery	0.19
Chicory	0.21
Chives	1.48
Collards	0.45
Coriander	0.01
Corn, sweet	0.01
Cucumbers	0.02
Eggplant	0.19
Endive	0.11
Garlic	0.36
Kale	0.02
Lettuce	0.33
Okra	0.05
Onion	0.05
Parsley	1.70
Parsnip	0.04
Pea	0.05
Pepper	0.04
Potato	0.05
Purslane	1.31
Radish	0.48
Rutabaga	0.03
Spinach	0.97
Squash	0.02
Sweet potato	0.24
Tomato	0.05
Turnip	0.21
Turnip greens	0.05
Watercress	0.31

Antioxidants

Most plant sources have natural antioxidants and the soybean; a member of the oilseed family includes tocopherols, flavonoids and phenolic acids among its antioxidants. Soybean antioxidants were found in the form of tocopherols, predominantly γ- and δ-tocopherol (White and Xing 1997). One of the lipid soluble vitamins in soybeans, vitamin E, contains vitamin activity in the form of α-tocopherol, since α-tocopherol alone is used for estimating vitamin E requirements and recommended intake because the other naturally occurring forms of vitamin E are not converted to α-tocopherol in the human body (The National Academy of Sciences 2000). Each of the tocopherol forms has a different vitamin E and antioxidant activity. Soybean tocopherol content varied in α-, γ-, and δ-tocopherol content, ranging from 10.9-191 μg/g dry matter in the soybean cultivars reported by Guzman and Murphy (1986). Even though there was a loss of total tocopherol when processing soybeans into tofu, the tofu was a better source of tocopherols than soybeans on a dry weight basis and the tofu tocopherol content was not affected by commercial storage conditions (Guzman and Murphy 1986).

The general structural components of natural antioxidants found in soybeans include phenols and flavonoids (Pokorny and others 2001). The primary flavonoids are isoflavone glucosides, which are the 7-glucosides including the isoflavones genistein, daidzein and glycitein, which are moderate antioxidants and much poorer antioxidants than tocopherols.

Isoflavone content may vary among soybean cultivars, crop year and location, as reported by Wang and Murphy (1994). The study found that crop year, rather than location, had a greater effect on isoflavone content. There were also varietal effects among American and Japanese soybean cultivars, as indicated by different ratios of the malonyl family of

isoflavones to the glucoside family and the distribution patterns of individual isoflavones. A more recent study by Hoeck and others (2000) found that environment and genotype played a significant role on isoflavone content in soybeans.

Isoflavones have also been shown to hydrolyze during soybean storage (Hou and Chang 2002). Hou and Chang (2002) demonstrated that conversion of malonylglucosides to aglucons during storage, especially at high temperature and humidity storage conditions (30°C and 84% RH).

Long-term storage may result in degradation of soybeans through lipid oxidation reactions. Antioxidants are inhibitors that prevent formation of hydroperoxides by scavenging the free radicals in soy. Soybeans that have been exposed to various levels of stress during transit and storage may contain oxidation products, which can alter the quantity and quality of food produced from such beans. Oxidative stress can influence the antioxidant level in the food and its shelf life.

An antioxidant may function as a reactive oxygen species (ROS) scavenger by sacrificing itself to stop free radical chain reactions, or as a preventative antioxidant by inhibiting formation of reactive oxidants. Dietary antioxidants broadly include radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors and antioxidant enzyme cofactors. There are many scientific papers assaying for antioxidant content which interchangeably use terms such as, capacity, potential, activity, efficiency and potency. However, it is only significant to use such terms if a specific condition is applied in an individual assay of the reactivity of an antioxidant (Huang and others 2005).

Major antioxidative capacity assays can be grouped into hydrogen atom transfer (HAT) reactions and electron transfer (ET) reactions. HAT assays measure the competitive

reaction kinetics whereas ET assays measure radical scavenging capacity (Huang and others 2005). Examples of HAT assays are oxygen radical absorbance capacity (ORAC) and total radical trapping antioxidant parameter (TRAP), whereas ET assays include Trolox equivalent antioxidant capacity (TEAC) and diphenyl-1-picrylhydrazyl (DPPH).

The Photochem® (analytikjenaAG, Germany) uses photochemiluminescence to evaluate the end products of a free radical reaction. A photosensitizer substance is optically excited by UV-light in the system to produce superoxide anion radials. The free radicals are detected by means of a chemiluminogenic substance, which emits light which is detected in the Photochem® by a photomultiplier. As such, the antioxidative potential is determined based on the radical scavenging capacity of the antioxidants in the sample. The remaining radicals are then quantified by comparing such numbers with the values for a phenolic antioxidant standard, Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid).

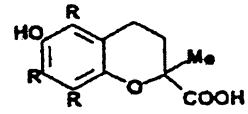

Figure 2: Chemical structure of Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (R=H or Me)

SOYBEAN STORAGE STUDIES

Soybeans are subjected to changes after harvest, during storage and transportation, before processing into soy products. These changes are especially critical during commercial storage and shipment of soybeans in the summer. Since soybeans are a major agricultural export of the United States to the Asian markets (Table 3), soybean storage and transport conditions are an important factor to consider for optimal end product quality besides genetic modification.

Table 3: U.S. Soybean Exports - By Region & Total Value (metric tons)
Source: 2004 Soya & Oilseed Bluebook (USDA FAS, FATUS Reports)

Region	2001	2002
North America	5,883,708	5,682,786
South America	254,590	259,715
Europe	6,523,134	6,069,578
Former Soviet Union	120,227	66,498
Middle East	911,102	1,113,302
Africa	332,188	356,168
Asia	14,698,791	14,113,792
Australia & Oceania	41	12,698

Several model storage studies on soybeans have been done to determine different functional properties. Two of the most comprehensive studies were done by Saio and others (1980, 1982). In their 1980 study, soybeans were stored at 25 and 35°C at two different humidities, 60 and 90% RH for each temperature. Severe quality changes were observed in the soybeans stored under high temperature and humidity. Some physical changes included decreased lightness of the soybeans after six months, mold growth at the high humidity samples, and damaged beans. Saio and others (1980) also observed for products from the stored soybeans, the ease of separation of the water and oil phases in soymilk, as well as the decreases in tofu hardness at the high temperature and humidity storage condition. In fact, the tofus produced from six months of storage at these conditions almost did not coagulate.

In the other study by Saio and others (1982), increased temperature and humidity during storage decreased solubility of soybean proteins, therefore, making the glycinin and β-conglycinin portions difficult to extract, as well as causing increased acid value of the oil. They also found that whole beans were more resistant to deterioration compared to defatted soybean meals, followed by full fat soybean meals. In other words, the functional quality of

the soybeans would be retained if the cellular organization of the soybeans were intact. Therefore, whole soybeans would have a better storage quality.

In addition to the properties discussed in the studies by Saio and others (1980, 1982), soymilk and tofu properties were also influenced by soybean storage conditions. Thomas and others (1989) reported in their study that relative humidity significantly influenced protein extractability, and there was an interaction between their storage time and humidity conditions. They found that at 85% RH, curds would settle at the bottom of the container while coagulating and would form a non-uniform mass. Several other researchers (Saio and others 1980, 1982; Yanagi and others 1985) have found changes in protein solubility that also were influenced by temperature and humidity.

An accelerated storage study by Murphy and others (1997) was modeled after the study by Saio and others (1982). The study found a decreased nitrogen solubility index (NSI) at the high temperature and humidity conditions. Although the extractabilities of glycinin and β-conglycinin decreased linearly with the temperature and humidity conditions, extractability of these proteins was also cultivar dependent. Such protein changes are important since tofu yield, texture, and quality are affected, since tofu is sold by weight. Consumer preference was correlated with soybean storage conditions too. At a higher temperature, the beans were darker; hence, producing darker soymilk, and, subsequently, tofu (Wilson and others 2004). These dark products were less preferred by the consumers.

Another comprehensive study by Narayan and others (1988) indicated that the changes of soybean physico-chemical properties affected sensory quality of soy products made there from. In that study, with increased storage time, soybean color changed from yellow to brown, increased peroxide values due to formation of peroxides from unsaturated

fatty acids, and increased free fatty acids from hydrolytic changes in fat compounds were measured. Among the physico-chemical properties observed with increased storage time were increased browning due to enzymatic and non-enzymatic browning (Maillard browning), decreased carotenoid pigment content from autoxidation, decreased reducing sugars from Maillard reaction and decreased non-reducing sugars from enzyme hydrolysis.

Tolerances to different storage conditions were soybean cultivar specific. Lambrecht and others (1995) discovered that Century–L2L3 (lacking lipoxygenase isozymes 2 and 3) yielded better stability towards storage under adverse conditions, as well as producing more desirable tofus.

Proto cultivar is grown in the upper northern plains of the United States and is high in protein compared to other soybean cultivars. A study by Wang and Chang (1995) showed that Proto soybeans had a tofu yield greater than other soybeans when calcium sulfate was the coagulant. The increased tofu yield was attributed to increased protein content, which also contributed to the increased firmness and springiness. A storage study done by Hou and Chang (1998) using Proto cultivar indicated reduced tofu yield when the Proto soybeans were stored at 85% RH and 30°C. The decreased yield was attributed to a decrease in solids and protein extractability from the beans to the milk.

SOYMILK AND TOFU PROCESSING

Soymilk is a common non-fermented beverage product made from the water-soluble extract of soybeans and is a very good nutritional alternative for people with lactose intolerance. Various methods have been employed to produce soymilk from soybeans, such as the traditional method (Chinese or Japanese), Cornell method, Illinois method or Rapid Hydration Hydrothermal Cooking (RHHTC).

The most common method used for soymilk production is the traditional method, which requires the soybeans to be soaked overnight for 8-12 hours in cold water, before being ground into slurry with water added during the grinding step (Shurtleff and Aoyagi 1983). In the Chinese method the resulting slurry is filtered before being heated to 95°C, which allows the LOX enzyme activity to increase resulting in more beany flavors. In the Japanese method however, the resulting slurry is filtered before being heated. The heating process denatures TI and LOX, the sources of reduced protein digestion and the source of beany off-flavors in the soymilk, respectively.

The Cornell method utilizes a hot-grinding process in which unsoaked, dehulled soybeans are ground using hot water. The slurry is then heated to between 80-100°C for 10 minutes to inactivate LOX enzymes that cause off-flavors in soymilk. In this method, hot grinding improves flavor but the initial heat denatures protein, thus decreasing extractability (Wilkens and others 1967).

The Illinois method (Nelson and others 1976) employs a carbonate presoaking and blanching procedure. The higher protein quality from these carbonate presoaked and blanched preparations are due to the unfolding of the protein molecule, because of the combined action of alkali and heat, which subsequently makes them more digestible.

The RHHTC method uses steam infusion into a ground soybean slurry that mixes it with hot water, minimizing amino acid degradation, while providing adequate inactivation of TI in the soymilk under a high-temperature-short-time (HTST) heating process as of this method. RHHTC process produces soymilks of increased protein recovery as a result of a more optimal heat exposure and the effects of dissociation of the protein bodies by the infused steam and the shear force it creates (Johnson and others 1981).

Trypsin inhibitor (TI), which is one of the antinutritional factors in soybean, can be effectively destroyed by moist heat to yield a product with improved nutritional value. These protease inhibitors are important in another aspect in that although they only represent a small part of the bean protein content (2.5%), they contain 30-40% of the cysteine amino acid in these inhibitors. Therefore, TI in its inactivated form can be a better source of sulfur containing amino acid. Inactivation of TI in soymilk production can be done by blanching whole soybeans before grinding with water, cooking the soy flour in a water slurry, or by heating the soymilk before or after filtration to remove the fibrous residue (okara). As such, precise control of the heating process is critical for the preparation of soy protein products with maximum nutritional value. The extent of destruction of TI in soymilk for maximal value or protein efficiency ratio was reported to be 90% (Hackler and others 1965). In essence, the D value, the time for 90% inactivation of TI at a specific temperature (~95°C), depends on the come up time and process efficiency.

Temperature is an essential factor when cooking soymilk, as Johnson and Snyder (1978) have shown that lower heat would increase the percent solids (from soluble proteins) as well as its yield of solids. Percent solids are important in tofu production since tofu texture may vary due to percent solid fluctuations. Although soymilk solids may differ depending on soybean variety, processing conditions were also found to affect the solids content, an important factor in tofu production (Johnson and Wilson 1984).

After the soymilk is cooked, it is filtered through a nylon mesh bag to separate the okara, which is a high moisture and fiber byproduct. This step is equally important since soymilk is an intermediate to tofu processing.

Tofu, a relatively soft bean curd obtained by coagulating soymilk proteins, is another Asian soy product that is fast gaining popularity in the Western countries (Soyatech 2004). Textural and sensory qualities are important in consumer acceptance. Asian consumers prefer a softer tofu whereas Western consumers prefer tofu with a firmer texture. Watanabe and others (1964) and Saio and others (1979) reported that increased soymilk solids and coagulating temperature would yield a hard tofu. Tofu texture can also be affected by the type of coagulant used, coagulant concentration, stirring speed, and pressure applied when pressing the tofu (deMan and others 1986).

Once soymilk solids have been determined (Johnson and Wilson 1984), the amount of coagulant to be used can be calculated for addition to the soymilk that is then heated to 85°C, and quickly stirred before letting it sit. Once coagulation is complete, the curd is then cut to separate the whey. Finally, the curds are carefully ladled into a tofu box lined with cheesecloth for pressing.

Gandhi and Bourne (1988) reported that with increased pressing pressure, texture profiles such as hardness, chewiness, and gumminess increased. They also reported that with increased storage time, hardness and gumminess of the tofu also increased. This finding however, was in contrast with the model storage study by Saio and others (1980) that indicated decreased hardness with storage at increased temperature and humidity. Murphy and others (1997) found that there were correlations between storage proteins and their subunits extractability with tofu texture in aged soybeans.

SOYMILK AND TOFU QUALITY

Quality of soymilk can be measured by color, flavor, and viscosity, whereas tofu quality can be quantified in terms of color, flavor, and texture. Sight, touch, sound, and taste influence our perception towards a food product.

Light and color perception first occur in the retina of the eye, where retinal receptors in the shape of rods and cones, discriminate between colors and send the information to the brain (MacDougall 2002).

Color

Color is one of the most important attributes of food appearance, which can influence its quality and palatability. Foods exhibit different appearance characteristics and surface qualities, such as haziness, opacity, translucence, transparency, glossiness, matte or porosity (MacDougall 2002). Most food products are classified as translucent, whereby light may be diffused or passed through the food object. Sample preparation is essential since every food has unique surface properties. Potential problems that arise from preparing a sample, such as slicing, compressing or trapping air bubbles could yield inaccurate color readings from the instrument (Hutchings 1999).

Accurate and precise color measurements depend on the viewing angle, light source, detector, aperture size, sample preparation, and sample presentation. Commonly used color measurement instruments employ a D65 illuminant, an illuminant that has an ultraviolet component and a color temperature of 6500°K, the average temperature of light on an overcast day, and it assumes a 10° standard observer to increase the diameter of the viewed object.

Uniform color spaces have been developed for color measurement, most notably the HunterLab and CIELAB color spaces. In a Hunter color system, color can be defined by the *L, a* and *b* parameters of the scales shown in Figure 3. *L* indicates lightness or darkness of and object or product (with 100 being white and 0 being black), whereas '*a*' indicates greenness as a negative value while a positive value measures its redness. Last but not least, a positive '*b*' value indicates yellowness, whereas its blueness is indicated by a negative value. The CIELAB color spaces define color in terms of *L**, *a** and *b**, where *L** indicates visually uniform lightness, *a** and *b** indicate visually uniform chromaticness coordinates (MacDougall 2002).

Instrumental measurement of color can be done in many ways using different apparatus or instruments. The terms colorimeter and spectrophotometer are often confused since the two instruments are used to measure color, although both treat color data obtained very differently. Among their differences are the colorimeter measures psychophysical data, which has been correlated with human eye-brain perception, whereas a spectrophotometer provides a wavelength spectral analysis of the object without human interpretation. Most notably, a colorimeter has a set observer and illuminant combinations, whereas a spectrophotometer has many observer and illumination combinations that may be used for calculating tristimulus data and the metamerism index (Hunter and Harold 1987).

Figure 3: HunterLab color solid (Source: www.hunterlab.com/pdf/labcolorsolid.pdf)

Texture

Texture as defined by the International Organization for Standardization as "all the mechanical, geometrical, and surface attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual, and auditory receptors". Food texture can be associated with the structure of a food product and ultimately the functional properties from which the structure is derived.

All said though, foods have a wide range of textural and rheological attributes, and one may find it hard to categorize a food product as just solid, liquid, or gel-like. It is more useful to classify food texture by the type of test that is used, i.e. whether it is a fundamental, empirical or imitative test. Instrumental texture measurements have a few disadvantages in that they are hard to correlate with human sensory data since there are different speeds at

which the jaw and tongue move, how temperature changes and salivation in mouth, as well as the combined sensation from the food product influenced the outcome of the value.

As described previously, texture is an important feature in tofu quality. There are many methods and instruments used to determine texture, but most notable and popular of the imitative test methods such as the texture profile analysis (TPA), which was developed at General Foods in the mid-1960s (Szczesniak 1963, Bourne 1978). The test consists of a two-bite cycle that imitates the action of the jaw. The principle of the TPA is the compression and decompression of a flat-based plunger twice to resemble the human jaw action. In order to mimic the chewing action, a high compression force, between 50-90% compression should be used in the TPA (Bourne 2002).

The TPA differentiates between texture attributes in a product, and is typically used to correlate its values with sensory analyses of texture. Table 4 lists the instrumental and sensory definitions of all attributes in the TPA. Most commercial texture instruments such as the Instron (Canton, MA) or the TA-XT2i Texture Analyzer (Texture Technologies Corporation; Scarsdale, NY), has a TPA program built into the software. The TPA gives a wide range of textural attributes diagrammed in Figure 4, such as hardness, fracturability, chewiness, gumminess, cohesiveness, adhesiveness, and springiness (Bourne 2002).

Table 4: Instrumental and sensory definitions of attributes in a Texture Profile Analysis (Szczesniak 1963, Bourne 1978)

Attribute	Instrumental Definition	Sensory Definition
Hardness	Peak force of the first compression of the product	Force required to compress a food between the molars
Fracturability	Force at the first significant peak during the first compression of the product	Force at which the food fractures
Cohesiveness	Area Peak 2 / Area Peak 1	How well a product withstands a second deformation, relative to how it behaved under the first deformation. Related to the strength of the internal bonds making up the food
Springiness	Distance of detected height of second compression / original compression distance Length 2 / Length 1	Extent to which a compressed food returns to its original size when the load is removed
Adhesiveness	Negative force area of first bite	Work required to pull a food away from the surface
Stringiness	Distance of adhesive peak, indicating the extension of the food before it separates from compression plate	Distance food extends before it separates from surface to which it is adhering
Gumminess (semi-solid foods)	Hardness * Cohesiveness	Energy required to disintegrate a semisolid food so that it is ready for swallowing
Chewiness (solid foods)	Hardness * Cohesiveness * Springiness	Energy required to chew a solid food until it is ready for swallowing

Fluid food products are characterized by their apparent viscosity. There are many types of fluid flow based on their viscosity behavior under stress. Figure 5 lists the various types of fluid flow behavior, which were modeled using the general viscosity equation $\sigma = b\ \gamma^{s} + C$, where σ is shear stress, b is proportionality factor, C is yield stress and s is pseudoplasticity constant (Bourne 2002). A fluid with Newtonian flow exhibits a straight line

that begins at the origin and does not comprise yield stress. As such, the equation for a Newtonian flow is $\sigma = \eta \gamma$, where η is apparent viscosity. Figures 6(a) and (b) further describe the viscosity of Newtonian fluids. Soymilk assumes a Newtonian fluid flow since it is a water extract of the soybeans.

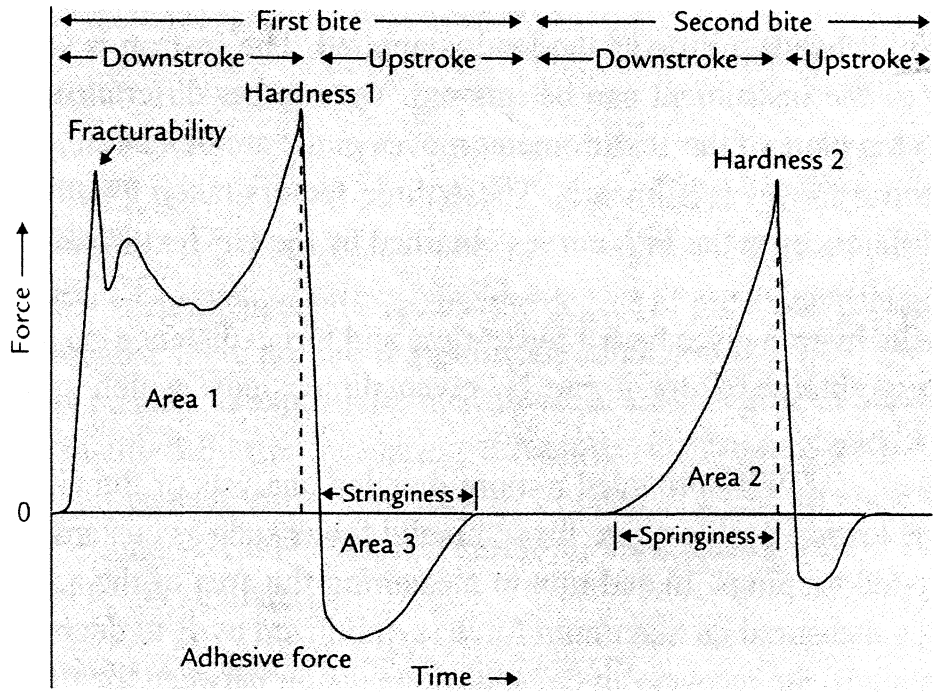

Figure 4: Common Texture Profile Analysis curve (Source: Bourne 2002)

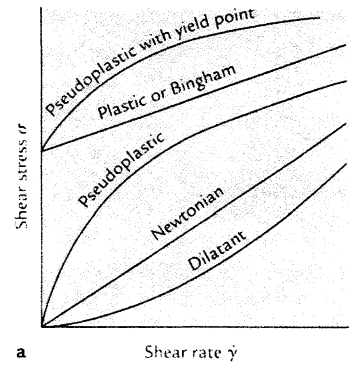

Figure 5: Various types of fluid flow behavior (Source: Bourne 2002)

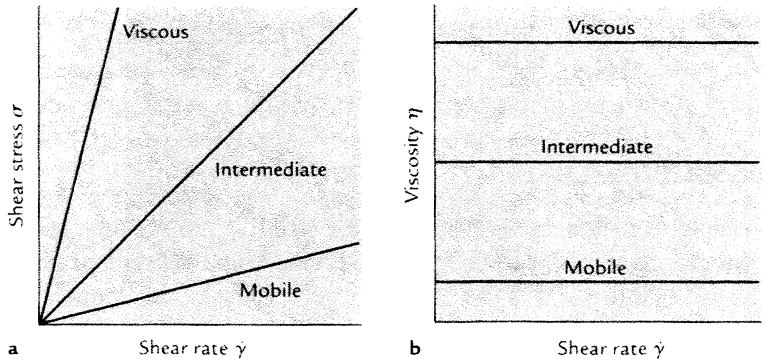

Figure 6: Newtonian flow behavior: (a) shear stress vs. shear rate, lines start at origin; (b) viscosity vs. shear rate, viscosity remains unchanged at different shear rates (Source: Bourne 2002)

CONCLUSION

Based on the studies surveyed in this literature review, many factors relating to storage conditions and soymilk and tofu quality have yet to be studied. The objectives of this study are (1) ascertain the oxalic acid content of soybeans under different storage conditions and the role of total and soluble oxalates in the coagulation of soymilk to form tofu, (2) ascertain antioxidant potential in stored soybeans and its relation to the quality of the soybean seed, and (3) evaluate the quality of soymilk and tofu produced from these soybeans stored under different storage time, temperature, humidity conditions.

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CHANGES OF OXALATE LEVELS IN SOYBEANS UNDER DIFFERENT STORAGE CONDITIONS

A paper to be submitted to the Journal of Food Science

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ABSTRACT

The rationale behind this study is to establish whether the storage conditions, time, temperature and humidity, would have an effect on oxalate levels in soybeans, thus influencing the level of coagulant needed for optimum tofu yield. Soybeans of three different cultivars and two crop years were stored at 20, 30 and 40°C and humidities of 32 and 75% RH, and were sampled every three months. Oxalates were assayed using a modified protocol from the SIGMA / Trinity Biotech Oxalate Kit Procedure No. 591. Each sample was assayed for total, soluble and insoluble oxalates using colorimetric absorbance data. Soybeans were then processed into tofu for quality analyses. Soybean oxalate content in this study were regarded as a high-oxalate food. There was a difference in total oxalates among cultivars in both crop years, but not in soluble oxalates. Tofu yields of both crop years decreased over time, but no correlation was observed among total or soluble oxalates and tofu texture. As such, oxalate content did not affect tofu yield, even at different storage conditions. Oxalates do not seem to play a role in the coagulation of soymilk to form tofu as no tofu yields or textures were affected by the oxalate concentration.

INTRODUCTION

Oxalic acid (Figure 1) and its metal ion salts are widely found in higher plants, vegetables and legumes. Oxalic acid when synthesized by plants may occur as a free acid or as a soluble salt (Simkiss and Wilbur 1989).

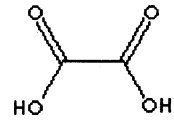

Figure 1: Chemical structure of oxalic acid

Oxalic acid exists in soluble or insoluble forms. The soluble forms include the potassium and sodium (K^+ and Na^+) salts of oxalic acid, whereas the insoluble form is present as calcium oxalate (Stephen and Stephen 1963). Calcium oxalate, being the least soluble form of oxalic acid (Stephen and Stephen 1963), occurred principally as a monohydrate (Whewellite) or dihydrate (Weddelite), although the monohydrate is more stable in plants (Hodgkinson 1977). Since calcium oxalate constitutes the largest part of the insoluble oxalate fraction, and soluble oxalates are absorbed more readily than insoluble oxalates, it is useful to know the concentrations of both the soluble and insoluble oxalates (Hodgkinson 1977).

The oxalic acid content of selected vegetables is published in the USDA nutrient data laboratory (USDA-ARS Nutrient Data Laboratory 1984), and values ranged from 0.01g/100g of oxalic acid in sweet corn to 1.70 g/100 g oxalic acid in parsley. The published values are limited to selected food items and data for soybeans are not available in the database. A survey of total oxalate content in 80 commonly consumed foods and beverages ranged from 0.2 mg/100 g of oxalate in corned beef to 1450 mg/100 g of oxalate in tea leaves (Hodgkinson 1977).

Human consumption of soybeans and products made from them have been increasing due to their many nutritional benefits. The FDA approved a nutrition label health claim recommending 6.25 g of soy protein per serving to reduce the risk of heart diseases (Henkel 2000). Despite the soy nutrient content, the nutritional consequences of oxalate content of soybeans and its binding with calcium have been overlooked due to the lack of extensive scientific research on the oxalate content of soybeans. Presently, there is very little information regarding the oxalate content in mature soybean seeds as well as information on the effects of oxalic acid on tofu coagulation.

Some commonly consumed soy foods have been found to contain 0.11-2.0 mg of oxalate per g of soy food (Massey and others 2002). Soy foods are considered high-oxalate foods, since foods containing more than 0.08 mg of oxalate per g of food are considered high-oxalate foods for patients with CaOx kidney stones (The Chicago Dietetic Association and others 2000). While recommendations for oxalate intake are generally based on the total oxalate content, it is important to realize that bioavailability of oxalate in food does not necessarily correspond with the oxalate content, i.e. a high oxalate food could have low bioavailability.

Oxalic acid and soluble oxalates are capable of forming an insoluble salt with calcium and thus interfere with its absorption by the body (Massey and others 2001). Hyperoxaluria, increased urinary oxalate excretion, and renal stone diseases are some common pathological conditions associated with increased urinary oxalate levels.

Phytates and oxalates are two constituents in soybeans that have been associated with decreased calcium absorption in humans (Heany and others 1991). In addition to binding calcium in the body, oxalates have also been hypothesized to bind minerals in soybeans. This

occurrence could be an area of concern if oxalates would compete with calcium-based coagulants to form a tofu curd, thus decreasing tofu yield. Even though tofu formed with these coagulants were found to contain 69% more oxalates than magnesium-coagulated tofu, one form of tofu is not better than the other since both would increase the likelihood of calcium oxalate kidney stone formation (Massey and others 2002).

The objectives of this study are to determine (1) whether the oxalate content in soybean is affected by change in time, temperature, and humidity storage conditions, and (2) the role of oxalates in the coagulation of soymilk to form tofu.

MATERIALS AND METHODS

Soybean cultivars and storage conditions

Soybeans of three food grade, non-GMO cultivars, Vinton 81 (Pattison Brothers, Fayette, IA), IA2032 LS (Stonebridge Ltd., Cedar Falls, IA) and Proto (Sinner Brothers & Bresnahan Company, Cassleton, ND), from the 2002 and 2003 harvest season were used. Vinton 81 is a high-protein, large-seeded soybean that is one of the dominant beans used by the U.S. soy food industry. IA2032LS is a large-seeded, lipoxygenase-free (triple null) soybean. Proto soybean is a high-protein cultivar with a dark hilum and smaller seed size, which is grown in the upper northern plains of the United States.

The soybeans were placed into nylon mesh bags with each lot weighing about 1200 g. These bags were then placed into tightly sealed five-gallon HDPE buckets (Berry Plastics, Evansville, IN). The soybeans were equilibrated in two extreme relative humidity (RH) conditions, 32% RH and 75% RH, and stored in different Isotemp® (Model 304R, Fisher Scientific, Pittsburgh, PA) large capacity incubators at 20, 30, and 40°C. Each bucket contained a saturated salt solution mixture prepared from standards according to the

American Society for Testing and Materials (ASTM) (Table 1) in a glass beaker and covered with a perforated plastic container, on which the bags of soybeans rested.

Table 1: Equilibrium relative humidity (ERH) values for selected saturated aqueous salt solutions

Temperature (°C)	Magnesium Chloride $MgCl_2.6H_20$, (ERH, %)	Sodium Chloride NaCl, (ERH, %)
20	33.1 ± 0.2	75.5 ± 0.1
30	32.4 ± 0.1	75.1 ± 0.1
40	31.6 ± 0.1	74.7 ± 0.1

Modified after ASTM D: E104-85 "Standard Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions," ASTM International

The storage conditions were monitored periodically using a set of three remote thermo-hygrometers with a multi-channel traceable sensor (Fisher Scientific, Pittsburgh, PA). Control soybeans were packaged and stored in the freezer (25% RH, -9°C). The samples in each temperature and relative humidity storage condition were then taken out of the incubators every three months for analysis. Soybeans from the 2002 crop year were sampled at 0, 3, 6 months, whereas the 2003 crop year soybeans were sampled at 0, 3, 6, 9, 12 months. The difference in the lengths of soybean storage was based on failure of the extracted soymilk to coagulate, which occurred at the highest temperature and humidity storage condition. The soybeans were evaluated for total, soluble and insoluble oxalate, and tofu yield.

Oxalate determination

Soybean seeds were ground using a coffee grinder (Mr. Coffee, Palm Beach, FL), then sieved through a 0.58 mm metal mesh sieve. Samples were placed in marked aluminum weighing pans and incubated in a convection oven for three days at 60°C. The ethylenediamine tetraacetic acid (EDTA) buffer solution and other oxalate reagents were

prepared according the protocol from the SIGMA Urinalysis Diagnostics Kit Procedure No. 591 (Sigma Diagnostics, St. Louis, MO) / Trinity Biotech Oxalate Procedure No. 591 (Trinity Biotech PLC, Co. Wicklow, Ireland). Oxalate was determined according to methods by Ilarslan and others (1997) and by Horner and others (personal communications).

The principle of the assay for oxalate is based on the oxidation by oxalate oxidase and the subsequent reaction of hydrogen peroxide generated with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-(dimethylamino)benzoic acid (DMAB) in the presence of peroxidase to yield an indamine dye (Figure 2). The intensity of the color produced is directly proportional to the concentration of oxalate in the sample.

$$\text{Oxalic acid} + O_2 \xrightarrow{\text{Oxalate oxidase}} 2CO_2 + H_2O_2$$

$$H_2O_2 + \text{MBTH} + \text{DMAB} \xrightarrow{\text{Peroxidase}} \text{Indamine dye} + H_2O$$

Figure 2: Enzymatic assay of oxalic acid

Absorbances were determined using a GENESYS™ 20 spectrophotometer (Thermo Spectronic, Rochester, N.Y.) at 590 nm. The values obtained were converted to oxalate concentration according to the formula in the oxalate kit procedure No. 591 and protocols from Horner and others (personal communications).

Tofu preparation

The method by Moizuddin and others (1999) was used to obtain a 7° Brix soymilk and the optimum coagulant concentration for tofu production. Three hundred grams of soybeans were soaked overnight at room temperature in a 10:1 (v:v) water to bean ratio. The soybeans were rinsed and ground in a Stephan Microcut Type MC15 grinder (Stephan Machinery Corporation, Columbus, OH) twice using 2 different grinder blade sizes, 0.5

inches initially and subsequently 0.05 inches. Water was added continuously during the grinding process. The slurry was cooked in a steam-jacketed kettle (Groen Model TDB/7-40, Jackson, MS), heated to 95°C, held for 7 minutes to inactivate the Kunitz trypsin inhibitors and lipoxygenase, as well as to reduce its microbial load. The heated soymilk was filtered and squeezed using a 100-mesh nylon filter-sack to separate the insoluble residue, okara, from the soymilk.

The soymilk was reheated in the kettle to 85°C, the coagulant was added, while initially increasing the mixing speed of the automated kettle stirrer to ensure uniform dispersion. Calcium sulfate dihydrate ($CaSO_4.2H_20$) was used as the coagulant and concentration was calculated using the formula: [$CaSO_4.2H_20$ (g)] = N x Tv x M, where N = Normality of calcium sulfate dihydrate, Tv = Total volume (L) of soymilk to be coagulated, and M = half molar weight of calcium sulfate dihydrate (Moizuddin and others 1999).

The mixture was then allowed to stand for 5 minutes before cutting the curd to release some of the whey. The coagulum mixture was poured into a stainless steel press box (13 cm x 10 cm x 9 cm), which have been lined with 2 layers of cheesecloth. The cheesecloth was folded into the top of the each box; a plate was added to seal the top, and a 2 kg press weight placed on the plate. The whey was released during pressing. After 15 minutes another 2 kg press weight was also placed on the plate. Fifteen minutes later the tofu curd was removed from the press box and the tofu was stored in water and refrigerated overnight before running color and texture tests. All processing was done in the Center for Crops Utilization and Research pilot plant (Iowa State University, Ames, IA).

Tofu quality analyses

Quality was evaluated in terms of tofu yield percentages and tofu texture. The tofu yield was expressed as kg fresh tofu weight (wet weight) produced from the starting dry weight of the soybeans in kg.

$$\% \text{ tofu yield} = (\text{tofu fresh weight} / \text{dry soybean weight}) * 100$$

Texture was evaluated using the TAXT-2 Texture Analyzer (Texture Technologies Corporation; Scarsdale, NY) equipped with a 6 cm cylindrical probe (TA-30). The Texture Profile Analysis (TPA) procedure was used to compare the different textural parameters of the different tofu samples as measured by the TAXT-2 Texture Analyzer. Three 2 cm^3 cubes from each tofu sample were obtained from the inside of the tofu block and subjected to 50% compression (compressed to 1 cm) at a speed of 1.7 mm/s. Attributes of interest in relation to tofu texture were hardness, cohesiveness, springiness and gumminess.

Statistical analyses

The statistical design is a randomized study of storage time, storage temperature, storage humidity and soybean cultivar over two crop years. Because many two-way, three-way and four-way interactions were statistically significant ($p<0.05$) between storage treatment factors, data are summarized graphically and are complemented by an ANOVA table to demonstrate the high-order interactions. Exploratory data analysis and regression coefficients were conducted using a statistical computing environment called R (R Development Core Team 2004). Data were analyzed with the General Linear Model procedure on SAS System 9.0 (SAS 2004) using ANOVA for a five-way factorial treatment structure (cultivar, humidity, temperature, time, and year). The standard error of the means was calculated based on the highest-order interaction. The results are presented using trellis

plots, displays that contain one or more panels that are arranged in a grid-like structure (Cleveland 1993), that compactly show patterns across all treatment factors in combination.

RESULTS AND DISCUSSION

Total oxalates were significantly different between storage temperatures, humidities, soybean cultivars, length of storage time, and crop years, which is reported by the p-values in Table 2. At 0 time of storage, total oxalates were different in all three soybeans cultivars in the 2002 crop year, but not in 2003, as illustrated in Figure 3. While it should be noted that there were fewer sampling times in 2002 and the oxalate assay kits for both years were obtained from different consignments, other components of soybean, such as proteins, glycinin and β-conglycinin content, isoflavones, and total oxalates, vary among cultivars, crop years and growing locations (Hughes and Murphy 1983, Johnson and Wilson 1984, Murphy and Resurreccion 1984, Schaefer and Love 1992, Wang and Murphy 1994, Wang and Chang 1995, Hoeck and others 2000, Horner and others 2005). Furthermore, plant breeding, harvest practices as well as post-harvest treatments have been shown to influence oxalate content in many crop plants (Libert and Franceschi 1987).

Table 2 lists the significant 3-way interaction of storage time and crop year in all three soybean cultivars, Vinton 81, IA2032 LS, and Proto, on changes in total oxalate (mg/g). The interaction is further illustrated in the trellis plots in Figure 3, which were averaged over all the storage temperatures and humidities. Pattern consistencies across both years were not discernable, but very significant cultivar difference ($p<0.0001$) in total oxalate content was observed. The difference in total oxalate content over storage time could be attributed to the soybean maturity at time of harvest. It had been observed in another study on the effect of maturity on spinach oxalates, which indicated that the total oxalate content of the spinach

leaves decreased with time (Kitchen and Burns 1965). In their study, they also reported a highly significant interaction of plant parts and harvest dates on the oxalate content of the spinach, which could be attributed to variations in environmental conditions between harvest dates.

The published values for oxalic acid in selected vegetables in the USDA nutrient data laboratory ranged from 10 mg/100 g (or 0.1 mg/g) for oxalic acid in sweet corn to 1700 mg/100 g (or 17 mg/g) oxalic acid in parsley. Assuming that those values were total oxalate for comparison to this study, which reports oxalates ranging from 0.5-2.3 mg/g, this would classify soybeans as reported here to be a high-oxalate containing food (The Chicago Dietetic Association 2000), regardless of storage conditions.

Within the 2003 crop year (Figure 4), there were statistical differences ($p < 0.05$) between storage temperatures and humidities. The trellis plots in Figure 4 illustrates the 4-way interaction of soybean cultivar, storage time, temperature, and humidity on the total oxalate content (mg/g) for the 2003 crop year soybeans. While the IA2032 LS and Proto cultivars showed a decreasing trend of total oxalate with increasing storage time at all temperatures and humidities, the Vinton 81 soybeans demonstrate a significant humidity effect after 9 months of storage (Figure 4), because at 40°C the total oxalate increases at the 75% RH storage. The variation of total oxalate in the soybeans could be attributed to the fact that most calcium oxalate compartmentalization remains unchanged in developing plants for a long period. However, not all deposits remain fixed, even though calcium is maintained in the form of calcium oxalate crystals. When plant physiological need arises, the crystals are degraded to release calcium. Although the soybeans in this study were dry, mature, and non-germinating, the storage condition at high temperature and humidity condition could

stimulate germination. Hodgkinson (1977) has observed that small calcium oxalate crystals in lupin seeds tend to erode and then disappear when the seeds germinated. Similar phenomena were also observed in ripening seeds and in shells of nuts (Hodgkinson 1977).

The IA2032 LS soybeans had a higher total oxalate content from 0.9-2.3 mg/g than the Proto soybeans, which ranged from 0.7-1.6 mg/g (Figures 3 and 4). The values for Proto total oxalate content were in line with a study by Horner and others (2005), in which Proto had the lowest concentration in the 86 soybean cultivars examined. That investigation also yielded a wide range of oxalate concentrations in the soybean cultivars, thus corresponding to the highly significant ($p<0.0001$) cultivar effect on oxalate in this study. A lower total oxalate concentration in the Proto soybeans could be due to the association of seed processes for calcium storage and seed storage protein synthesis during these observations as suggested by Ilarsan and others (1997).

The data for soluble oxalates (mg/g) is obtained by subtracting the values of insoluble oxalates from total oxalates. While the increasing length of storage time on decreasing soluble oxalates were statistically significant, the effect of storage temperature and humidity were not significant by themselves, but the combined interaction of storage temperature and humidity on soluble oxalates were statistically significant (Table 2, Figure 5). A decreasing trend for total and soluble oxalates over prolonged storage time was rather inconsistent in the 2003 crop year, although it should be noted that there were fewer sampling times in 2002 (Figures 3 and 5). Soybean cultivars, however, did not play a role in affecting soluble oxalate content during storage (Table 2).

The trellis plots in Figure 6 illustrate the 4-way interaction of storage temperature, relative humidity and soybean cultivar at each of the storage times for the 2003 crop year.

While Table 2 lists the interaction as significant, there were no discernable patterns of storage on the soluble oxalates as observed from Figure 6. Since the variation of soluble oxalate in the soybeans could be due to plant calcium regulation and seed storage protein synthesis process (Franceschi and Loewus 1995, Ilarslan and others 1997), the proportion of soluble and insoluble oxalates may vary widely in plants.

The total and soluble oxalate content in soybean seeds, along with other seed components as stated earlier, may vary considerably, and large differences can be noted even within the same species, depending on age of the plant, seasonal variation, climate, type of soil and even the anatomical site of the plant (Kitchen and Burns 1965, Hodgkinson 1977). The oxalate content may increase as soybean seeds mature, with some seeds showing a fast rise in oxalate content during early stages of growth, but decreased content as the seed matures (Ilarslan and others 1997; 2001).

Figure 7 illustrates the interactions of storage time and crop year for all three soybean cultivars, Vinton 81, IA2032 LS, and Proto, and the change in tofu yield (%). This data was averaged over all the storage temperatures and humidities. There was a decreasing trend for tofu yields at each of the 2 crop years. Tofu yields from soybeans stored at the highest temperature and humidity (40°C, 75% RH) decreased over storage time, with changes evident at 6 months in the 2002 crop year while the difference did not appear until 12 months of storage in the 2003 crop year soybeans. The finding of decreased tofu yields in 2002 was consistent with the model storage study by Saio and others (1980), in which soybeans stored for 6 months at 35°C and 80% RH did not coagulate to form tofu. The trellis plots in Figure 8 illustrate the decrease in tofu yield at increasing storage temperature in all 3 soybean cultivars across all time, temperature and humidity conditions. These data show about the

same level of tofu yields, from 103 to 245%, but Proto cultivar was consistently lower in yields for both years, ranging from 117-205%. It was followed by Vinton 81, 103-235%, while the IA2032 LS cultivar had the largest tofu yields, with a range of 133-245% (Figures 7 and 8). The highest temperature and humidity storage conditions, 40°C and 75% RH, respectively, had the largest impact on tofu yield, decreasing considerably over time in the 2003 crop year soybeans for all cultivars (Figure 8).

Both total and soluble oxalates were not significantly correlated to tofu yield (Table 3). As such, neither total nor soluble oxalate content affected tofu yield. A study by Horner and others (2005) indicated that there was no significant relationship between total oxalates and calcium content in soybeans grown at one location. Therefore, the oxalate content would not be a factor to compete with the calcium-based coagulant used to produce tofu in this study, even though the tofu yield is decreasing.

For all the tofu textural attributes observed, hardness, springiness and chewiness correlated with total oxalate whereas cohesiveness and chewiness correlated with soluble oxalate (Table 3). Even though the values for total oxalates and hardness, springiness and chewiness, and soluble oxalates and cohesiveness and chewiness, indicated statistical significance, the correlation coefficient is too low to take into account this relationship. There was a trend of decreased textural values at the higher temperature and humidity storage conditions (Figure 8), if a tofu were formed. However, none of the tofu textural attributes correlated greatly with either total or soluble oxalate (Table 3).

CONCLUSION

The oxalate content in soybean is not affected by storage temperature or humidity for up to 1 year of storage. Likewise, total and soluble oxalates apparently do not play a role in

the coagulation of soymilk to form tofu as none of the tofu yields or texture parameters were affected by the oxalate concentration. Nevertheless, oxalate content was found from this study to be specific for each of the 3 soybean cultivars used in this study.

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TABLES AND FIGURES

Table 2: Storage effects on total and soluble oxalates ($p<0.05$ is significant)

Effect	Total oxalate	Soluble oxalate
Temperature	0.0019	0.0523
Humidity	0.0206	0.1924
Temp x hum	0.0011	0.0116
Cultivar	<0.0001	0.1164
Cultivar x temp	0.0020	0.0152
Cultivar x humidity	0.0304	0.1194
Cultivar x temp x hum	0.0118	0.0484
Time	0.0006	0.0011
Time x temperature	0.0018	0.0110
Time x humidity	0.0394	0.1336
Time x temp x hum	0.0064	0.0578
Cultivar x time	0.0181	0.1527
Cultivar x time x temp	0.0398	0.1012
Cultivar x time x hum	0.0217	0.0317
Cultivar x time x temp x hum	0.0124	0.0347
Year	<0.0001	0.2258
Year x temperature	0.0083	0.0098
Year x humidity	0.0019	0.0160
Year x temp x hum	0.2368	0.2202
Cultivar x year	0.0004	0.0274
Cultivar x year x temp	0.0022	0.0257
Cultivar x year x hum	0.0037	0.2279
Cultivar x year x temp x hum	0.0342	0.3142
Time x year	0.0101	0.0218
Time x year x temp	0.0007	0.0105
Time x year x hum	0.3030	0.7029
Time x year x temp x hum	0.0048	0.0600
Cultivar x time x year	0.0012	0.0052
Cultivar x time x year x temp	0.0112	0.0840
Cultivar x time x year x hum	0.0774	0.5375

Table 3: Correlation of total and soluble oxalates with tofu yield and texture ($p<0.05$ is significant)

	Total oxalate	Soluble oxalate
Tofu yield		
R^2	0.0546	0.0338
(p-value)	(0.539)	(0.704)
Hardness		
R^2	-0.289	-0.169
(p-value)	(0.000913)	(0.0556)
Springiness		
R^2	-0.206	-0.0472
(p-value)	(0.0192)	(0.595)
Cohesiveness		
R^2	-0.171	-0.239
(p-value)	(0.0529)	(0.00644)
Chewiness		
R^2	-0.280	-0.210
(p-value)	(0.00133)	(0.0167)

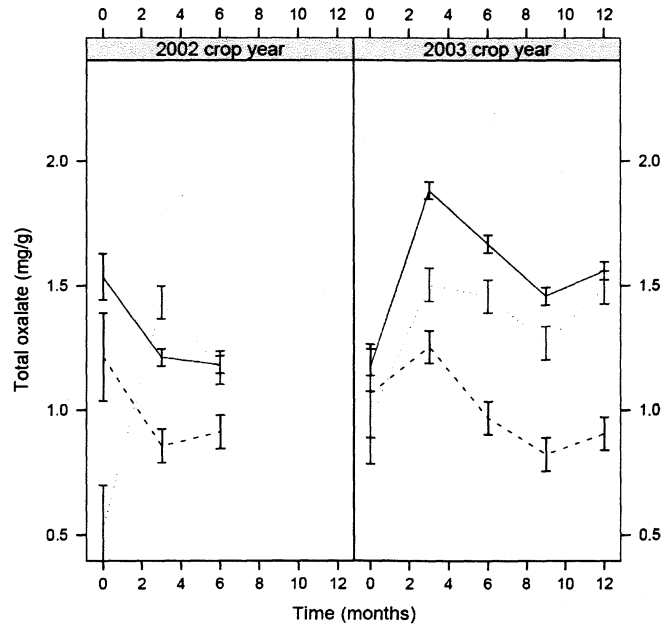

Figure 3: Mean total oxalate content (± standard error of means, SEM) for three soybean cultivars averaged for all storage temperatures and humidities. —— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

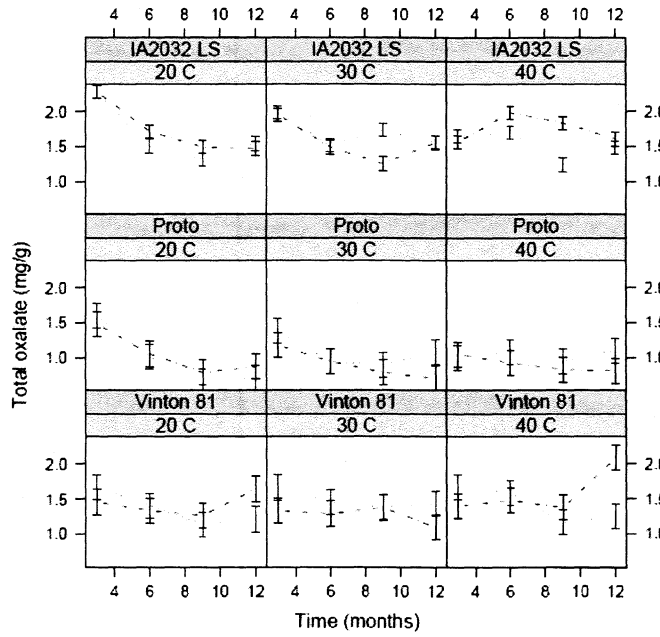

Figure 4: Mean effect of storage time and relative humidity on total oxalate (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32% RH and -·-· = 75% RH.

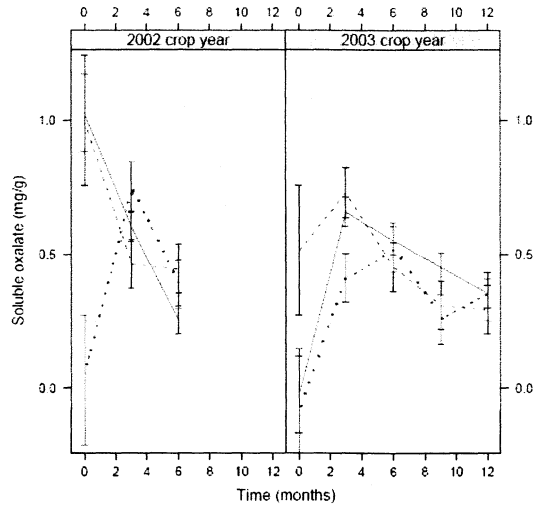

Figure 5: Mean soluble oxalate content (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, - - - = Proto, and ····· = Vinton 81

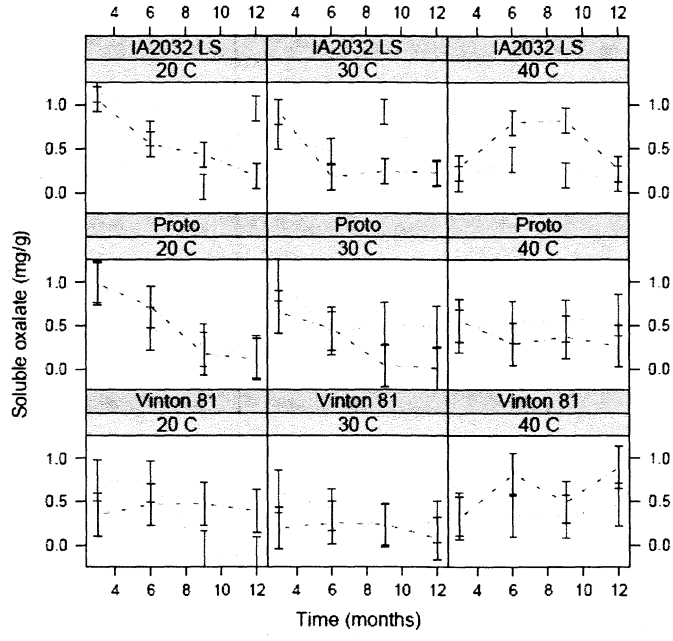

Figure 6: Effect of storage time and relative humidity on soluble oxalate (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ····· = 32%RH and -·-· = 75%RH.

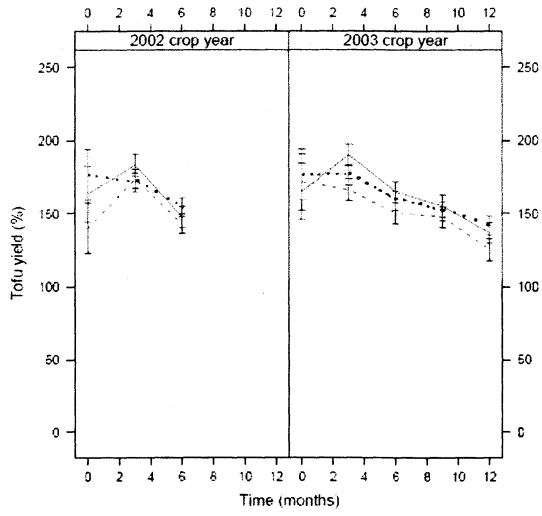

Figure 7: Mean % tofu yield (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, - - - = Proto, and ···· = Vinton 81

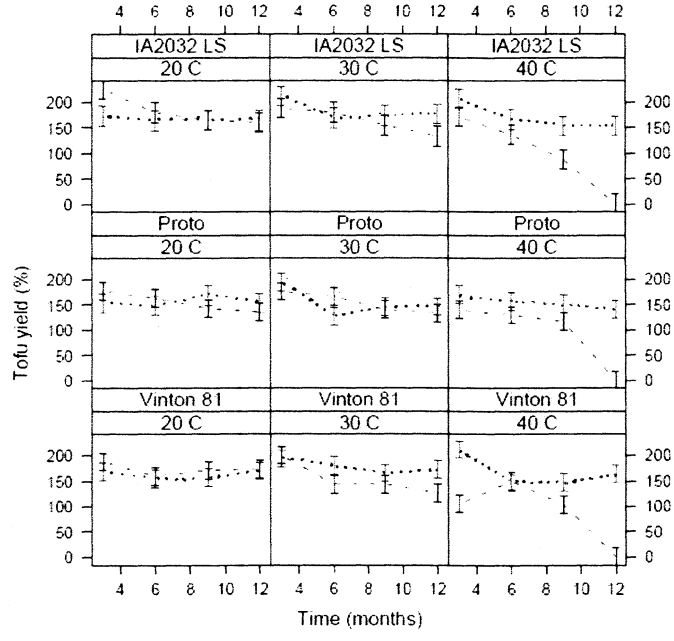

Figure 8: Effect of storage time and relative humidity on % tofu yield (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

DETERMINATION OF SOYBEAN ANTIOXIDANT POTENTIAL UNDER DIFFERENT STORAGE CONDITIONS

A paper to be submitted to the Journal of Food Science

J. Y. L. Kuan, H. Wickham, P. M. Dixon, and L. A. Wilson

ABSTRACT

The objective of this study was to ascertain the antioxidant capacity in soybeans under different storage conditions. Total assay of soybean antioxidants were quantitated by photochemical luminescence. The free radicals generated reacted with a photosensitizer dye, and detected by their reaction with a chemiluminogenic agent through measurement of the emitted light. This technique was used to determine the antioxidant capacity in three different soybean cultivars that had been stored at 20°C, 30°C and 40°C, in two humidity conditions, 32 and 75% RH, for up to 6 months. Soybeans were evaluated every 3 months. There was a difference in antioxidant potential due to storage conditions and soybean cultivars. The results indicated that the higher protein soybean cultivar had a higher antioxidant potential. A lower storage temperature resulted in lower luminescence since the soybeans were less prone to degradation, whereas higher temperature storage increased its luminescence. Storage conditions affect soybean antioxidant capacity as observed by an increased antioxidative capacity over time, and there was a decrease in color lightness of soybeans due to storage conditions, but antioxidant capacity was not directly related to soyfood quality. However, more studies need to be done on the prolonged storage at lower humidity and temperature, as well as the nature of soybean antioxidants as measured through photochemiluminescence.

INTRODUCTION

Antioxidants, naturally occurring or added in food products, are inhibitors that prevent the formation of hydroperoxides by scavenging free radicals or function as singlet oxygen quenchers. Most plant sources have natural antioxidants and the soybean, a member of the oilseed family; has tocopherols, flavonoids (occurring as isoflavones), phenolic acids, phospholipids, phytic acid and peptides that potentially function as antioxidants (Graf and Eaton 1990, Chen and others 1995, Shahidi 1997, Pokorny and others 2001).

Most soybean antioxidants are found in the form of tocopherols and tocotrienols (White and Xing 1997). The primary tocopherols in soybean are γ- and δ-tocopherol, although the α- and β- forms also exist. A range of tocopherols between 900-1200 -μg/g has been reported. γ-Tocopherol accounts for 60% of the total tocopherol content in soybeans, followed by δ- at 27%, α- at 12%, and β- at 1% (Pokorny and others 2001). One of the lipid soluble vitamins in soybeans, vitamin E, contains vitamin activity in the form of α-tocopherol, since α-tocopherol alone is used for estimating vitamin E requirements and recommended intake because the other naturally occurring forms of vitamin E are not converted to α-tocopherol in the human body (The National Academy of Sciences 2000). Each of the tocopherol forms has a different vitamin E and antioxidant activity. Soybean tocopherol content varied in α-, γ-, and δ-tocopherol content, ranging from 10.9-191 μg/g dry matter in the soybean cultivars reported by Guzman and Murphy (1986). Even though there was a loss of total tocopherol when processing soybeans into tofu, the tofu was a better source of tocopherols than soybeans on a dry weight basis and the tofu tocopherol content was not affected by commercial storage conditions (Guzman and Murphy 1986).

The major flavonoids are the isoflavone family, which have 12 isomers. They are the aglycons: genistin, genistein, daidzin, daidzein, glycetin, glycetein; the acetylglucosides: 6"-O-acetyldaidzein, 6"-O-acetylgenistein, 6"-O-acetylglycetein; and the malonylglucosides: 6"-O-malonyldaidzein, 6"-O-malonylgenistein, 6"-O-malonylglycetein (Kudou and others 1991). These isoflavones have moderate antioxidant activity and are much poorer antioxidants than tocopherols. Isoflavone content may vary among soybean cultivars, crop year and location, as reported by Wang and Murphy (1994). The study found that crop year, rather than location, had a greater effect on isoflavone content. There were also cultivar effects among American and Japanese soybean cultivars, as indicated by different ratios of the malonyl family of isoflavones to the glucoside family and the distribution patterns of individual isoflavones. A more recent study by Hoeck and others (2000) found that environment and genotype played a significant role on isoflavone content in soybeans.

Phenolic acids as antioxidants in soybeans include chlorogenic, syringic, vanillic, ferulic, and caffeic acids, vanillin, *m*-ferulic acid, 3,5-demethoxy-4-hydroxycinnamic acid, gentisic acid, salicylic acid, *trans*-cinnamic acid, *p*-hydroxybenzoic acid, syringaldehyde, *p*- and *o*-coumaric acid. Chlorogenic acid or its hydrolysis product, caffeic acid, may be the major natural phenolic antioxidant of soybean (Pratt and Birac 1979; White and Xing 1997). Phenolic acid content of soybeans has been reported to be 69 mg/100 g, with syringic acid making up 39% of the phenolic acids (Dabrowski and Sosulski 1984).

Long-term storage may result in degradation of soybeans through lipid oxidation reactions. Increased free fatty acids have been observed with increased storage time, due to hydrolysis of triglycerides (Narayan and others 1988). Soybeans that have been exposed to various levels of stress during transit and storage may contain oxidation products that could

affect the quantity and quality of foods produced from such beans. Oxidative stress can influence the antioxidant level in the resultant foods and their shelf life.

Isoflavones have been shown to hydrolyze during soybean storage at extremes of humidity (Hou and Chang 2002). Hou and Chang (2002) demonstrated the conversion of malonylglucosides to aglucons during storage, especially under high temperature and humidity storage conditions (30°C and 84% RH).

Soybeans are subjected to changes after harvest, such as storage environment and transportation conditions, before being processed into soy food products. These factors are especially critical during commercial storage and shipment of soybeans in the summer months. Human consumption of soybeans and products made from them has been increasing due to their many nutritional benefits. Since oxidative stress can influence the antioxidant level in the food and its shelf-life, the main objective of this study is to ascertain the antioxidant potential in soybeans under different storage conditions.

MATERIALS AND METHODS

Soybean storage

Three food grade soybeans, non-GMO cultivars, Vinton 81 (Pattison Brothers, Fayette, IA), IA2032LS (Stonebridge Ltd., Cedar Falls, IA) and Proto (Sinner Brothers & Bresnahan Company, Cassleton, ND), from the 2002 harvest season were used. Vinton 81 is a high-protein, large-seeded soybean that is one of the dominant beans used by the U.S. soy food industry. IA2032LS is a large-seeded, lipoxygenase-free (triple null) soybean. Proto soybean is a high-protein cultivar with a dark hilum and smaller seed size, which is grown in the upper northern plains of the United States.

Soybeans were placed into nylon mesh bags with each lot weighing about 1200 g. These bags were then placed into tightly sealed five-gallon HDPE buckets (Berry Plastics, Evansville, IN). The soybeans were equilibrated in two extreme humidity conditions, 32% RH and 75% RH, and stored in different Isotemp® (Model 304R, Fisher Scientific, Pittsburgh, PA) large capacity incubators at 20°C, 30°C and 40°C. Each bucket contains a saturated salt solution mixture prepared from standards according to the American Society for Testing and Materials (ASTM) (Table 1) in a glass beaker and covered with a perforated plastic container, on which the bags of soybeans rested.

Table 1: Equilibrium relative humidity (ERH) values for selected saturated aqueous salt solutions

Temperature (°C)	Magnesium Chloride $MgCl_2.6H_20$, (ERH, %)	Sodium Chloride NaCl, (ERH, %)
20	33.1 ± 0.2	75.5 ± 0.1
30	32.4 ± 0.1	75.1 ± 0.1
40	31.6 ± 0.1	74.7 ± 0.1

Modified after ASTM D: E104-85 "Standard Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions," ASTM International

These buckets were then placed into 20, 30 and 40°C incubators, and conditions were monitored periodically using a set of three remote thermo-hygrometers with a multi-channel traceable sensor (Fisher Scientific, Pittsburgh, PA). A set of soybeans was stored in the freezer in an uncontrolled environment (25%RH, -9°C) as a control for this study. The samples in each storage condition of temperature and relative humidity were taken out of the incubators every 3 months for analysis. Soybeans from the 2002 crop year were sampled at 0, 3, and 6 months. At each interval samples were evaluated for antioxidant capacity.

Antioxidative capacity determination

The Photochem® (analytikjenaAG, Germany) uses photochemiluminescence to evaluate the end products of a free radical reaction. A photosensitizer dye (luminol) was optically excited by UV-light in the system to produce superoxide anion radicals. The free radicals were detected by means of a chemiluminogenic substance, and the emitted light was detected in the Photochem® by a photomultiplier. From this measure, the antioxidative capacity is determined based on the radical scavenging capacity of the antioxidant source in the sample. The remaining radicals are then quantified by comparing the reading to that measured from the use of a phenolic antioxidant, Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), a cell–permeable, water-soluble derivative of vitamin E (Figure 1) with potent antioxidant properties, standardized curve.

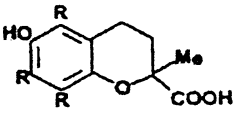

Figure 1: Chemical structure of Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), R= Me

One gram of the ground soybean (to pass through ASTM Sieve #40) was extracted using 10 mL of HPLC grade methanol in a water bath shaker at room temperature for 5 mins. The solution was filtered through a syringe with a 0.45 μm cellulose acetate filter. A 15 μL sample was then used as specified in the Analytik Jena's protocol for the determination of antioxidative capacity of lipid soluble compounds with Photochem® (ACL-Kit Protocol) instrument. Each sample was run in duplicate.

Calculation of antioxidative capacity

$$\text{Concentration } (\mu g/mg) = \frac{\text{Quantity*Dilution*M*Volume}}{\text{Pipetted Volume*Weighted Sample}}$$

Quantity:	Trolox equivalents in nmol
M:	Molar mass of Trolox (250.3 ng/nmol)
Pipetted volume:	15 μL
Weighted sample:	1000 mg
Volume:	10 mL
Dilution:	10 (at 1:10 dilution factor)

Color

Soybean color was measured using the Hunter LabScan XE Spectrophotometer (HunterLab; Reston, VA). Soybeans were placed in a small plastic petri dish and filled to the level brim. The spectrophotometer was standardized using a black and white (X=79.43, Y=84.32, Z=90.39) tile, D65 illuminant with a 10° standard observer. The port size used was 0.4 inches with 0.25 inches view area and an average of three measurements was taken for each sample. L = 100 indicates lightness and L = 0 indicates darkness, whereas + a = red and – a = green, and + b = yellow and – b = blue.

Statistical analyses

The statistical design is a randomized study of storage time, storage temperature, storage humidity and soybean cultivar in one crop year. Because many two-way and three-way interactions were statistically significant ($p<0.05$) between storage treatment factors, data are summarized graphically and are complemented by an ANOVA table to demonstrate the high-order interactions. Exploratory data analysis and regression coefficients were conducted using a statistical computing environment called R (R Development Core Team 2004). Data were analyzed with the General Linear Model procedure on SAS System 9.0 (SAS 2004) using ANOVA for a four-way factorial treatment structure (cultivar, humidity,

temperature, and time). The standard error of the means was calculated based on the highest-order interaction. The results are presented using trellis plots, displays that contain one or more panels that are arranged in a grid-like structure (Cleveland 1993), that compactly show patterns across all treatment factors in combination.

RESULTS AND DISCUSSION

Antioxidative capacity

Antioxidant capacity was significantly different within the soybean cultivars (Table 2). At 0 time storage, Vinton 81 soybeans had a lower capacity, 0.61 μg/mg, compared to the Proto cultivar, which has the highest antioxidant capacity at 1.7 μg/mg (Figure 2). This variability among cultivars is similar to that as previously reported by Wang and Murphy (1994), who found isoflavone content to vary among soybean cultivars, crop year and location. In that study, there were cultivar effects among American and Japanese soybean cultivars, which were indicated by the distribution patterns of individual isoflavones. A recent study by Hoeck and others (2000) found that environment and genotype played a significant role on isoflavone content in soybeans.

For the 2002 crop year, the antioxidant capacity increased over storage time, even when averaged across all storage temperatures and humidities (Table 2, Figure 2). Although the effect of relative humidity was not significant, the combination of storage temperature and humidity were significant, as observed in Table 2 and trellis plot in Figure 3. In the storage study by Saio and others (1980), the combination of high temperature and relative humidity caused severe quality changes in the soybeans. A high antioxidant capacity value indicates that there are more antioxidants to trap the free radicals. Table 2 indicates a significant effect ($p < 0.05$) of storage temperature, as well as the interaction of soybean

cultivar and storage temperature, and results are presented in the trellis plots in Figure 4. Storage at 40°C showed the biggest antioxidant increment in cultivars IA2032 LS (2.46 μg/mg) and Vinton 81 (2.25 μg/mg). As illustrated in Figure 3, soybeans antioxidant capacity increased over time, especially at 75% RH and showed the largest increase when stored at 40°C. The increase in antioxidant capacity could be attributed to the hydrolysis of isoflavones from the malonylglucoside to the aglycone form. Hou and Chang (2002) observed that storage affected soybean β-glucosidase with hydrolysis of isoflavone glucosides to aglycones after nine months at 84% RH and 30°C.

Color

Given that the antioxidant compounds such as tocopherols or phenolic acids could be a substrate for browning reactions, correlation coefficients were used to determine the association of soybean color and antioxidant potential under different storage conditions. The scatter plot matrix in Figure 5 illustrates that antioxidant capacity were not correlated to soybean color, when averaged over storage time, temperature and humidity. Table 3 lists the Hunter *L* and *b* values were negatively correlated to antioxidant capacity, whereas the *a* values were positively correlated. The correlations were not robust even though they were statistically significant at the $p < 0.05$ level. Negative *L* and *b* correlations indicate that as soybeans become increasingly darker and less yellow, the antioxidative capacity increased, whereas positive *a* correlation value suggests redder soybeans with increasing antioxidative capacity. The discoloration of such beans, especially at high temperature and humidity conditions, would contribute to the color of food produced made therefrom, such as soymilk and tofu (Wilson and others 2004).

Enzymatic browning occurs when a phenolic substance reacts with oxygen, catalyzed by the enzyme polyphenol oxidase, to produce brown pigments. Non-enzymatic or Maillard browning occurs with the reaction of free amino acid groups with reducing sugars and other carbonyls. Degradation of Amadori compounds in the Maillard reaction form intermediates which act as antioxidants (Shahidi 1997).

The soybeans stored at a higher humidity (75% RH) and temperature (40°C) had a more pronounced change in color, as was also observed in previous storage studies, in which the color of the soybeans changed from pale yellow to brown (Saio and others 1980, Narayan and others 1988). Saio and others (1980) indicated a possibility of the interaction of proteins with carbohydrates since reducing sugar contents increased markedly after water immersion of the stored soybeans. Narayan and others (1988) found that the reduction in reducing sugars was attributed to their participation in Maillard browning reactions, whereas the reduction in non-reducing sugars may be attributed to enzymatic hydrolysis.

Friedlander and Navarro (1972) investigated the role of phenolic acids in the browning and deterioration of stored soybeans, and found that the phenolic acid content of the acidic 'browned' fraction of deteriorated soybeans, increased with increasing storage temperature. They concluded that phenolic acid content could be used an indicator of deterioration long before substantial discoloration can be observed on the soybeans. They also found a correlation between phenolic acid content and germination capacity.

CONCLUSION

Storage conditions affect soybean antioxidant capacity as observed by an increased antioxidative capacity over time, but are not directly related to soyfood quality. Nevertheless, there was a decrease in color lightness of soybeans due to storage conditions. It is necessary

to control the temperature and moisture in storage to maintain functionality and quality of soy foods made from these stored soybeans. However, more studies need to be done on the prolonged storage at lower humidity and temperature, as well as the nature of soybean antioxidants and its increased capacity at higher temperature and humidity storage. A repetition for the next crop year should also be conducted to gauge crop year effects on antioxidative capacity.

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TABLES AND FIGURES

Table 2: Storage effects on soybean antioxidant capacity ($p < 0.05$ is significant).

Effect	p-values
Temperature	0.0009
Humidity	0.2275
Temp x hum	0.0007
Cultivar	0.0206
Cultivar x temp	0.0170
Cultivar x hum	0.3321
Cultivar x temp x hum	0.3251
Time	<0.0001
Time x temperature	0.0092
Time x humidity	0.4326
Time x temp x hum	0.0076
Cultivar x time	0.1238
Cultivar x time x temp	0.4029
Cultivar x time x hum	0.0864
Cultivar x time x temp x hum	0.8329

Table 3: Correlation coefficients of soybean color with antioxidant capacity (μg/mg).

Color	Antioxidant capacity
L	-0.595*
a	0.353*
b	-0.528*

* $P < 0.05$

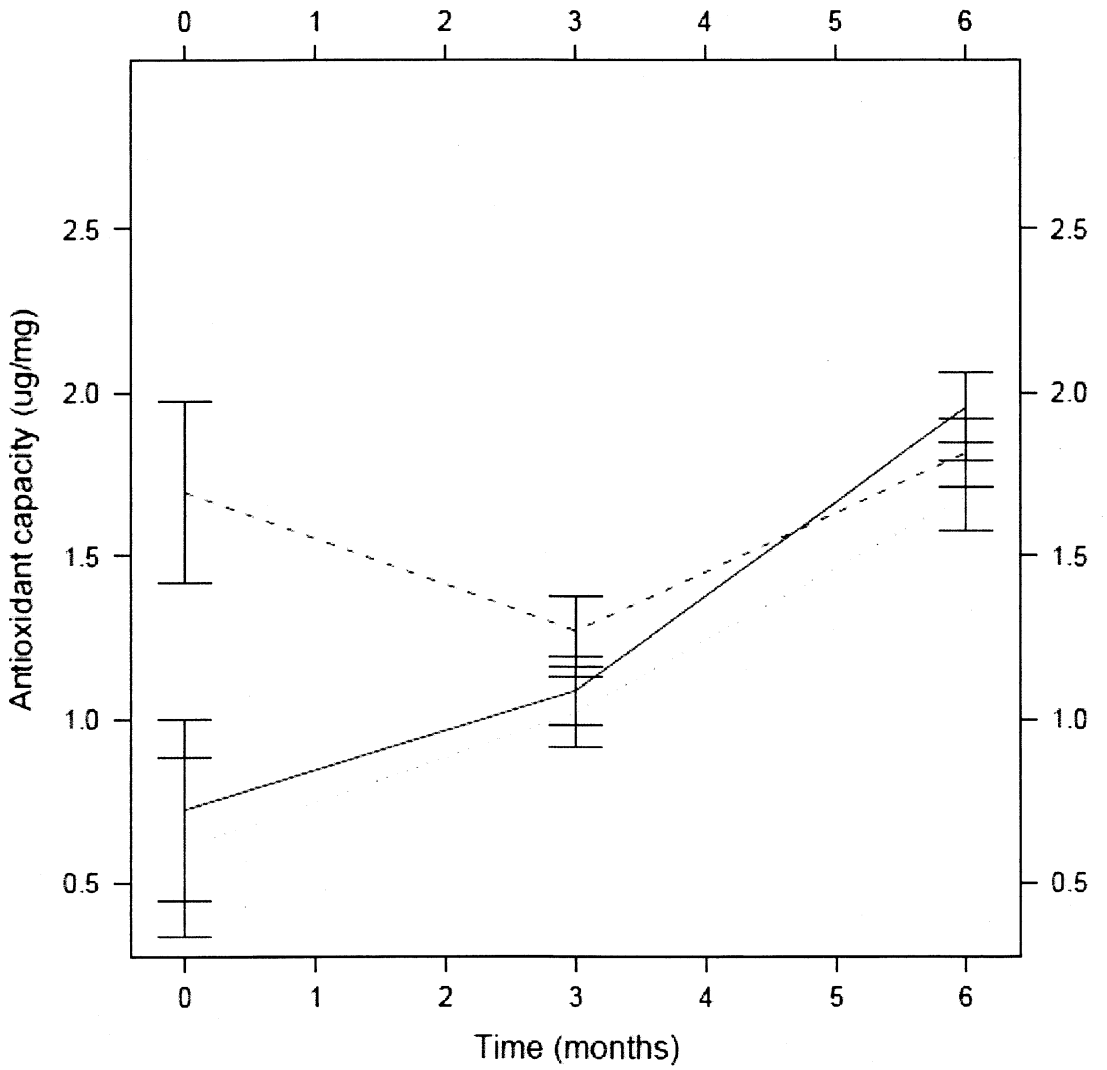

Figure 2: Mean antioxidant capacity (± standard error of means, SEM) for three soybean cultivars from the 2002 crop year, averaged for all storage temperatures and humidities. ——— = IA2032 LS, - - - - = Proto, and ···· = Vinton 81.

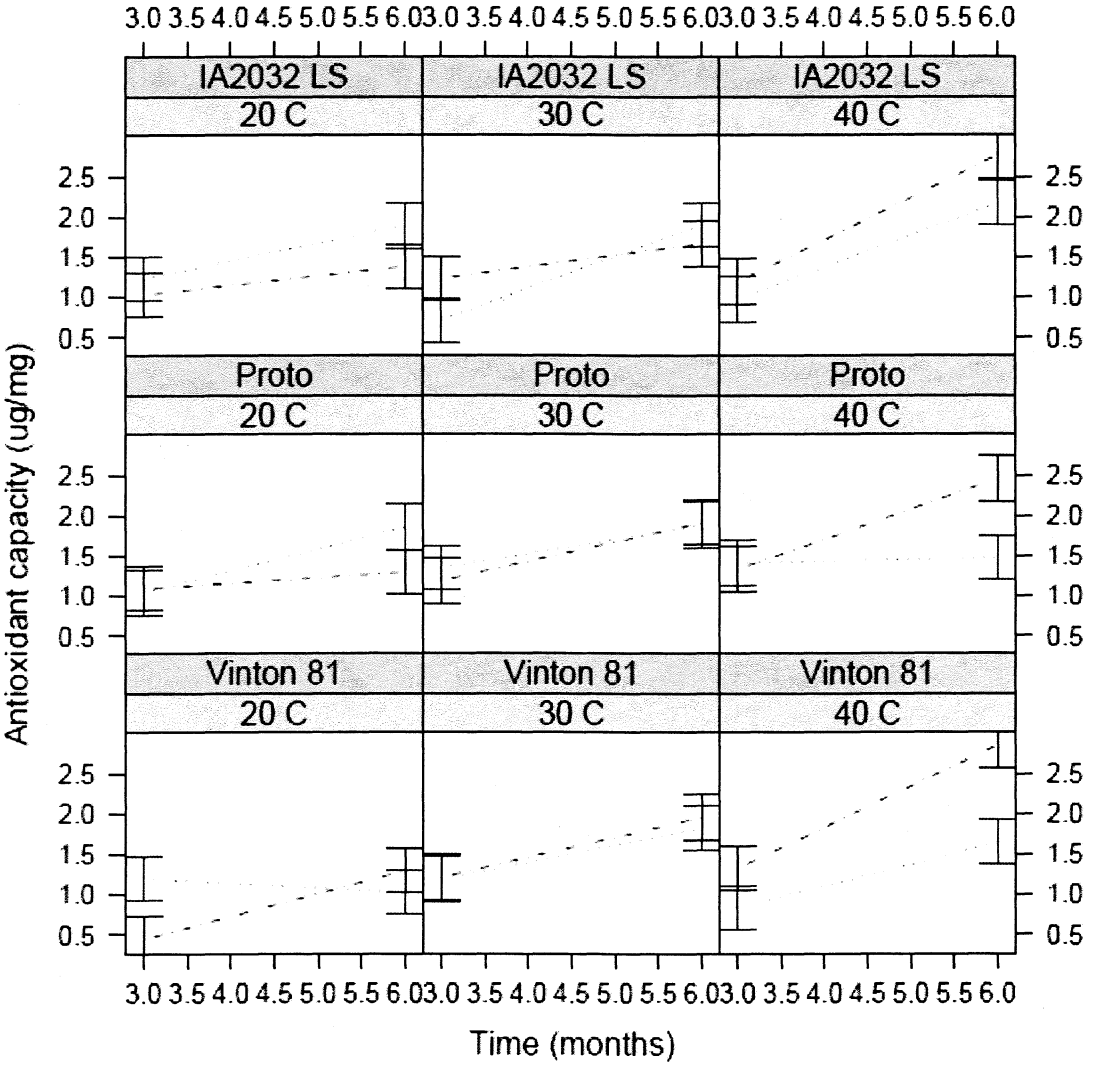

Figure 3: Mean effect of storage time and relative humidity on antioxidant capacity (± SEM) for 2002 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32% RH and -·-· = 75% RH.

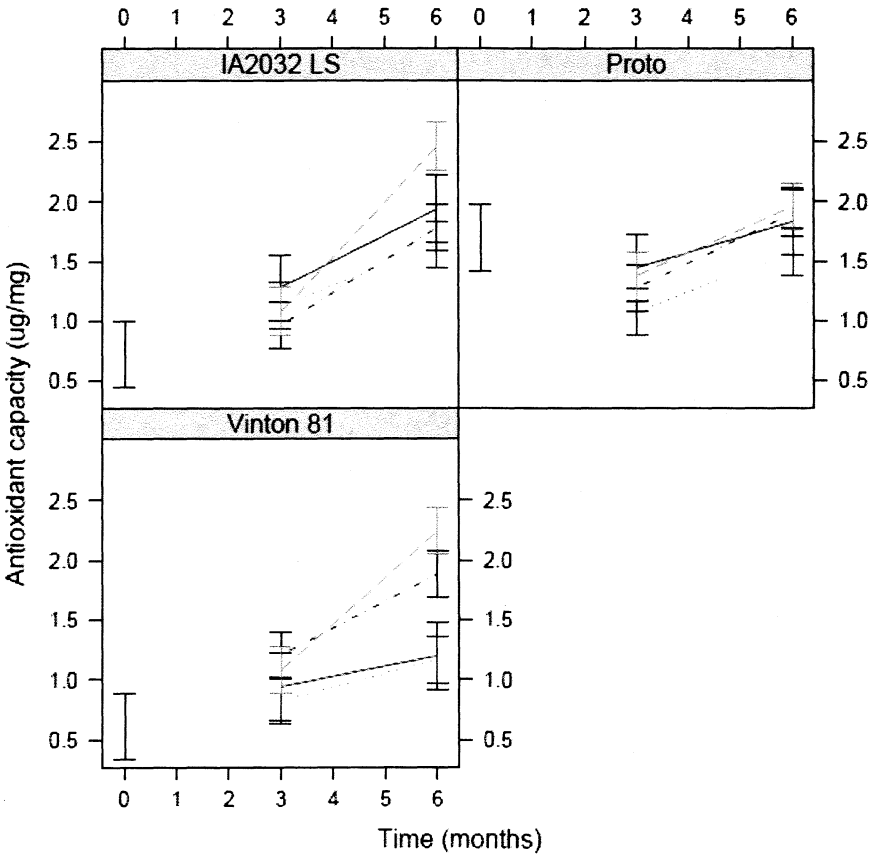

Figure 4: Mean effect of storage time and temperature on antioxidant capacity (± SEM) averaged over all storage humidities. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81). —— = -9°C (Control), ···· = 20°C, -·-· =30°C and - - - - = 40°C.

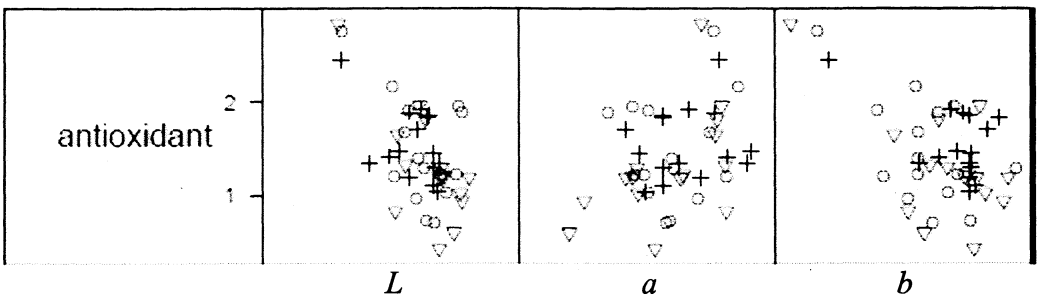

Figure 5: Correlations between antioxidant capacity and soybean color, *L*, *a*, and *b* respectively. ∘ = IA2032 LS, + = Proto, and ∇= Vinton 81.

BIOCHEMICAL CHANGES OF STORED SOYBEANS AND ITS EFFECT ON SOYMILK AND TOFU QUALITY

A paper to be submitted to the Journal of Food Science

J. Y. L. Kuan, H. Wickham, P. M. Dixon, and L. A. Wilson

ABSTRACT

The objective of this study was to observe the biochemical changes that occur during soybean storage and its effects on soymilk and tofu quality. Three different soybean cultivars from two crop years were stored at 20°C, 30°C, and 40°C, in two humidity combinations of 75% RH and 32% RH, for 0-12 months. Soybeans were analyzed for composition, color, soluble sugars, and processed into soymilk and tofu using the traditional Japanese method. With increased storage time, temperature and humidity, soybeans had lower Hunter L values, as browning of the soybean seed coat was observed. This in turn carried over into soymilk and tofu produced from these soybeans. At elevated temperatures and humidities, the soymilk failed to coagulate at 6 months of storage in 2002, and 12 months of storage in 2003. Clear segregation of soybean protein and oil, were maintained between cultivars through the storage conditions and crop years. Soybean soluble sugars are an important factor to consider in seed quality since glucose was only found under high temperature and humidity storage conditions, which suggests hydrolysis of oligosaccharides from other sugar molecules, proteins, or isoflavones. Soymilk solids level is a good indicator of its ensuing tofu quality. Color difference was very pronounced in soybean cultivars and can be used as a predictor of soybean storage condition and its resulting product quality. Based on these results, storing

soybeans under low moisture and temperature conditions are recommended in order to have quality soy products with good functional properties and economic viability.

INTRODUCTION

The market for soy-based foods in the United States was worth almost $4 billion in 2003 alone (Soyatech 2004), therefore soy food sales and demand for soybeans are predicted to rise, thus quality of soybeans and soy foods are very important. Soybeans are considered one of the most valuable agricultural commodities since they have both economic and nutritional value with their multifaceted components. After harvest, soybeans are stored in farm grain elevators or processing facilities, and as such, are subjected to changes during storage and transportation, before processing into soy products. Post-harvest modification of soybeans is very pronounced in the summer months, especially during storage and transporting across continents. Over prolonged storage, soybean seed quality and quality of edible products made thereof decreases.

Several model storage studies on soybeans have been done to determine different functional properties. Saio and others (1980, 1982) did one of the most comprehensive studies. In those studies, severe quality changes were observed in the soybeans stored under high temperature and humidity. Some physical changes include decreased lightness of the soybeans after 6 months, mold growth at the high humidity storage and damaged beans. Decrease in ability of protein and oil to emulsify in soymilk was also observed. Temperature and relative humidity play a significant role in protein extractability during soybean storage (Thomas and others 1989). Researchers have also found changes in protein solubility that is influenced by temperature and humidity (Saio and others, 1980, 1982; Yanagi and others 1985). Such protein changes are important since tofu yield and quality are affected in terms

of sales (tofu is sold by weight) and consumer preference, whereby soybeans stored at a higher temperature are darker; hence the color is carried into the soymilk and subsequently tofu (Wilson and others 2004).

Most deterioration has been attributed to protein functionality. There are also some studies on oil quality from storage-stressed soybeans (Spencer 1976, List and others 1977, Narayan and others 1988, Dornbos Jr. and others 1989). However, few studies have dealt with the effect of storage on other components that may or may not be nutritionally valuable. Compositional analysis of soybeans should be used to monitor quality in agricultural or food industry. Wet chemistry analysis can be time consuming, labor intensive, expensive and requires sample destruction for such purposes. Near infrared reflectance (NIR) spectroscopy can be used to rapidly analyze grains and oilseeds, such as soybeans, for moisture, protein, oil and fiber, nondestructively. Advances in NIR technology has even allowed for the analysis of amino and fatty acid composition in soybean seeds (Pazdernik and others 1997).

Soybean composition can be obtained by way of reflectance from whole seeds in a non-destructive manner, rapidly and accurately. NIR spectroscopy analysis of whole seeds has been adopted as approved methods by the American Association of Cereal Chemists (Method 39-21). Measurements occur in the near-IR spectral region of 700-2500nm. Seed characteristics are obtained by way of reflectance from whole soybean seeds and the absorption bands observed in the NIR region, arising from the functional groups in the sample. The system is calibrated by an ideal absorbance curve obtained from analyzing a large number of seed or grain samples (Hardy and others 1996). A study by Takahashi and others (1996) using NIR spectroscopy indicated there was no influence of seed size or seed coat color, thus allowing for use on a wide array of soybean breeding lines.

Total soluble sugars in soybeans include glucose, arabinose, sucrose, raffinose, stachyose, fructose and galactose (Liu 1997, Locher and Bucheli 1998). Most notable oligosaccharides in soybeans are raffinose and stachyose, due to their flatulence effects in some people. The human digestive system does not possess the enzyme α-galactosidase necessary to break down the α-galactosidic linkages found in these oligosaccharides. However, there have been some recent interests in soy oligosaccharides as anticarcinogenic and a functional food (Messina 1999). Locher and Bucheli (1998) looked at the soluble sugar degradation in soybean seeds under simulated tropical storage conditions. They found that hydrolysis of oligosaccharides to glucose and galactose is linked to seed germination and determination of glucose can a good indicator of soybean seed quality under extreme climatic conditions.

There are many studies describing different methods for oligosaccharide determination in soybeans. However, most methods are cost and labor intensive, in addition to the different types and concentration levels of sugars observed. High-pressure liquid chromatography (HPLC) is fast gaining popularity as a means of sugar separation. Nevertheless, previous studies on the use of HPLC to separate soy oligosaccharides are limited and specific to the protocols of each researcher (Havel and others 1977, Black and Bagley 1978, Locher and Bucheli 1998).

Therefore, the objectives of this study were to examine how storage conditions influence biochemical components in soybeans, as well as quantify its effect on the quality of soymilk and tofu produced from such soybeans.

MATERIALS AND METHODS

Soybean cultivars and storage conditions

Three food grade soybeans, non-GMO cultivars, Vinton 81 (Pattison Brothers, Fayette, IA), IA2032LS (Stonebridge Ltd., Cedar Falls, IA) and Proto (Sinner Brothers & Bresnahan Company, Cassleton, ND), from the 2002 and 2003 harvest season were used. Vinton 81 is a high-protein, large-seeded soybean that is one of the dominant beans used by the U.S. soy food industry. IA2032LS is a large-seeded, lipoxygenase-free (triple null) soybean. Proto soybean is a high-protein cultivar with a dark hilum and smaller seed size, which is grown in the upper northern plains of the United States.

Soybeans were placed into nylon mesh bags with each lot weighing about 1200 g. These bags were then placed into tightly sealed five-gallon HDPE buckets (Berry Plastics, Evansville, IN). Each bucket contained a saturated salt solution mixture prepared from standards according to the American Society for Testing and Materials (ASTM) (Table 1) in a glass beaker and covered with a perforated plastic container, on which the bags of soybeans rested. The soybeans were equilibrated in two humidity conditions, 32% RH and 75% RH, and stored in different Isotemp® (Model 304R, Fisher Scientific, Pittsburgh, PA), large capacity, incubators at 20°C, 30°C and 40°C.

Table 1: Equilibrium relative humidity values for selected saturated aqueous salt solutions

Temperature (°C)	Magnesium Chloride $MgCl_2.6H_20$, (ERH, %)	Sodium Chloride NaCl, (ERH, %)
20	33.1 ± 0.2	75.5 ± 0.1
30	32.4 ± 0.1	75.1 ± 0.1
40	31.6 ± 0.1	74.7 ± 0.1

Modified after ASTM D: E104-85 "Standard Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions," ASTM International

The storage conditions were monitored periodically using a set of three remote thermo-hygrometers with a multi-channel traceable sensor (Fisher Scientific, Pittsburgh, PA). Control soybeans were packaged and stored in the freezer (25%RH, -9°C). The samples in each storage condition of temperature and relative humidity were taken out of the incubators every 3 months for analysis. Soybeans from the 2002 crop year were sampled at time zero, three months and six months, whereas the 2003 crop year soybeans were sampled at zero, three, six, nine and twelve months. The difference in the lengths of soybean storage was based on failure of the extracted soymilk to coagulate, which occurred at the highest temperature and humidity storage condition.

The soybeans were evaluated for composition on NIR analyzer, fatty acids using AOCS Official Method Ca 5a-40, and soluble sugars using HPLC. The soybeans were subsequently processed into soymilk and tofu, and were evaluated for yield, color and texture/viscosity.

Soymilk and tofu preparation

The method by Moizuddin and others (1999) was used to obtain a 7° Brix soymilk and the optimum coagulant concentration for tofu production. Three hundred grams of soybeans were soaked overnight at room temperature in a 10:1 (v:v) water to bean ratio. The soybeans were rinsed and ground in a Stephan Microcut Type MC15 grinder (Stephan Machinery Corporation, Columbus, OH) twice using 2 different grinder blade sizes, 0.5 inches initially and subsequently 0.05 inches. Water was added continuously during the grinding process. The slurry was cooked in a steam-jacketed kettle (Groen Model TDB/7-40, Jackson, MS) that was heated to 95°C, held for 7 minutes to inactivate the trypsin inhibitors and lipoxygenase enzymes, as well as to reduce its microbial load. The heated soymilk was

filtered and squeezed using a 100-mesh nylon filter-sack to separate the insoluble residue, okara, from the soymilk.

The total soymilk volume was measured along with its % soluble solids (measured as °Brix, % sucrose at 20°C) on a refractometer (Milton Roy Company, Rochester, NY) (Johnson and Wilson 1984). A portion of the soymilk was reheated in the kettle to 85° C, the coagulant was added, while initially increasing the mixing speed of the automated kettle stirrer to ensure uniform dispersion. Calcium sulfate dihydrate ($CaSO_4.2H_20$) was used as the coagulant and concentration was calculated using the formula: [$CaSO_4.2H_20$ (g)] = N x Tv x M, where N = Normality of calcium sulfate dihydrate, Tv = Total volume (L) of soymilk to be coagulated, and M = half molar weight of calcium sulfate dihydrate (Moizuddin and others 1999).

The mixture was then allowed to stand for 5 minutes before cutting the curd to release some of the whey. The coagulum mixture was poured into a stainless steel press box (13 cm x 10 cm x 9 cm), which have been lined with 2 layers of cheesecloth. The cheesecloth was folded into the top of the each box; a plate was added to seal the top, and a 2 kg press weight placed on the plate. The whey was released during pressing. After 15 minutes another 2 kg press weight was also placed on the plate. Fifteen minutes later the tofu curd was removed from the press box and the tofu was stored in water and refrigerated overnight before running color and texture tests. All processing was done in the Center for Crops Utilization and Research pilot plant (Iowa State University, Ames, IA).

Soymilk and tofu quality analyses

Quality was evaluated in terms of yield percentages, color using the LabScan XE Spectrophotometer (HunterLab; Reston, VA) and texture using the TAXT-2 Texture

Analyzer (Texture Technologies Corporation; Scarsdale, NY) and HAAKE RheoStress 150 (Thermo Electron Corporation; Karlsruhe, Germany), for tofu and soymilk, respectively.

Yield

Soymilk yield is expressed as weight or volume of soymilk produced (kg) from the original soybeans (kg), which is normally 6-10 times that of soybeans processed.

% soymilk yield = (soymilk weight / dry soybean weight) * 100

Tofu yield is expressed as kg fresh tofu weight (wet weight) produced from the starting dry weight of the soybeans in kg.

% tofu yield = (tofu fresh weight / dry soybean weight) * 100

Color

Color of soybeans, soymilk and tofu were measured using the Hunter LabScan XE Spectrophotometer. Samples were placed in a small plastic petri dish and filled to the level brim. The spectrophotometer was standardized using a black and white (X=79.43, Y=84.32, Z=90.39) tile, D65 illuminant with a 10° standard observer. The port size used was 0.4" with 0.25" view area and an average of three measurements was taken for each sample.

Texture

The Texture Profile Analysis (TPA) was used to compare the different textural parameters of the different tofu samples as measured on the TAXT-2 Texture Analyzer with a 6 cm cylindrical probe (TA-30). Three 2 cm^3 cubes from each tofu sample were obtained from the inside of the tofu block and subjected to 50% compression (compressed to 1cm^3) at a speed of 1.7mm/s. Attributes of interest in this study in relation to tofu were hardness, cohesiveness, springiness and gumminess.

Viscosity

Soymilk viscosity was measured using a HAAKE RheoStress 150 (Thermo Electron Corporation; Karlsruhe, Germany) rheometer. A cone-plate sensor system with a 2° angle spindle (C60/2, 222-1274, d=60 mm, angle=2°) was used to obtain apparent viscosity when data for shear stress was plotted against shear rate.

Soybean compositional analysis

NIR spectroscopy was used to analyze soybean samples for moisture, protein, and oil. Protein and oil are expressed on a 13% moisture basis. The Foss/Infratec 1229 Grain Analyzer (Foss North America, Eden Prairie, MN) measures the potential energy of the vibrations of atoms in the molecules after excitation with near-infrared electromagnetic energy. The analyzer is calibrated by an ideal absorbance curve obtained from analyzing a large number of grain samples (Hardy and others 1996). A sample cell holding approximately 250 g of soybean seeds was scanned and the reflectance spectra were recorded at 8 nm intervals from 810.5-1075nm.

Soluble sugar analysis using high performance liquid chromatography (HPLC)

Ground soybean flour was defatted with n-hexane (Fisher Scientific, Pittsburgh, PA) and 10 g of the defatted sample was extracted in 100 mL of 80% ethanol. Samples were extracted in a water bath shaker for 2 h at 75-80°C, and subsequently centrifuged at room temperature under 9000 rpm (SLA 3000 centrifuge holder for Sorvall RC5B Plus centrifuge, Kendro Laboratory Products, Newton, CT) for 30 minutes. The supernatant was removed and ethanol vacuum evaporated to concentrate remaining soluble sugars to a syrup-like consistency. The sugar samples were then dissolved in 30 mL of water and extracted into

HPLC vials using 0.45 μm cellulose acetate filter fitted with glass pre-filters (VWR International, Chester, PA).

Soluble sugars were then analyzed on a Waters HPLC system with a refractive index detector (Waters Associates, Milford, MA). Each run was calibrated with a set of external sugar standards; D-glucose, D-fructose, sucrose (all Fisher Scientific, Pittsburgh, PA), D (+)-raffinose pentahydrate, and stachyose hydrate (Fluka, Buchs, Switzerland). A Prevail Carbohydrate ES carbohydrate column (250 mm x 4.6 mm ID) coupled with an All-Guard Cartridge System (Alltech, Deerfield, IL) at 30°C, along with an acetonitrile:water (75:25, v:v) mobile phase was used to separate the soluble sugars. Acetonitrile was of HPLC grade (Fisher Scientific, Pittsburgh, PA) and water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). Calculations are based on area of sample curve versus area and concentration of known standards.

Statistical analyses

The statistical design is a randomized study of storage time, storage temperature, storage humidity and soybean cultivars over two crop years. Because many two-way, three-way and four-way interactions were statistically significant ($p<0.05$) between storage treatment factors, data are summarized graphically and are complemented by an ANOVA table to demonstrate the high-order interactions. Exploratory data analysis and regression coefficients were conducted using a statistical computing environment called R (R Development Core Team 2004). Data were analyzed with the General Linear Model procedure on SAS System 9.0 (SAS 2004) using ANOVA for a five-way factorial treatment structure (cultivar, humidity, temperature, time, and year). The standard error of the means was calculated based on the highest-order interaction. The results are presented using trellis

plots, displays that contain one or more panels that are arranged in a grid-like structure (Cleveland 1993), that compactly show patterns across all treatment factors in combination.

RESULTS AND DISCUSSION

Compositional analysis using NIR spectroscopy

At time 0 of storage, there was a significant difference in moisture, protein and oil between soybean cultivars, over both crop years, and storage time (Table 2). Soybeans that were stored at 75% RH had higher moisture content and showed an increasing trend over prolonged storage time, regardless of storage temperature or soybean cultivar (Table 2, Figure 2). Although temperature was found to significantly affect moisture content (Table 2) with a clear separation between samples stored at 40°C, versus 20°C and 30°C, it did not have as much effect as did humidity. The trellis plot in Figure 3 further illustrates the interaction of storage time, temperature and humidity on moisture content of the soybean seeds for the 2003 crop year of soybeans. The storage study by Saio and others (1980) found a drop in moisture content when soybeans were stored at 60 %RH for six months, whereas soybean moisture content rose from 10.61% to 14.8% after storage at 80 %RH for six months. In that study, soybeans stored under high humidity conditions showed fungal growth, discoloration, and increased acid value of more than tenfold its original value. There was also a difference in moisture content for soybeans of two crop years, 1977 and 1978, in a follow up storage study by Saio and others (1982). This further establishes that seed moisture content is a critical factor affecting the deterioration of stored soybeans.

Storage temperature and humidity by themselves significantly affected the protein content, as indicated by the p-values in Table 2. Crop year effect on protein content was very pronounced in the 2003 crop year on the IA2032 LS cultivar soybeans, over all temperatures

and humidities. Cultivar variation was very significant for protein content, as observed from Table 2, even for both crop years, as illustrated in Figure 4. At time 0 of storage in the 2002 crop year, the triple null soybeans, IA2032 LS, had higher protein content at 39%, whereas Proto started out having lower protein content at 38.2%. In the 2003 crop year however, IA2032 LS had lower protein content, 37.6%, whereas the Proto had the highest protein content at 39.6%. Differences in crop years could be due to environmental factors, and crop year effect was also observed in the storage study by Saio and others (1982), with 47.84% to 41.82% protein in 1977 and 1978 crop year of soybeans, respectively. Humidity influenced the protein content more than temperature (Figure 5) and this was also observed by Saio and others (1980; 1982). Hou and Chang (2004) showed changes in the structure of glycinin and β-conglycinin of soybeans stored under adverse conditions (30°C, 84 %RH), after 3 months and 6 months respectively. Such protein structural changes are important in soyfood quality, especially tofu, since it forms a gel from glycinin. Protein changes was also observed in previous storage studies by Saio and others (1980; 1982), whereby soybean stored under adverse conditions (35°C, 80 %RH) showed a marked decrease in nitrogen solubility index (NSI) and total extractable protein, be it in the form of whole soybeans or defatted soybean meal.

Although oil trends were different for both crop years, clear indication of cultivar differences was observed. Table 2 lists the significant effect ($p<0.0001$) of cultivars and crop year on the oil content. Separation of cultivars was maintained for both years, even though the range of oil content was larger in 2003 (Figure 6). This was in line with the observation that Proto cultivar had a higher protein content (as described in previous paragraph) would have a lower oil content. The opposite effect was observed for the IA2032LS (triple null)

soybeans, which would be more stable with higher oil content, thus less protein. No significant temperature and humidity interaction was observed on the oil content (Table 2), but the trellis plot in Figure 7 illustrates the significant cultivar effect.

This study was not able to characterize the changes in oil or fatty acid composition under different storage temperature and humidity conditions as measured on the NIR analyzer. Perhaps different reflectance spectra should be used to increase sensitivity of the NIR analyzer to measure oil content in these stored soybeans. Decreased extractability of total lipids, decreased processing yield of oil, poor oil flavor and increased refining loss of oil, and increased free fatty acids have been reported for storage damaged soybeans (Spencer 1976, List and others 1977, Saio and others 1980, Narayan and others 1988). Mounts and others (1979) observed that there was no effect of decreased moisture in quality of extracted soybean oil in different soybean shipments.

The scatter plot matrix in Figure 8 illustrates the cultivar interaction between protein and oil content. There was a clear segregation of cultivars based on whether the cultivar was high in oil (thus low in protein) such as the IA2032 LS, or high protein (low oil) cultivars such as Proto or Vinton 81, regardless of storage conditions. These results further demonstrate the breeding interactions (Figure 1) as derived by Smith (1989). The same negative interaction was observed in Proto and Vinton 81 soybeans had higher protein and less oil but vice versa for the IA2032 LS soybeans. The interactions along with genetic variability are used to develop soybean germplasm with increased potential for food or industrial uses. Schaefer and Love (1992) found significant correlations of soybean and soymilk components, indicating that soybean composition was a good predictor of soymilk composition prepared from such beans.

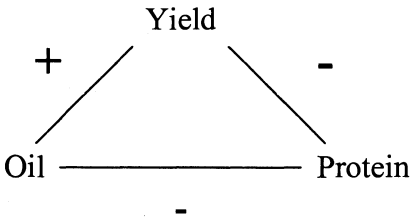

Figure 1: Soybean breeding interactions (Source: Smith 1989)

Soluble sugar HPLC analysis

Sucrose remained relatively stable at all storage conditions, indicating that it was not hydrolyzed or used in a Maillard browning reaction. Raffinose and stachyose remained stable as well, since it has a sucrose group in its structure. Although the total sugar content was not significant for most storage conditions (Table 3), most notable, however, was the appearance of a glucose peak only on samples that were stored at a high temperature and humidity condition (40°C, 75% RH).

Presence of glucose at those conditions could be attributed to the hydrolysis of more complex sugar molecules, compared to those stored at a lower temperature and humidity (Table 3). In the study of Locher and Bucheli (1998) on soluble sugars in stored soybeans, they concluded that even small differences in glucose and galactose would allow for the prediction of soybean storage stability and assessment of seed deterioration since these sugars would reflect germination capacity.

Another point of interest was the differences in % total sugar (discounting all storage conditions) among the three soybean cultivars. IA2032LS soybeans had the lowest % total sugar (possibly from a lesser sucrose concentration) than Vinton 81 or Proto. There may be a possibility of soluble sugar cultivar differences based on protein or oil content in these cultivars. Given that only five basic sugars molecules were analyzed in this study, there could

be a possibility of hydrolysis of other sugar molecules such as arabinose, galactose, and verbascose.

Soymilk and tofu yield

The p-values in Table 4 indicate that the soymilk yields were significantly different between both crop years. There was a decrease in yield in 2002 whereas in 2003 the yields increased (Figure 9). The trellis plots in Figures 9 and 10 illustrate the 4-way interaction of storage time, crop year, temperature and humidity as well as cultivar, storage time, temperature and humidity, respectively, on soymilk yields. The difference in soymilk yield may also be due to differences in processing resources in the pilot plant, such as personnel or batch-to-batch variation.

Even though Saio and others (1980) observed that soymilk made from beans stored in adverse conditions separated easily into distinct water and oil phases, our soymilk yields were not correlated to moisture, protein or oil content. While soybean cultivars were specific for protein and oil content as measured on the NIR analyzer, none of these factors translated to soymilk yields.

Johnson and others (1984) reported that the same water: bean ratio will not amount to equal % solids as soymilk solids have been shown to affect tofu texture, Moizuddin and others (1999) as well as Wilson (1995) have determined an optimum coagulant concentration at different soymilk solids level for tofu manufacturing. Nevertheless, the solids level of soymilk in this study was fixed at 7° Brix using 10:1 water: bean ratio and a coagulant concentration of 0.023 N. A decrease in soymilk solids was a good indicator of decreased protein quality and subsequently, non-formation of tofu curd, especially at the high temperature and humidity storage (Table 8). The trellis plot in Figure 11 illustrates the 3-way

interaction of storage time, temperature and humidity on the soymilk solids level from the 2003 crop year soybeans. As temperature increases from 20 to 40 °C, % solids of soybeans stored at 75% RH decreased over time. The interaction of storage time, crop year and storage temperature on soymilk solids level is illustrated in the trellis plot in Figure 12. Solids level of soybeans for all cultivars stored at 40 °C decreased over time, and was different in both crop years (Figure 13). In 2002, when the soymilk made from soybeans stored at 75%RH and 40°C would not coagulate, the % solids ranged from 2.1-2.8 °Brix, whereas in 2003, the % solids ranged from 4.7-5.0 °Brix. The difference in crop years could be attributed to the differences in environmental factors.

Tofu yields showed a significant decrease over time, especially at high temperature and humidity storage conditions of 40°C and 75% RH (Figure 14). When averaged over all storage temperatures and humidities, tofu yields for both crop years show a decreasing trend (Figure 15). A combination of high temperature and humidity had the largest effect on decreased tofu yield across all soybean cultivars, and this is illustrated in the trellis plot for the 2003 crop year (Figure 16). While Lambrecht and others (1996) reported that tofu yield was not affected by storage time, our study has found that tofu yield decreases with increasing storage time, regardless of storage conditions, at both crop years. However, they have also found a large tofu yield decrease when soybeans were stored at 70% RH, 50° C for 3 months, in two soybean cultivars, along with reduced curd yields.

Texture

As observed in Table 7, even though most interactions of storage conditions were not significant on the textural properties of tofu, the major factor was that storage at the high temperature and humidity (40°C, 75% RH) condition causes breakdown of the tofu texture

over time, with regards to the decrease in soymilk solids level and inability of soymilk to coagulate. The trellis plot in Figure 17 further illustrates the breakdown over storage time of the 75% RH tofu texture quality made from the 2003 crop year soybeans when stored at 40°C. This data was consistent with previous storage studies since the adverse storage conditions would yield a poor quality tofu, possibly due to the loss of protein solubility and differing soy protein ratio, thus losing its ability to coagulate or hold water (Saio and Arisaka 1978, Saio and others 1980, Thomas and others 1989, Lambrecht and others 1996, Hou and Chang 2004). Figures 18 and 21 show that while tofu hardness and chewiness were greatly affected by humidity at the 40°C storage, tofu springiness and cohesiveness were affected more by temperature (Figures 19 and 20). No observable correlations were noted for texture except for hardness and chewiness (Figure 22), which had an excellent correlation. This would follow since the instrumental definition of chewiness includes properties of hardness, cohesiveness and springiness, and the property of tofu hardness overrode tofu cohesiveness and springiness.

Saio and Arisaka (1978) found that soybeans stored at 75.2% RH and 40°C for one month, produced tofu that had less hardness, even with increasing the concentration of GDL coagulant. They have also found such soybeans yielded soymilk with decreased soymilk solids, thus producing a softer tofu, in addition to the higher β-conglycinin to glycinin ratio in soak water of stored soybeans. A follow up study by Saio and others (1981) also found that adverse soybean storage (85% RH, 35 °C) causes a loss in tofu hardness and cohesion, along with increased fragility. Thomas and others (1989) reported that adverse (85%RH, 20/30°C) soybean storage causes increased tofu hardness, and expelled more whey, especially at the 85% RH storage. They also reported a high negative correlation between protein content of

soymilk and volume of whey expelled, and observed that curds were not uniform in shape and tended to settle at the bottom of the container. Lambrecht and others (1996) also noticed poor coagulation and insufficient tofu curds made from soybeans stored at 70% RH and 50°C for 3 months. They found such tofu had increased fracturability and hardness at 2 months of storage. Our study was in line with the storage study by Thomas and others (1989) since we have also observed similar non-uniform shaped curds that settled at the bottom of the kettle and increased whey volume. The increasing volume of whey expelled can be elucidated by the decreased gel water holding capacity, thus causing a subsequent increase in tofu hardness.

Schaefer and Love (1992) reported that hardness of tofu related to amount of calcium retained in tofu. Significant negative relationship between % solids of tofu and tofu yield, increase yield primarily the result of increased water retention in tofu gel. Lambrecht and others (1996) observed poor coagulation and insufficient curds for texture analysis in tofu made from soybeans stored at 70% RH and 50°C for 3 months. They noticed increased fracturability and hardness at 2 months of storage, although they stated that it was difficult to compare storage conditions and soybean cultivars due the low precision of texture data. Hou and Chang (2004) reported that when soybeans were stored under adverse conditions (84%RH, 30°C), β-conglycinin were unextractable after 6 months storage, with a significant decrease in surface hydrophobicity, increase in total free SH, and total SH including SS content. The glycinin structure changed after 3 months under adverse storage, and since glycinin was associated with sugar, had decreased hydrophobic interactions, increased SH and SS interchange reactions due to decreased total free SH and increased SS content, which affected tofu quality.

The inconsistencies in literature could also be explained by a high variability in the tofu processing methods, some were lab scale and some were pilot plant scale but use different equipment, and also the measurement of textural properties due to different texture equipment and parameters.

Color

Soybeans

At high temperature and humidity storage conditions, a darkened tone was observed in the soybeans, indicated by a lower *L* value (decreased brightness), higher *a* value (increased redness) and lower *b* value (decreased yellowness). Table 7 lists the significant effects of storage temperature, humidity and cultivar on the *L* values. While the high-order (e.g. 2-, 3-, and 4-way) interactions were not significant for *L* values, the trellis plots in Figure 23 illustrate the decrease in soybean *L* value with increasing temperature at both humidity levels, 32% and 75% RH, for 3 different soybean cultivars, Vinton 81, IA2032 LS and Proto, for the 2003 crop year. The cultivar effect was observed, as Proto soybeans that have a dark hilum on its seed coat, would have a lower *L* value. Cultivar effect was also observed in the soybean *a* values, as was the storage temperature (Table 7). Trellis plots in Figure 24 illustrate the significant higher order interaction of soybean cultivar, storage time, temperature, and crop year (2003). The *a* values increased with increasing temperature, and Proto cultivar had higher *a* values, regardless of storage humidity, suggesting that storage temperature, along with soybean cultivar, have a larger effect on the redness of soybeans. Although Table 7 lists the significant 3-way interaction of storage time, temperature and humidity, the patterns illustrated in the trellis plot (Fig 25) were rather inconsistent. When

averaged for all storage temperatures and humidities, there was a significant cultivar and crop year interaction effect on the yellowness (*b* value) of soybeans (Table 7, Fig 26).

Soymilk

Table 8 lists the interactions of storage conditions on soymilk color. Storage temperature, humidity, time and crop year by itself were significant, as was the 3-way interaction of crop year, storage temperature and humidity, and 4-way interaction of storage time, crop year, temperature and humidity on the *L* values of soymilk. Although there was no significant cultivar effect on soymilk *L* values, the trellis plots in Figure 27 illustrate the crop year effect on cultivars when the values were averaged for all temperature and humidity storage conditions. Fig 28 further illustrates the interaction of storage time, temperature and humidity for the 2003 crop year on soymilk *L* values. Soymilk *a* values were all significant for all storage conditions, either by itself or in combination, as listed in Table 8. This would suggest that Hunter *a* value is a good predictor of soymilk quality since it was able to detect soymilk color changes in all the storage conditions of temperature, humidity, storage time, soybean cultivar and crop year. The trellis plots in Figure 29 shows the difference in *a* values in both crop years, a sharp increase and decrease in 2002 compared to a gradual increase in 2003, patterns were maintained for all cultivars in both years, even after averaging for storage temperature and humidity. Proto cultivar had higher *a* values, compared to IA2032 LS and Vinton 81, due to the dark hilum on its seed coat, and this higher *a* value cultivar effect was also observed in soybeans. Figure 30 further illustrates the interaction of storage time, temperature, humidity and cultivar for the 2003 crop year. It can be observed that as temperature increases, the *a* values increase as well, signifying a change in soymilk color from green to red (*-a* to *+a*). The humidity effect was also more pronounced as temperature

increased, with the higher humidity (75% RH) storage condition having a larger *a* value increase by the end of the storage time. The effects of temperature and/or humidity were not very significant on soymilk *b* values (Table 8). Soybean cultivar, storage time and crop year, however, were very significant, as was the interaction with a combination of these storage effects (Table 8). Figure 31 shows a distinct increase followed by a decrease in 2002, whereas 2003 had a gradual decrease, in soymilk *b* values over the storage times for both years. Proto cultivar was again significantly different, with a lower *b* value indicating that it is less yellow.

Tofu

Table 9 lists the effects of storage conditions on tofu *L*, *a* and *b* values. While storage time, temperature and humidity, by itself and in combination, were significant on tofu *L* values, cultivar and crop year were not. Figure 32 illustrates the decrease in tofu *L* values for all cultivars in both crop years, which were averaged for all storage temperatures and humidities. A decrease in *L* value over time indicates that the tofu is increasingly darker. The trellis plots in Figure 33 further illustrate the 3-way interaction of storage time, temperature and humidity on tofu L values made from the 2003 crop year soybeans. High humidity (75% RH) had a significant decreasing effect, on tofu L values, especially when soybeans were stored at the highest temperature, 40°C. The same decreasing trend was also observed at 30°C, although the range was not as large (Figure 33). Storage temperature, humidity, time, and soybean cultivar had a significant effect on tofu *a* values (Table 9), and Figure 35 illustrates the increase in *a* value with increasing temperature and humidity for all 3 soybean cultivars from the 2003 crop year. As the *a* value increases, the redness of the tofu also increases, and similar trends in *a* values were also observed for soybeans and soymilk. The

tofu *b* values were significant for most storage conditions, either by itself or in combination, as listed in Table 9. Figure 36 shows the interaction of soybean cultivars, storage time, and crop years, averaged over all temperatures and humidities, on the tofu *b* values. While 2002 showed a decreasing trend whereas 2003 had a very inconsistent trend, what was most obvious in the tofu b values was the cultivar effect. For both years, Proto had a lower *b* value, compared to IA2032 LS and Vinton 81, which were almost similar, indicating the dark hilum in the Proto soybean cultivars were translated into the tofu, thus making the tofu look less yellow regardless of temperature or humidity storage conditions.

The same trend of lower *L*, higher *a* and lower *b* values were observed for both the soymilk and tofu at the highest temperature and humidity, even though the patterns were rather inconsistent. Table 10 lists the correlation among color of soybean, soymilk and tofu at all storage conditions. While most of the correlations were significant, the correlations between soybean *a* values with soymilk and tofu, as well as soymilk *a* value with tofu, were the most robust. As such, the *a* value correlations for soybean, soymilk and tofu indicates that the red-green color was translated across all processing factors. The *b* values, however, picked up differences between each soybean cultivar. It should be noted as well that since there are considerable differences in surface properties of the soybean (smooth and round), soymilk (translucent liquid) and tofu (solid and opaque), the difference in the reflectance of light as measured on the Hunter LabScan spectrophotometer would be reasonable. It was interesting to note however, that color between each cultivar was more pronounced at each processing level (i.e. from soybean to soymilk to tofu), and that storage effects were significant in predicting changes in soybean *L* values, soymilk *a* values, and tofu *b* values.

Saio and others (1980) have also reported darkening of soybeans after 6 months of

storage at high temperature and humidity storage conditions. Thomas and others (1989) reported a significant increase in redness (*a* value) of soymilk made from soybeans stored at 65% RH and 30° C, and that pigments produced in the beans were extracted into soymilk. Lambrecht and others (1996) have observed a large decrease in lightness of tofu color made from soybeans stored at 70% RH and 50° C, even in 2 different soybean cultivars, and that a lipoxygenase containing soybean cultivar produced a significantly darker tofu from the other stored soybean cultivars.

Other findings

No observable difference was noted for soymilk viscosity, which was indicated by Newtonian fluid flow behavior regardless of storage time or condition, even with a lower % solids of soymilk (2 or 5 °Brix) compared to our fixed 7 °Brix soymilk. This was not surprising since soymilk is essentially a water extract of soybean seeds, with the insoluble solids such as okara removed in the filtering step. However, the insignificance of the viscosity data could be due to the low sensitivity and precision of the sensor used in the Rheometer. Large particles may interfere with the sensitivity of the attachment, which would explain why the Rheometer was not able to pick up differences in the lower % solids soymilk. A different sensor attachment that is more sensitive is currently being researched.

While we have insufficient data sets on lipid analysis, we have found that free fatty acids (FFA) increased with increasing temperature and humidity. This is also in line with observations by Saio and others (1980) where storage at a higher humidity had a higher acid value, while Narayan and others (1988) found an increase in FFA with increasing storage time, ranging from 1-9 years.

CONCLUSION

Relative humidity had a bigger influence on deterioration of soybeans than temperature. Soybean cultivars with different genotype for different components are important in determining yield and end product quality. Clear segregation of soybean traits such as moisture, protein and oil, were maintained between cultivars through the storage conditions and crop years. This cultivar effect cannot be discounted, especially with the emergence of specialty and identity preserved soybeans, and the NIR provides a rapid and accurate method for identifying these traits easily. Hydrolysis of carbohydrates from other sugar molecules, proteins, and isoflavones could be occurring at high temperature and humidity storage. Soymilk solids level is a good indicator of its ensuing product quality. Tofu yields were not correlated with textural quality whereas color difference was very pronounced in soybean cultivars. Color could also be used as a predictor of soybean storage condition and its resulting product quality.

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TABLES AND FIGURES

Table 2: Storage effects on soybeans characteristics as measured on the NIR analyzer ($p<0.05$ is significant)

Effect	Moisture	Protein	Oil
Temperature	0.0001	0.0132	0.0292
Humidity	<0.0001	0.0004	0.0808
Temp x hum	0.0006	0.9753	0.0626
Cultivar	0.0012	<0.0001	<0.0001
Cultivar x temp	0.0197	0.0987	0.0996
Cultivar x humidity	0.0305	0.3455	0.0793
Cultivar x temp x hum	0.7733	0.3470	0.4422
Time	0.0005	0.0004	0.0003
Time x temperature	0.0033	0.1026	0.1111
Time x humidity	<0.0001	0.0851	0.1086
Time x temp x hum	0.0093	0.3463	0.3987
Cultivar x time	0.3540	0.0027	0.0012
Cultivar x time x temp	0.0528	0.5644	0.3099
Cultivar x time x hum	0.2287	0.7102	0.4927
Cultivar x time x temp x hum	0.2024	0.7765	0.3750
Year	<0.0001	<0.0001	<0.0001
Year x temperature	0.0003	0.0117	0.5202
Year x humidity	0.0002	0.9161	0.0804
Year x temp x hum	0.0031	0.4463	0.1435
Cultivar x year	0.0017	<0.0001	<0.0001
Cultivar x year x temp	0.0055	0.1085	0.1002
Cultivar x year x hum	0.0458	0.2236	0.1812
Cultivar x year x temp x hum	0.2918	0.6496	0.3612
Time x year	0.0183	0.0308	0.7655
Time x year x temp	0.0703	0.2031	0.0944
Time x year x hum	0.0533	0.3699	0.2166
Time x year x temp x hum	0.0353	0.3468	0.5975
Cultivar x time x year	0.1552	0.6605	0.1348
Cultivar x time x year x temp	0.0934	0.4993	0.3551
Cultivar x time x year x hum	0.1666	0.1911	0.2596

Table 3: Mean soybean soluble sugar (%) as affected by storage temperature and humidity

Humidity (%RH)	Temperature (°C)	Fructose	Glucose	Sucrose	Raffinose	Stachyose	Total
25	-9	0.161		3.502	0.586	1.258	**5.507**
32	20	0.066		3.213	0.456	1.256	**4.991**
	30	-0.138		2.649	0.168	1.100	**3.779**
	40	0.151		3.165	0.502	1.227	**5.046**
75	20	0.203		3.440	0.589	1.296	**5.528**
	30	-0.001		3.487	0.518	1.405	**5.409**
	40	0.478	0.175	2.848	0.709	1.168	**5.300**

Table 4: Storage effects on % soymilk yield ($p<0.05$ is significant)

Effect	Soymilk yield
Temperature	0.0016
Humidity	0.0382
Temp x hum	0.0036
Cultivar	0.0084
Cultivar x temp	0.0120
Cultivar x humidity	0.0028
Cultivar x temp x hum	0.0955
Time	0.0035
Time x temperature	0.0092
Time x humidity	0.0066
Time x temp x hum	0.0059
Cultivar x time	0.0242
Cultivar x time x temp	0.0215
Cultivar x time x hum	0.1345
Cultivar x time x temp x hum	0.0088
Year	<0.0001
Year x temperature	0.0099
Year x humidity	0.0463
Year x temp x hum	0.0038
Cultivar x year	0.0532
Cultivar x year x temp	0.1203
Cultivar x year x hum	0.2035
Cultivar x year x temp x hum	0.0077
Time x year	0.0005
Time x year x temp	0.0090
Time x year x hum	0.0826
Time x year x temp x hum	0.0010
Cultivar x time x year	0.0312
Cultivar x time x year x temp	0.0412
Cultivar x time x year x hum	0.5457

Table 5: Storage effects on soymilk solids, ° Brix ($p<0.05$ is significant)

Effect	° Brix
Temperature	0.0002
Humidity	0.0003
Temp x hum	0.0020
Cultivar	0.3701
Cultivar x temp	0.1876
Cultivar x humidity	0.2477
Cultivar x temp x hum	0.4544
Time	0.0137
Time x temperature	0.0272
Time x humidity	0.0089
Time x temp x hum	0.0405
Cultivar x time	0.5294
Cultivar x time x temp	0.2300
Cultivar x time x hum	0.8363
Cultivar x time x temp x hum	0.4885
Year	0.0005
Year x temperature	0.0060
Year x humidity	0.0100
Year x temp x hum	0.0313
Cultivar x year	0.1300
Cultivar x year x temp	0.7212
Cultivar x year x hum	0.3781
Cultivar x year x temp x hum	0.4136
Time x year	0.4169
Time x year x temp	0.0054
Time x year x hum	0.0965
Time x year x temp x hum	0.0604
Cultivar x time x year	0.4436
Cultivar x time x year x temp	0.6849
Cultivar x time x year x hum	0.4312

Table 6: Storage effects on tofu textural characteristics – hardness, springiness, cohesiveness, and chewiness ($p<0.05$ is significant)

Effect	Hardness	Springiness	Cohesiveness	Chewiness
Temperature	0.9539	<0.0001	0.0052	0.9412
Humidity	0.2782	<0.0001	0.0023	0.2203
Temp x hum	0.2994	<0.0001	0.0018	0.1847
Cultivar	0.1313	0.4226	0.8094	0.1752
Cultivar x temp	0.8852	0.5644	0.5487	0.8132
Cultivar x humidity	0.9138	0.4029	0.9625	0.8833
Cultivar x temp x hum	0.5031	0.3497	0.3920	0.4589
Time	0.0438	<0.0001	0.1386	0.0629
Time x temperature	0.2883	<0.0001	0.0316	0.2832
Time x humidity	0.2206	<0.0001	0.0374	0.2064
Time x temp x hum	0.2497	<0.0001	0.0534	0.2139
Cultivar x time	0.7358	0.4446	0.5155	0.6981
Cultivar x time x temp	0.9965	0.5023	0.7685	0.9939
Cultivar x time x hum	0.8038	0.5295	0.4328	0.7562
Cultivar x time x temp x hum	0.9178	0.3749	0.6876	0.8723
Year	0.2343	<0.0001	0.0062	0.2122
Year x temperature	0.4591	<0.0001	0.0059	0.3933
Year x humidity	0.9166	<0.0001	0.0090	0.9981
Year x temp x hum	0.5958	<0.0001	0.0188	0.7010
Cultivar x year	0.4954	0.9054	0.1915	0.4041
Cultivar x year x temp	0.8930	0.9012	0.7335	0.9328
Cultivar x year x hum	0.5928	0.6269	0.4885	0.5408
Cultivar x year x temp x hum	0.6410	0.5781	0.5377	0.6428
Time x year	0.0514	<0.0001	0.1277	0.0897
Time x year x temp	0.3074	<0.0001	0.0114	0.3382
Time x year x hum	0.8092	<0.0001	0.0231	0.9585
Time x year x temp x hum	0.8392	<0.0001	0.0326	0.9909
Cultivar x time x year	0.5927	0.2734	0.4050	0.6031
Cultivar x time x year x temp	0.7456	0.6113	0.5386	0.7178
Cultivar x time x year x hum	0.7396	0.1217	0.4491	0.6632

Table 7: Storage effects on soybean color – Hunter L, a, b ($p<0.05$ is significant)

Effect	L	a	b
Temperature	0.0014	<0.0001	0.0011
Humidity	0.0145	0.0518	0.0013
Temp x hum	0.0654	0.1751	0.0068
Cultivar	0.0318	0.0001	0.0557
Cultivar x temp	0.6977	0.0107	0.0898
Cultivar x humidity	0.1447	0.0246	0.3594
Cultivar x temp x hum	0.7937	0.3688	0.5542
Time	0.0891	0.0201	0.0154
Time x temperature	0.2455	0.0500	0.1197
Time x humidity	0.2534	0.8119	0.0716
Time x temp x hum	0.1795	0.0758	0.0173
Cultivar x time	0.5081	0.0243	0.0927
Cultivar x time x temp	0.3851	0.0579	0.2410
Cultivar x time x hum	0.4259	0.0613	0.2538
Cultivar x time x temp x hum	0.3468	0.0092	0.1630
Year	0.0515	0.0001	0.3173
Year x temperature	0.4498	0.0291	0.0291
Year x humidity	0.9042	0.5620	0.4011
Year x temp x hum	0.6241	0.0303	0.6561
Cultivar x year	0.3051	0.0036	0.0139
Cultivar x year x temp	0.8875	0.0641	0.5370
Cultivar x year x hum	0.6385	0.1159	0.5097
Cultivar x year x temp x hum	0.8800	0.2627	0.5099
Time x year	0.4920	0.2900	0.2442
Time x year x temp	0.6792	0.2747	0.6222
Time x year x hum	0.5705	0.5081	0.9051
Time x year x temp x hum	0.7919	0.5336	0.4001
Cultivar x time x year	0.9934	0.0365	0.7336
Cultivar x time x year x temp	0.4007	0.0301	0.7537
Cultivar x time x year x hum	0.3669	0.2113	0.7525

Table 8: Storage effects on soymilk color – Hunter L, a, b ($p<0.05$ is significant)

Effect	L	a	b
Temperature	0.0041	<0.0001	0.4808
Humidity	0.0063	<0.0001	0.4749
Temp x hum	0.0254	<0.0001	0.2709
Cultivar	0.1958	<0.0001	0.0010
Cultivar x temp	0.2514	0.0004	0.6113
Cultivar x humidity	0.1930	0.0006	0.3564
Cultivar x temp x hum	0.4710	0.0005	0.2722
Time	0.0017	<0.0001	0.0010
Time x temperature	0.1051	0.0007	0.2998
Time x humidity	0.0710	<0.0001	0.2106
Time x temp x hum	0.0838	<0.0001	0.2904
Cultivar x time	0.8616	0.0046	0.8193
Cultivar x time x temp	0.5522	0.0012	0.8570
Cultivar x time x hum	0.5392	0.0158	0.8884
Cultivar x time x temp x hum	0.6690	0.0045	0.8559
Year	0.0043	<0.0001	0.0212
Year x temperature	0.0147	<0.0001	0.0730
Year x humidity	0.8286	<0.0001	0.6471
Year x temp x hum	0.0108	<0.0001	0.0185
Cultivar x year	0.3176	<0.0001	0.0288
Cultivar x year x temp	0.1159	0.0007	0.4898
Cultivar x year x hum	0.1315	0.0012	0.2550
Cultivar x year x temp x hum	0.3994	0.0052	0.2995
Time x year	0.0042	<0.0001	0.0007
Time x year x temp	0.3928	<0.0001	0.5112
Time x year x hum	0.7109	<0.0001	0.1098
Time x year x temp x hum	0.0478	0.0004	0.1371
Cultivar x time x year	0.6425	0.0017	0.9151
Cultivar x time x year x temp	0.2342	0.0073	0.5930
Cultivar x time x year x hum	0.4674	0.0016	0.4380

Table 9: Storage effects on tofu color – Hunter L, a, b ($p<0.05$ is significant)

Effect	L	a	b
Temperature	0.0027	0.0046	0.0185
Humidity	0.0051	0.0059	0.0041
Temp x hum	0.0059	0.0198	0.0192
Cultivar	0.0653	0.0247	0.0003
Cultivar x temp	0.2722	0.2468	0.0271
Cultivar x humidity	0.1108	0.3864	0.0659
Cultivar x temp x hum	0.6267	0.7812	0.0451
Time	0.0062	0.0281	0.0026
Time x temperature	0.0521	0.1561	0.0609
Time x humidity	0.0200	0.0511	0.0521
Time x temp x hum	0.0267	0.2441	0.0519
Cultivar x time	0.1380	0.2890	0.0136
Cultivar x time x temp	0.1748	0.6144	0.0552
Cultivar x time x hum	0.3676	0.6865	0.0527
Cultivar x time x temp x hum	0.2607	0.4706	0.0490
Year	0.9615	0.3047	0.0010
Year x temperature	0.0769	0.0997	0.0476
Year x humidity	0.6537	0.2090	0.2166
Year x temp x hum	0.1349	0.5172	0.0959
Cultivar x year	0.0248	0.3382	0.0031
Cultivar x year x temp	0.0827	0.7384	0.1444
Cultivar x year x hum	0.1955	0.6987	0.1043
Cultivar x year x temp x hum	0.1419	0.3017	0.0215
Time x year	0.0185	0.0457	0.0049
Time x year x temp	0.0530	0.2583	0.0482
Time x year x hum	0.2966	0.8947	0.1095
Time x year x temp x hum	0.1372	0.9403	0.0403
Cultivar x time x year	0.3862	0.6890	0.1116
Cultivar x time x year x temp	0.4471	0.1888	0.0213
Cultivar x time x year x hum	0.0682	0.6919	0.0347

Table 10: Correlations of soybean with soymilk and tofu color.

		Soymilk			**Tofu**		
		L	a	b	L	a	b
Soybean	L	0.407*			0.549*		
	a		0.688*			0.619*	
	b			0.236*			-0.0174
Soymilk	L				0.478*		
	a					0.805*	
	b						0.509*

* Significant at $p<0.05$

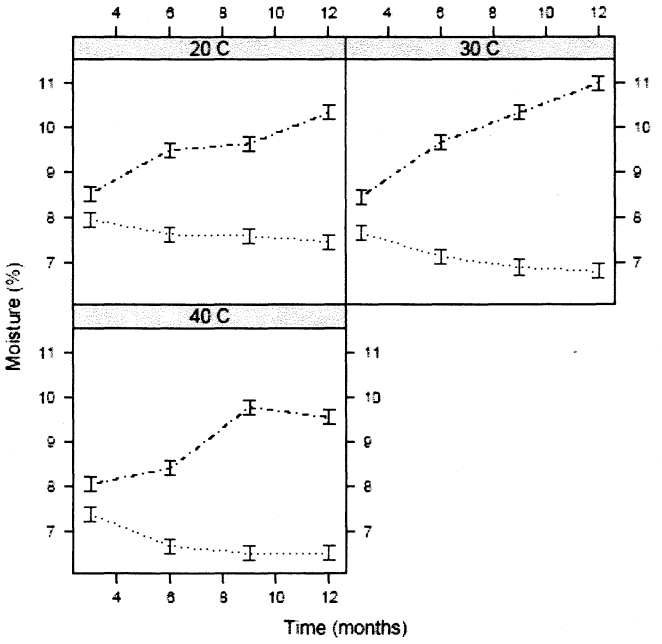

Figure 2: Effect of storage time and relative humidity on % moisture (± standard error of means, SEM) for 2003 crop year soybeans averaged for all soybean cultivars. The strip labels at the top of each panel indicate the storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

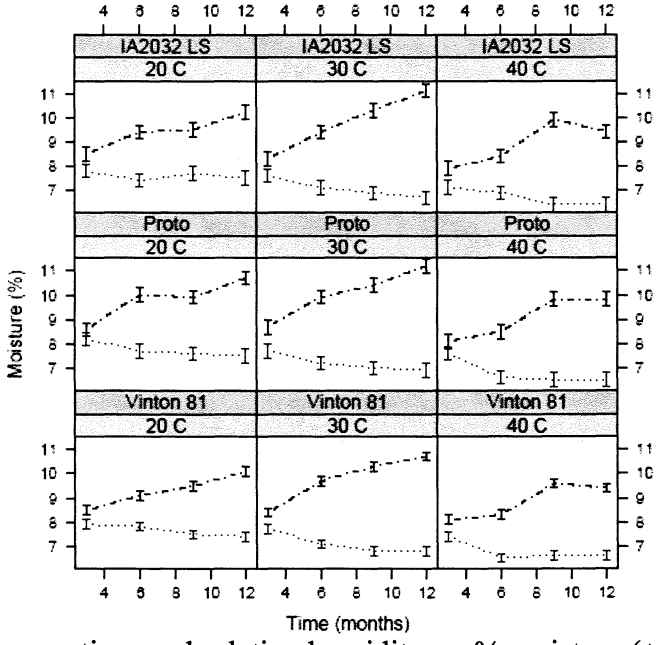

Figure 3: Effect of storage time and relative humidity on % moisture (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

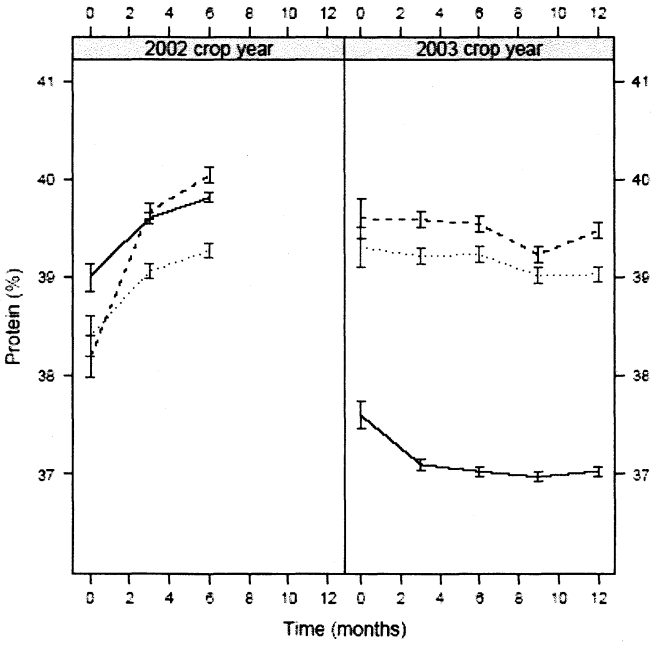

Figure 4: Mean protein content (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

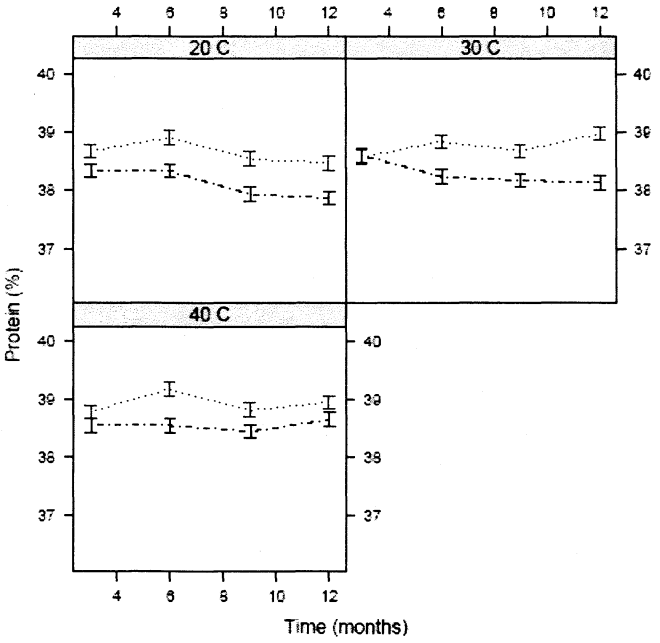

Figure 5: Effect of storage time, temperature and relative humidity on % protein (± SEM) for 2003 crop year soybeans averaged for all soybean cultivars. The strip labels at the top of each panel indicate the storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

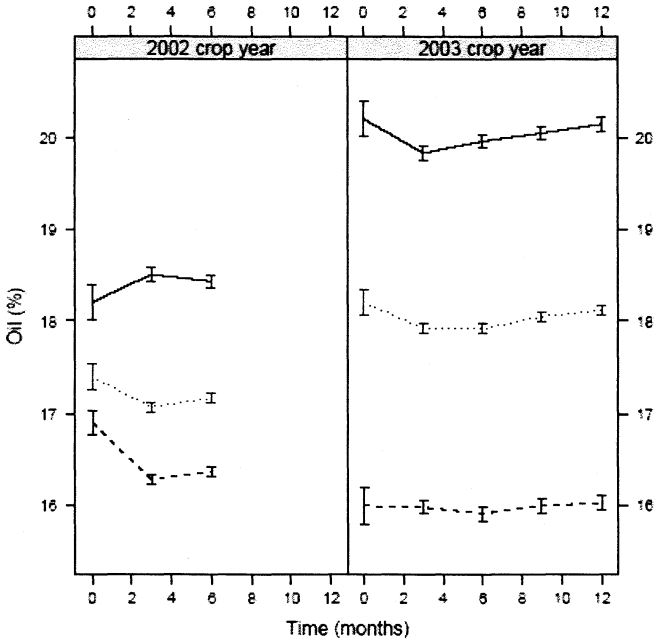

Figure 6: Mean oil content (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

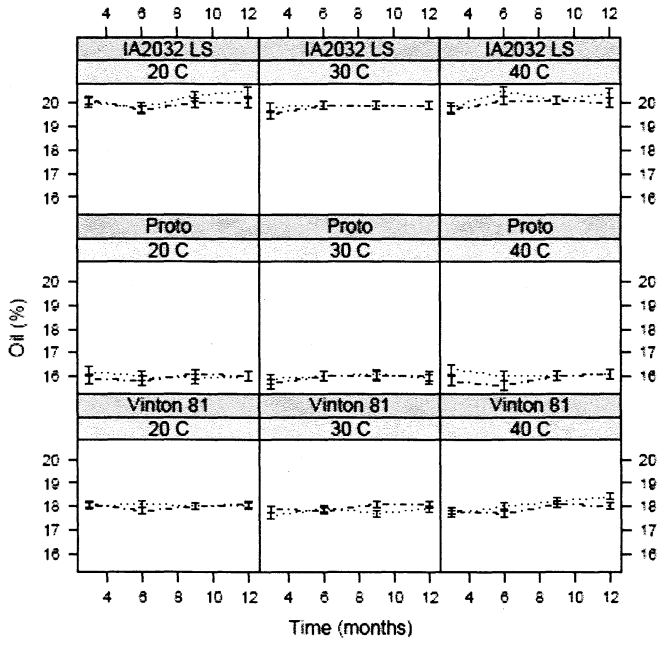

Figure 7: Effect of storage time and relative humidity on % oil (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

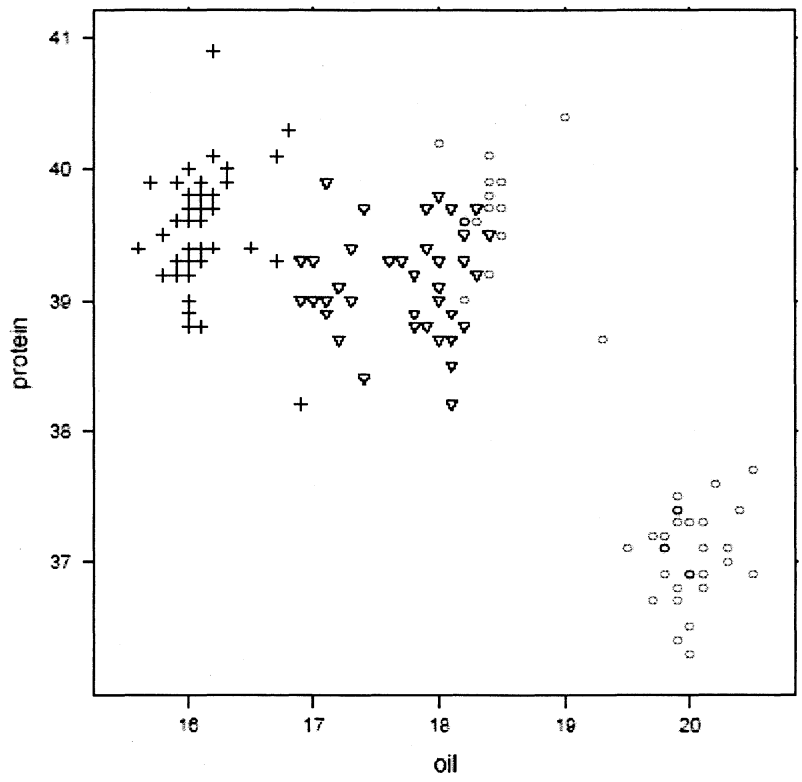

Figure 8: Protein and oil interaction of soybean cultivars, averaged for all storage conditions (time, temperature, humidity) and crop years. ° = IA2032 LS, + = Proto, and ∇= Vinton 81.

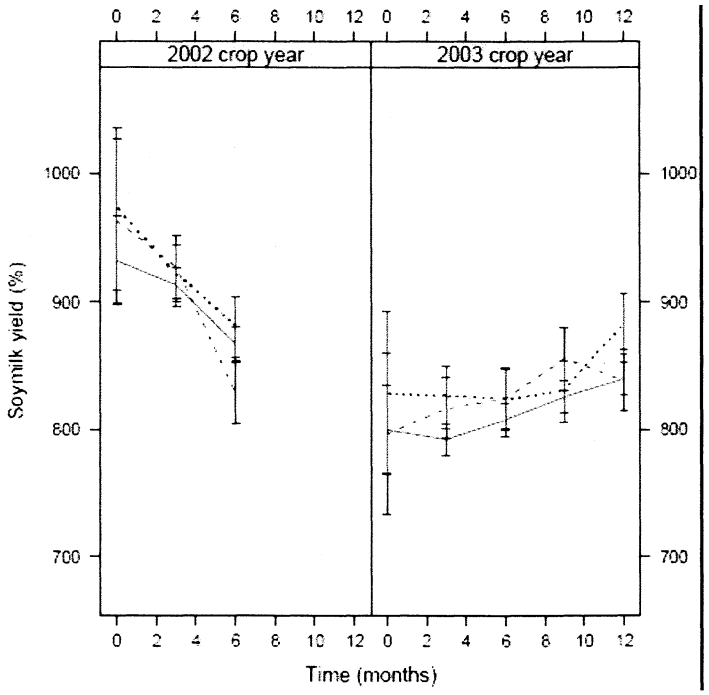

Figure 9: Mean % soymilk yield (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

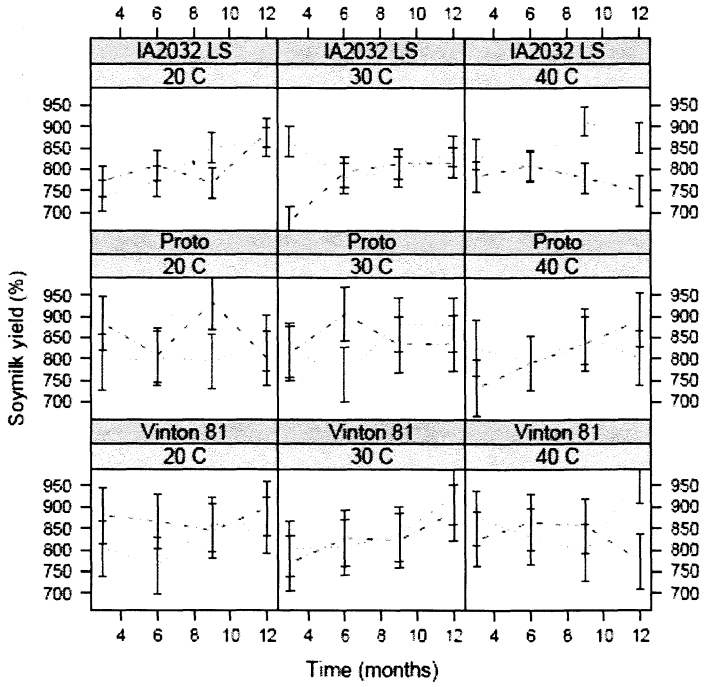

Figure 10: Effect of storage time and relative humidity on % soymilk yield (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

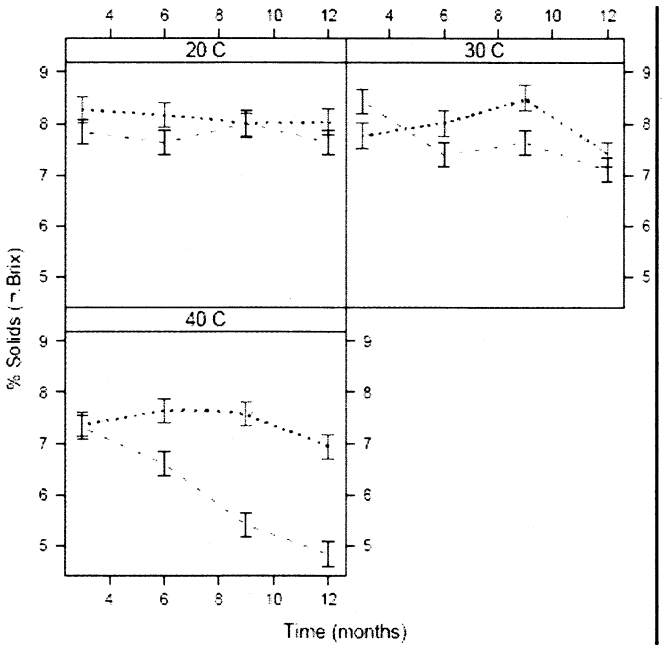

Figure 11: Effect of storage time, temperature and relative humidity on % solids (± SEM) for 2003 crop year soybeans averaged for all soybean cultivars. The strip labels at the top of each panel indicate the storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

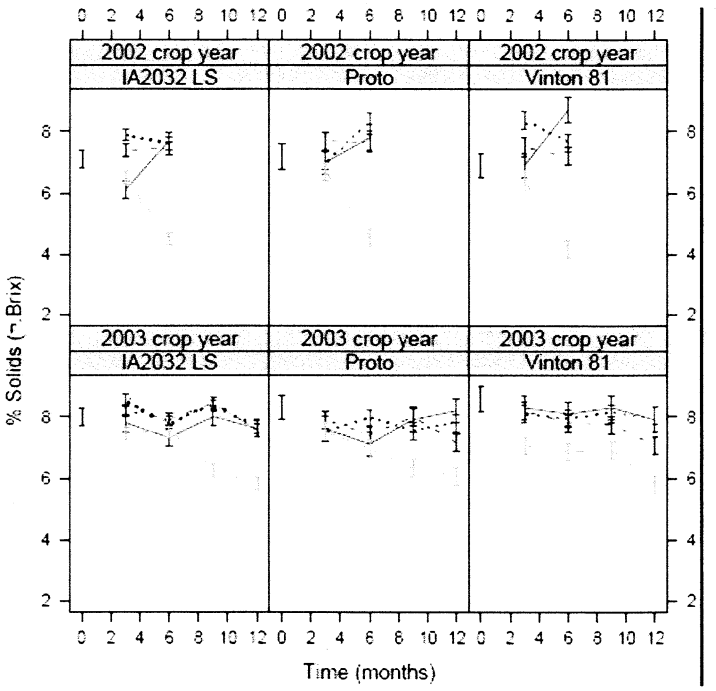

Figure 12: Effect of storage time, crop year and temperature on % solids (± SEM) of soymilk. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and soybean crop year (2002 and 2003). —— = -9°C, ···· = 20°C, -·-· = 30°C, and ---- = 40°C.

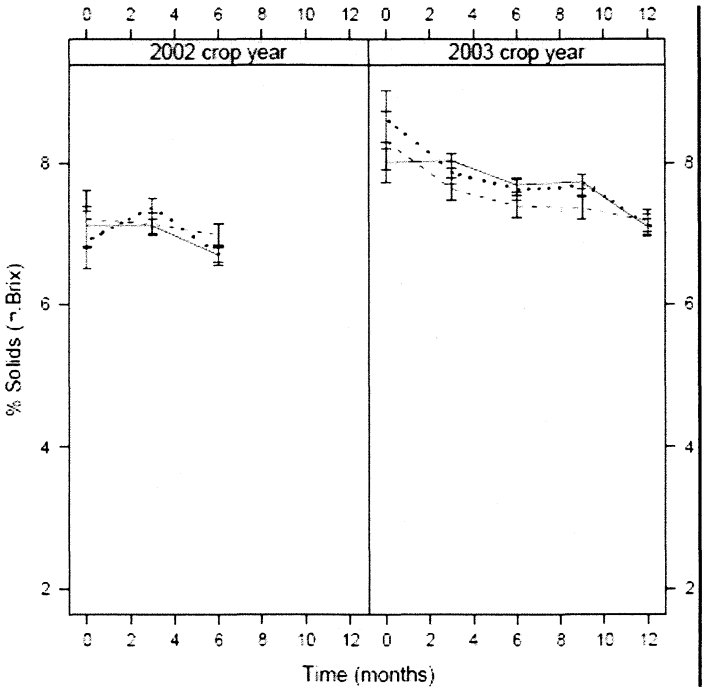

Figure 13: Mean % solids of soymilk (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

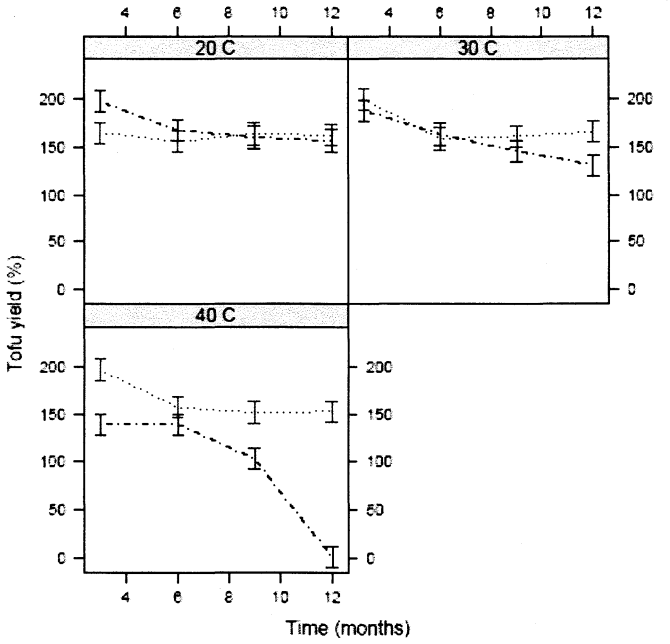

Figure 14: Effect of storage time, temperature and relative humidity on % tofu yield (± SEM) for 2003 crop year soybeans averaged for all soybean cultivars. The strip labels at the top of each panel indicate the storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

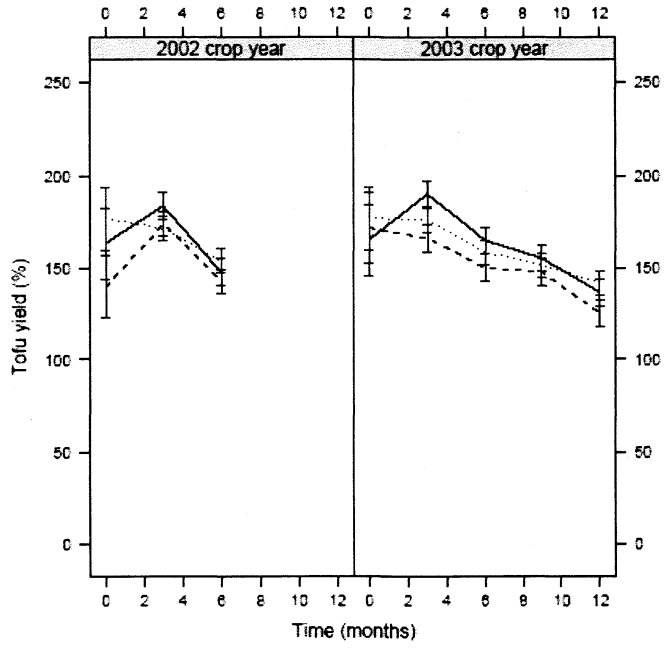

Figure 15: Mean % tofu yield (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

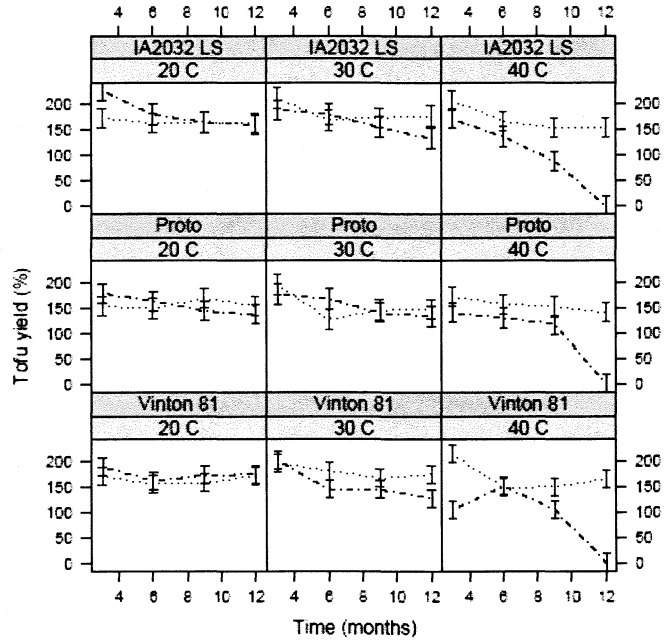

Figure 16: Effect of storage time and relative humidity on % tofu yield (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

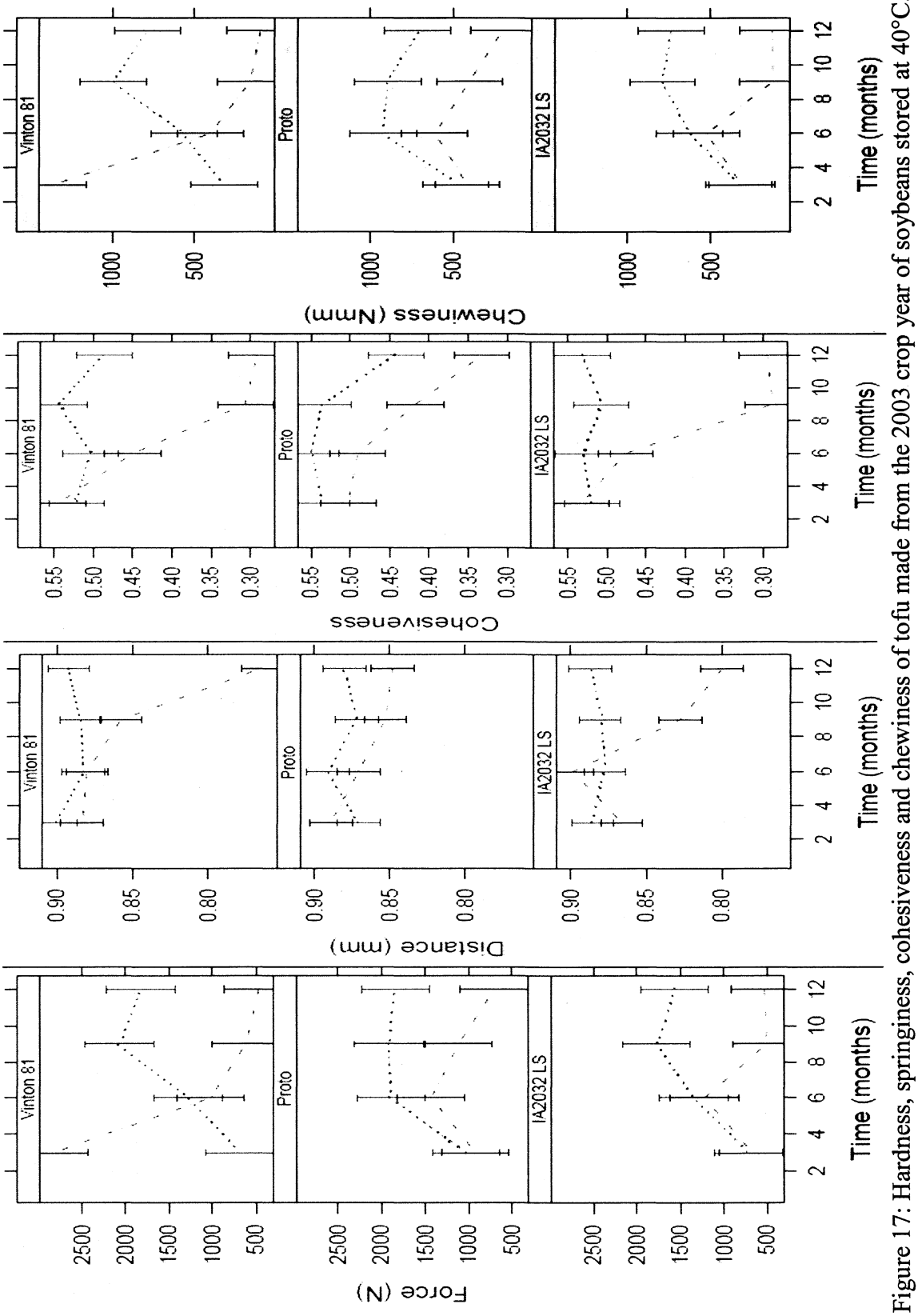

Figure 17: Hardness, springiness, cohesiveness and chewiness of tofu made from the 2003 crop year of soybeans stored at 40°C.
···· = 32%RH and - · - · = 75%RH.

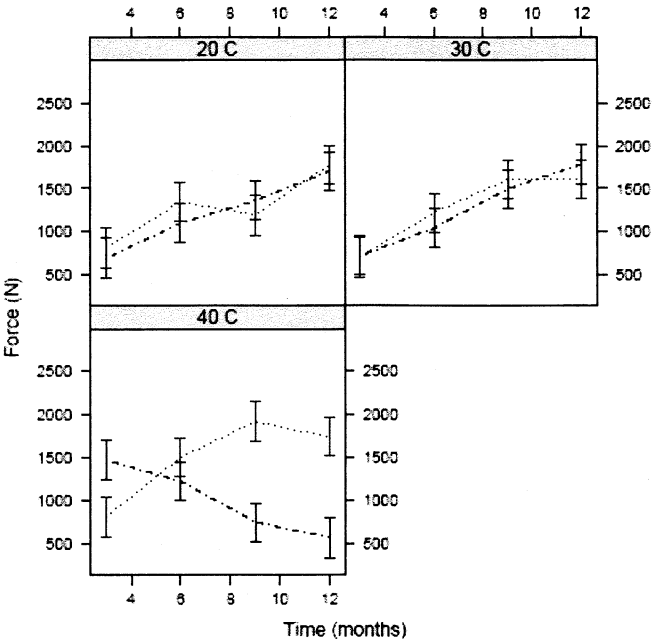

Figure 18: Effect of storage time, temperature and relative humidity on tofu hardness (measured as force (N)) ± SEM for 2003 crop year soybeans averaged for all soybean cultivars. The strip labels at the top of each panel indicate the storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

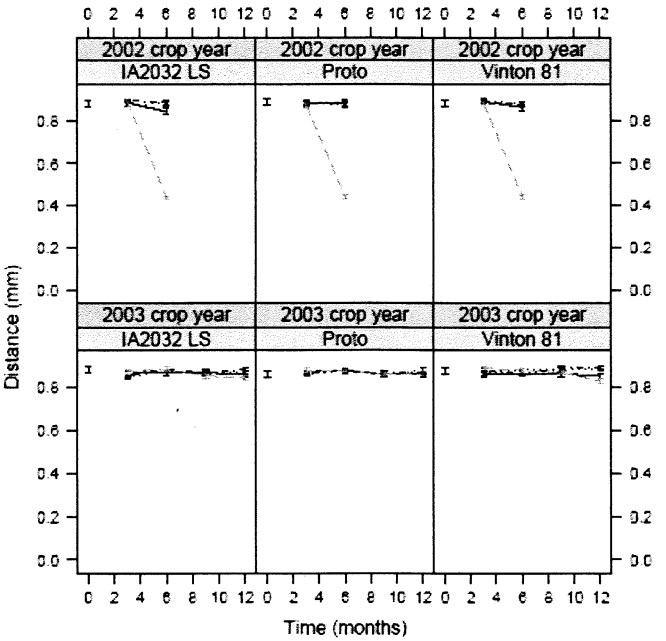

Figure 19: Effect of storage time, crop year and temperature on tofu springiness (measured as distance (mm)) ± SEM. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and soybean crop year (2002 and 2003). ——— = -9°C, ···· = 20°C, -·-· = 30°C, and ---- = 40°C.

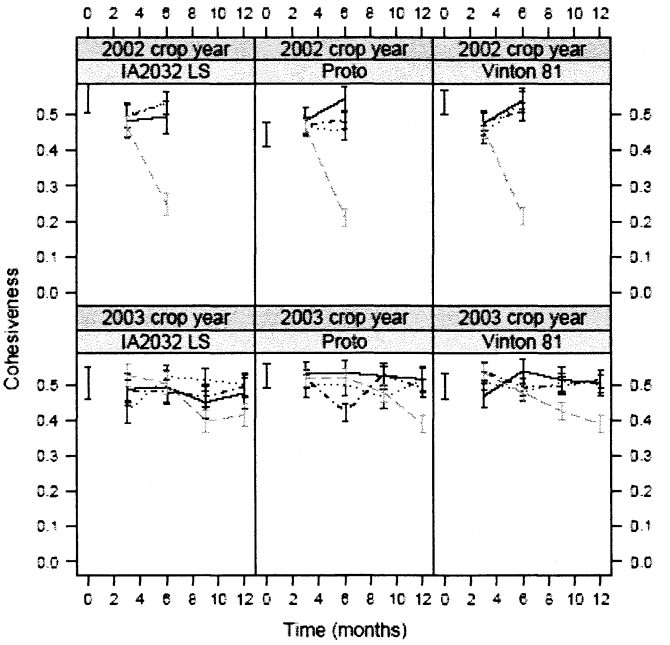

Figure 20: Effect of storage time, crop year and temperature on tofu cohesiveness (± SEM) of soymilk. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and soybean crop year (2002 and 2003). —— = -9°C, ···· = 20°C, -·-· = 30°C, and ---- = 40°C.

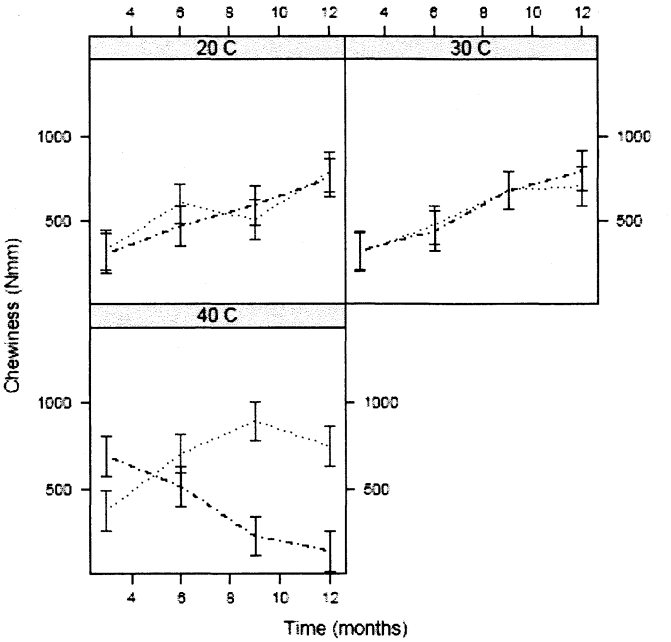

Figure 21: Effect of storage time, temperature and relative humidity on tofu chewiness (measured in Nmm) ± SEM for 2003 crop year soybeans averaged for all soybean cultivars. The strip labels at the top of each panel indicate the storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

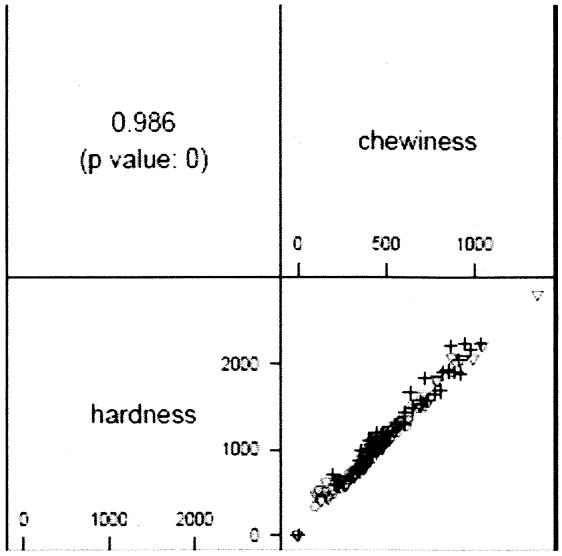

Figure 22: Correlation between tofu textural hardness and chewiness. ° = IA2032 LS, + = Proto, and ∇= Vinton 81

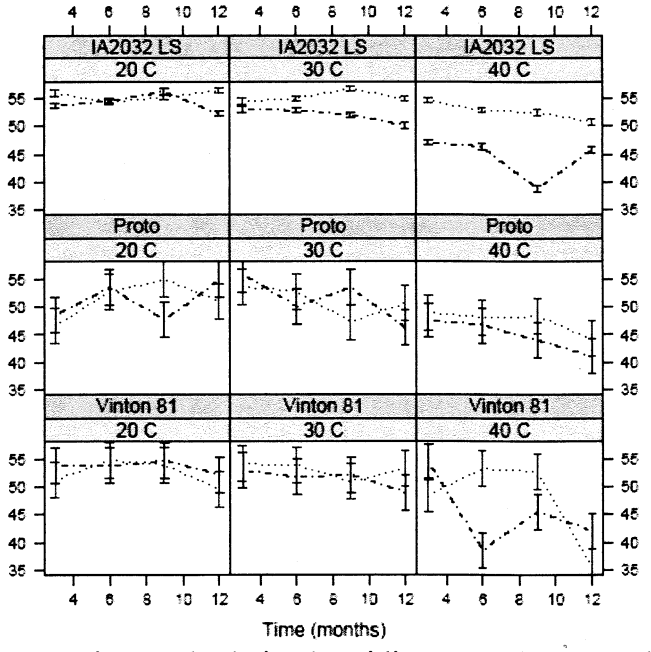

Figure 23: Effect of storage time and relative humidity on soybean L value (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

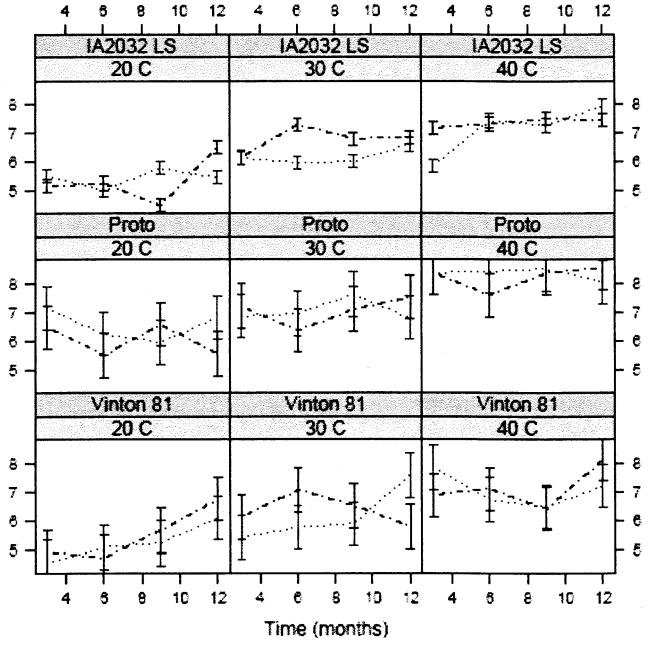

Figure 24: Effect of storage time and relative humidity on soybean a value (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

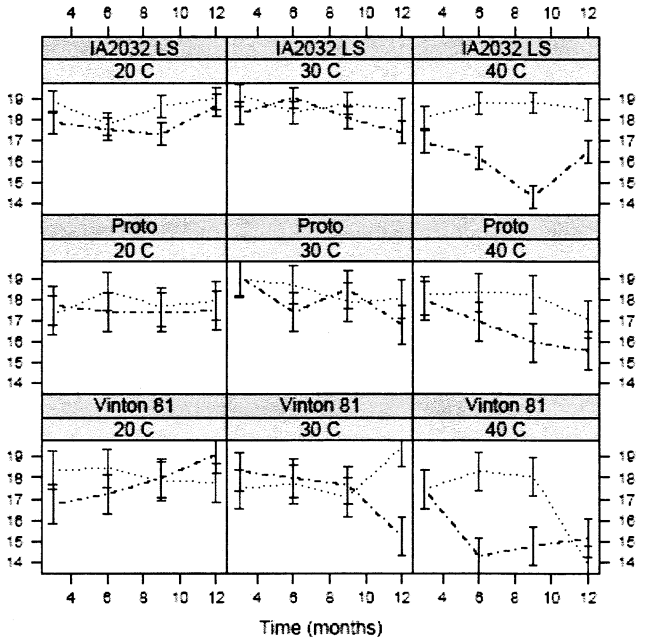

Figure 25: Effect of storage time and relative humidity on soybean b value (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

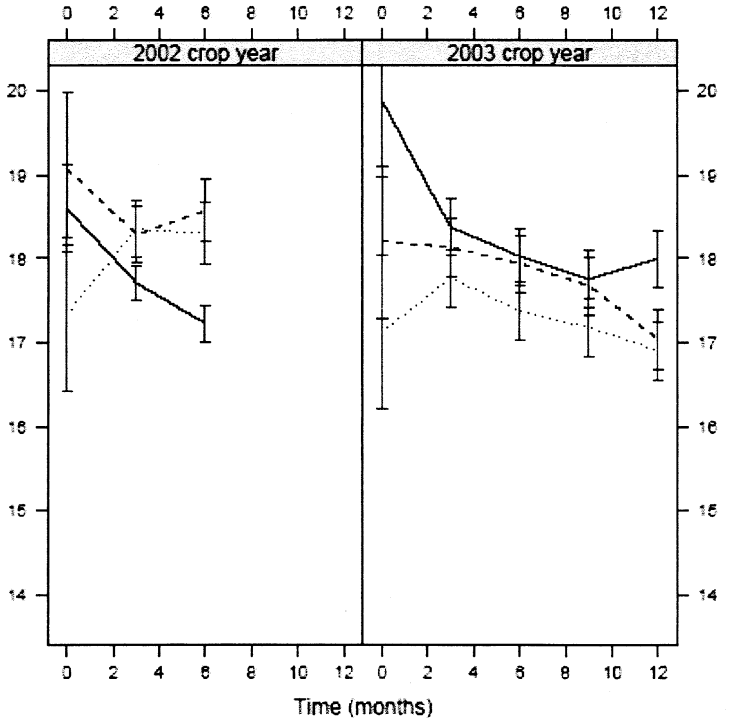

Figure 26: Mean soybean Hunter b value (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. —— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

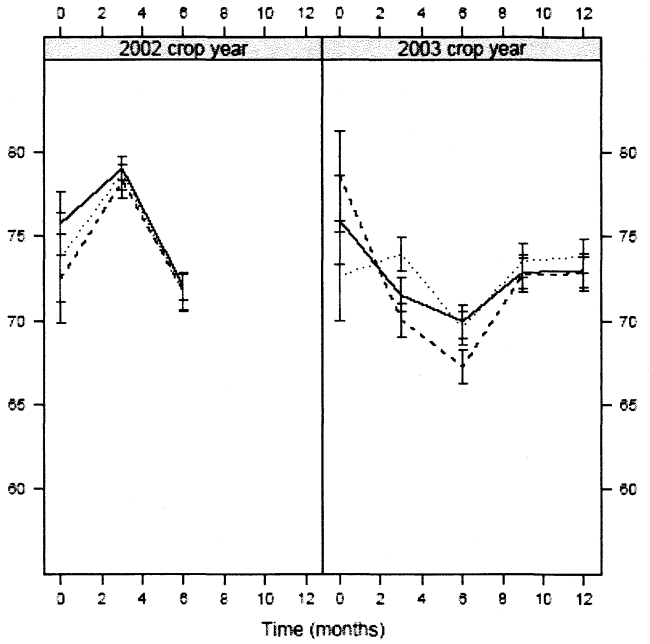

Figure 27: Mean soymilk Hunter L value (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. —— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

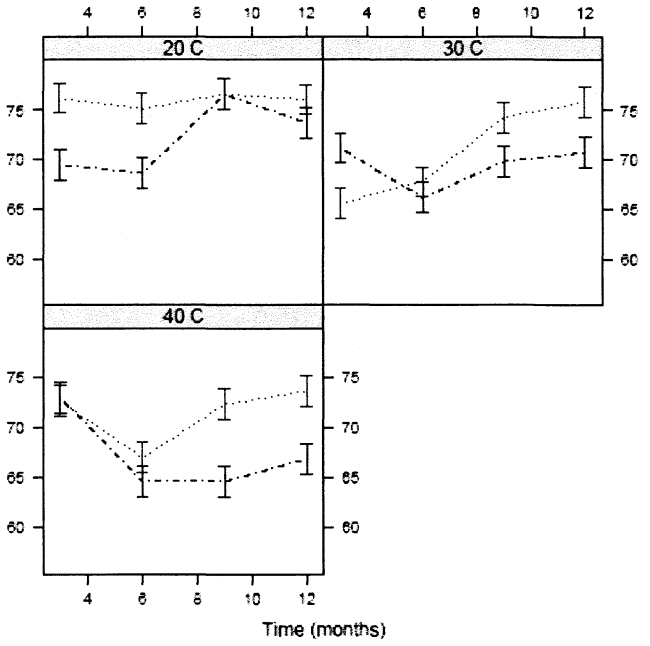

Figure 28: Effect of storage time, temperature and relative humidity on soymilk L value (± SEM) for 2003 crop year soybeans averaged for all soybean cultivars. The strip labels at the top of each panel indicate the storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

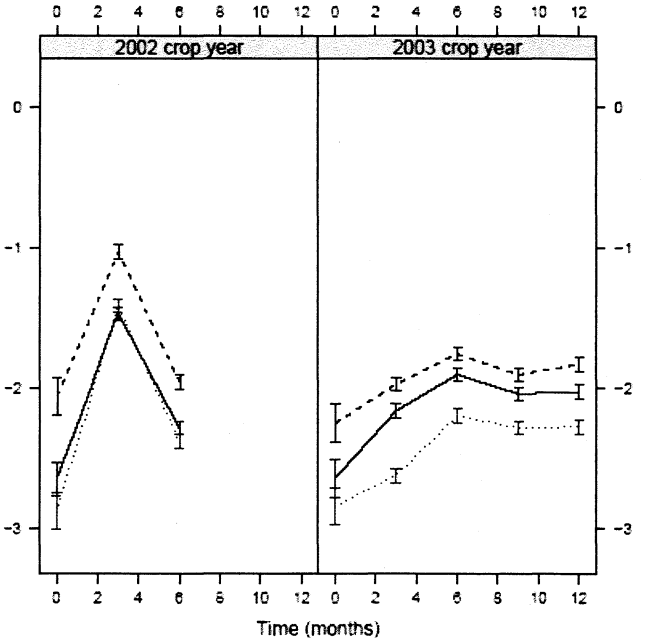

Figure 29: Mean soymilk Hunter a value (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

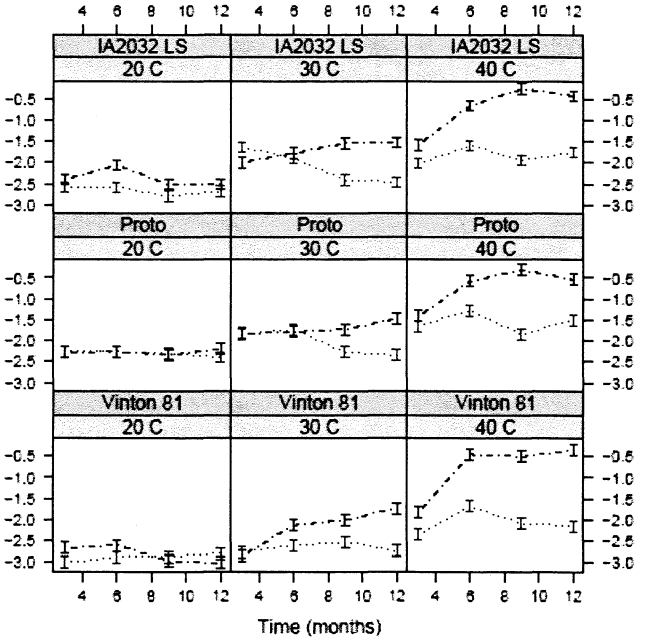

Figure 30: Effect of storage time and relative humidity on soymilk a value (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

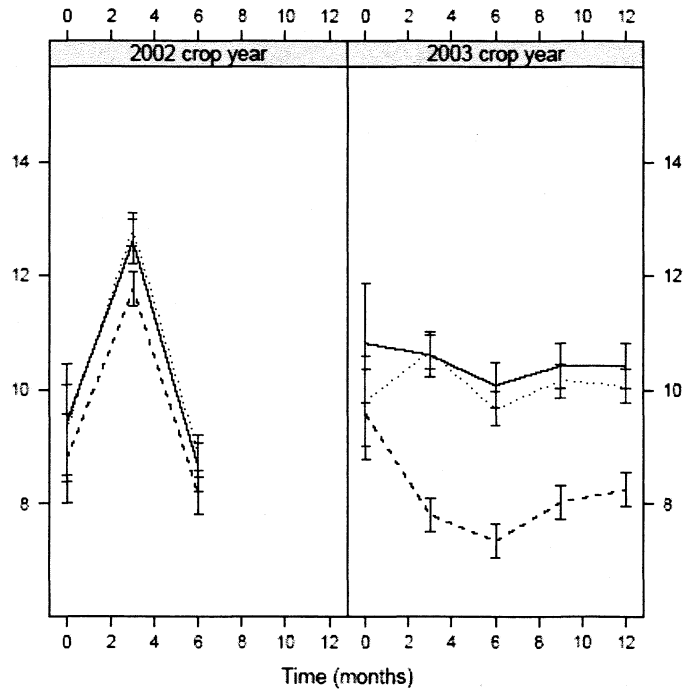

Figure 31: Mean soymilk Hunter b value (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. —— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

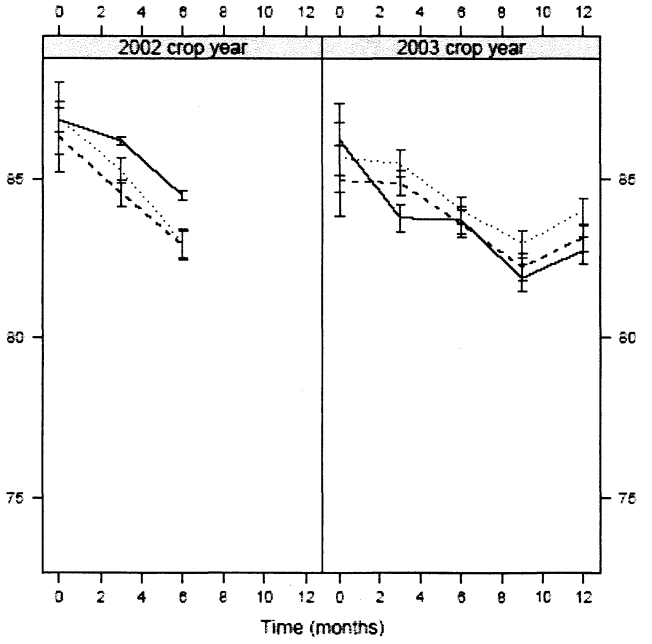

Figure 32: Mean tofu Hunter L value (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

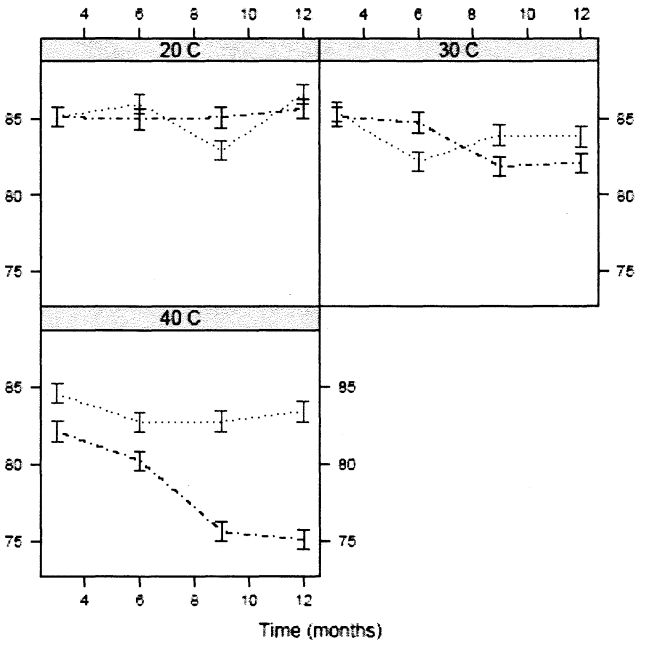

Figure 33: Effect of storage time, temperature and relative humidity on tofu L value (± SEM) for 2003 crop year soybeans averaged for all soybean cultivars. The strip labels at the top of each panel indicate the storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

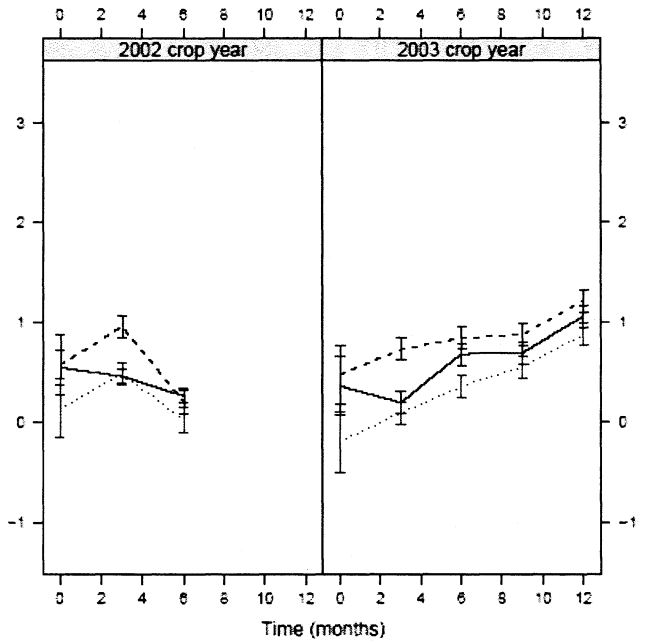

Figure 34: Mean tofu Hunter a value (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

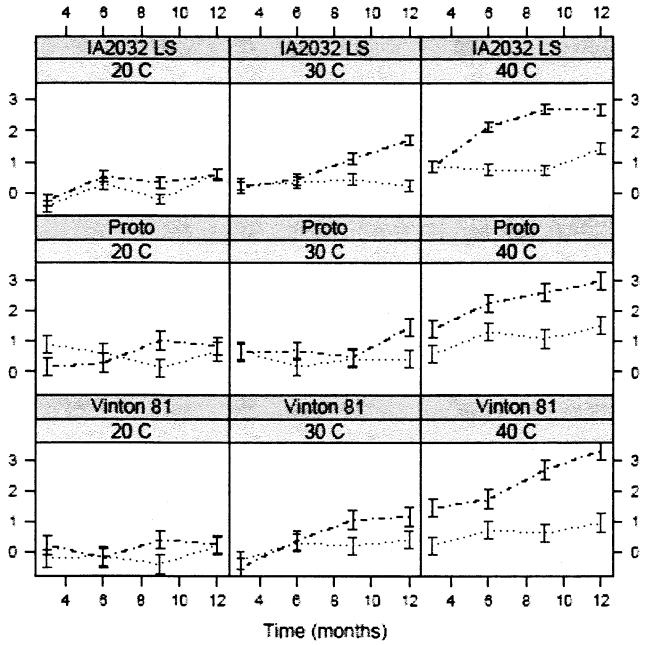

Figure 35: Effect of storage time and relative humidity on tofu a value (± SEM) for 2003 crop year soybeans. The strip labels at the top of each panel indicate the soybean cultivars (IA2032 LS, Proto, and Vinton 81) and storage temperature (20, 30, 40°C). ···· = 32%RH and -·-· = 75%RH.

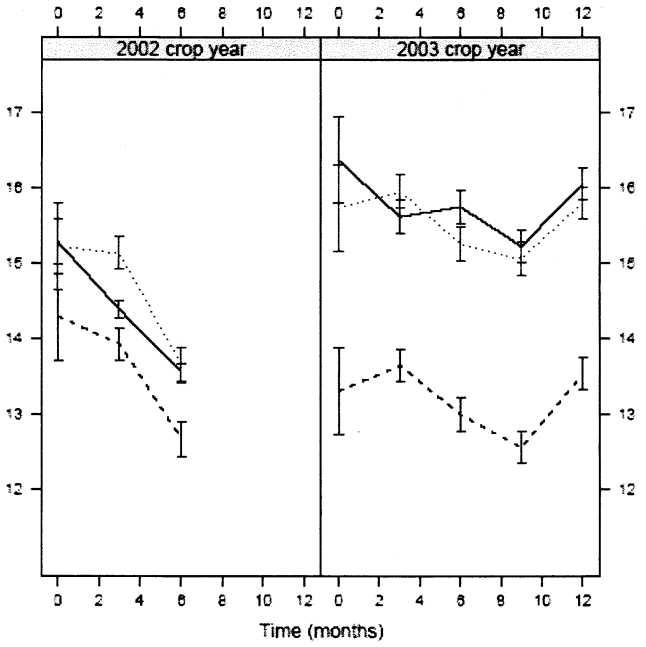

Figure 36: Mean tofu Hunter b value (± SEM) for three soybean cultivars averaged for all storage temperatures and humidities. ——— = IA2032 LS, ---- = Proto, and ···· = Vinton 81.

CONCLUSIONS

From the first study, it was concluded that the oxalate content was not affected by different storage conditions, although there was a difference between total oxalate content and soybean cultivars. Oxalate did not make a difference in tofu yield or texture, thus its function in calcium binding and protein coagulation is insignificant in this study.

While soybean storage at high temperature and high humidity affects the color of soybeans, there was no correlation between antioxidant capacity and quality of soybean color. However, storage at high temperature and relative humidity conditions increases antioxidant capacity. This phenomenon could be attributed to conversion of isoflavone forms from the malonylglucoside to the aglycone form.

In the final study on biochemical changes and soymilk and tofu quality, it was concluded that relative humidity had a bigger effect than storage temperature on deterioration. Soybean cultivars and its identifying traits, such as protein, oil, and soluble sugars, are a good indicator of seed quality. Soymilk solids level is a good indicator of its ensuing tofu quality. There was a very distinct color difference in different soybean cultivars at all storage conditions. Therefore, color can be used as a predictor of soybean storage conditions and its resulting product quality.

Based on these studies, several recommendations are suggested for future research. With increasing interests in antioxidants and oligosaccharides as functional foods, a better mechanism is needed to understand the changes of antioxidant capacity and soluble sugars over storage time, temperature and humidity, as well as its synergism with other soy components. Additionally, a predictive model for color could be constructed to estimate soybean seed and end product quality.

APPENDIX

RAW DATA

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Total oxalate (mg/g)	Soluble oxalate (mg/g)	Antioxidant capacity (ug/mg)
2002	Vinton 81	0	0	0	0.520	0.029	0.608
2002	IA2032 LS	0	0	0	1.533	1.026	0.724
2002	Proto	0	0	0	1.209	0.998	1.695
2002	Vinton 81	40	75	3	1.505	0.749	1.326
2002	IA2032 LS	40	75	3	1.148	0.625	1.184
2002	Proto	40	75	3	1.045	0.815	1.335
2002	Vinton 81	40	32	3	0.988	0.309	0.833
2002	IA2032 LS	40	32	3	1.020	0.412	0.968
2002	Proto	40	32	3	0.808	0.396	1.415
2002	Vinton 81	30	75	3	1.531	0.881	1.218
2002	IA2032 LS	30	75	3	1.243	0.679	1.226
2002	Proto	30	75	3	0.788	0.444	1.193
2002	Vinton 81	30	32	3	1.472	0.824	1.188
2002	IA2032 LS	30	32	3	1.282	0.741	0.706
2002	Proto	30	32	3	0.807	0.336	1.350
2002	Vinton 81	20	75	3	1.494	0.729	0.442
2002	IA2032 LS	20	75	3	1.234	0.611	1.025
2002	Proto	20	75	3	0.819	0.464	1.097
2002	Vinton 81	20	32	3	1.682	1.023	1.200
2002	IA2032 LS	20	32	3	1.163	0.479	1.225
2002	Proto	20	32	3	0.924	0.440	1.045
2002	Vinton 81	-9	25	3	1.326	0.748	0.939
2002	IA2032 LS	-9	25	3	1.351	0.670	1.275
2002	Proto	-9	25	3	0.784	0.357	1.440
2002	Vinton 81	40	75	6	1.284	0.404	2.845
2002	IA2032 LS	40	75	6	0.983	0.231	2.754
2002	Proto	40	75	6	0.797	0.325	2.447
2002	Vinton 81	40	32	6	0.874	0.153	1.646
2002	IA2032 LS	40	32	6	1.234	0.122	2.161
2002	Proto	40	32	6	1.079	0.636	1.470
2002	Vinton 81	30	75	6	1.339	0.717	1.955
2002	IA2032 LS	30	75	6	0.845	-0.072	1.661
2002	Proto	30	75	6	0.877	0.513	1.917
2002	Vinton 81	30	32	6	1.186	0.386	1.816
2002	IA2032 LS	30	32	6	1.067	0.015	1.897
2002	Proto	30	32	6	0.831	0.216	1.886
2002	Vinton 81	20	75	6	1.541	0.529	1.298
2002	IA2032 LS	20	75	6	1.706	0.762	1.377
2002	Proto	20	75	6	1.385	0.883	1.296
2002	Vinton 81	20	32	6	1.131	0.421	1.024
2002	IA2032 LS	20	32	6	1.314	0.448	1.887
2002	Proto	20	32	6	0.661	0.260	1.856
2002	Vinton 81	-9	25	6	0.798	0.092	1.193
2002	IA2032 LS	-9	25	6	1.090	0.273	1.935
2002	Proto	-9	25	6	0.741	0.288	1.828

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Total oxalate (mg/g)	Soluble oxalate (mg/g)
2003	Vinton 81	0	0	0	0.958	-0.097
2003	IA2032 LS	0	0	0	1.164	-0.026
2003	Proto	0	0	0	1.063	0.513
2003	Vinton 81	40	75	3	1.395	0.308
2003	IA2032 LS	40	75	3	1.561	0.278
2003	Proto	40	75	3	1.046	0.554
2003	Vinton 81	40	32	3	1.665	0.351
2003	IA2032 LS	40	32	3	1.639	0.159
2003	Proto	40	32	3	1.000	0.430
2003	Vinton 81	30	75	3	1.330	0.196
2003	IA2032 LS	30	75	3	1.951	0.921
2003	Proto	30	75	3	1.173	0.647
2003	Vinton 81	30	32	3	1.655	0.609
2003	IA2032 LS	30	32	3	1.979	0.623
2003	Proto	30	32	3	1.377	1.018
2003	Vinton 81	20	75	3	1.454	0.351
2003	IA2032 LS	20	75	3	2.278	1.058
2003	Proto	20	75	3	1.478	0.979
2003	Vinton 81	20	32	3	1.659	0.739
2003	IA2032 LS	20	32	3	2.276	1.171
2003	Proto	20	32	3	1.596	0.995
2003	Vinton 81	-9	25	3	1.341	0.319
2003	IA2032 LS	-9	25	3	1.473	0.401
2003	Proto	-9	25	3	1.074	0.485
2003	Vinton 81	40	75	6	1.473	0.815
2003	IA2032 LS	40	75	6	1.981	0.791
2003	Proto	40	75	6	0.923	0.287
2003	Vinton 81	40	32	6	1.581	0.342
2003	IA2032 LS	40	32	6	1.690	0.370
2003	Proto	40	32	6	1.078	0.536
2003	Vinton 81	30	75	6	1.286	0.255
2003	IA2032 LS	30	75	6	1.485	0.169
2003	Proto	30	75	6	0.942	0.461
2003	Vinton 81	30	32	6	1.440	0.398
2003	IA2032 LS	30	32	6	1.505	0.464
2003	Proto	30	32	6	0.938	0.407
2003	Vinton 81	20	75	6	1.330	0.466
2003	IA2032 LS	20	75	6	1.713	0.548
2003	Proto	20	75	6	1.052	0.710
2003	Vinton 81	20	32	6	1.396	0.723
2003	IA2032 LS	20	32	6	1.502	0.668
2003	Proto	20	32	6	1.005	0.458
2003	Vinton 81	-9	25	6	1.674	0.689
2003	IA2032 LS	-9	25	6	1.780	0.857
2003	Proto	-9	25	6	0.820	0.329
2003	Vinton 81	40	75	9	1.371	0.493
2003	IA2032 LS	40	75	9	1.833	0.821
2003	Proto	40	75	9	0.828	0.368
2003	Vinton 81	30	32	9	1.374	0.212
2003	IA2032 LS	30	32	9	1.727	0.916
2003	Proto	30	32	9	0.888	0.510
2003	Vinton 81	20	75	9	1.262	0.472
2003	IA2032 LS	20	75	9	1.491	0.426

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Total oxalate (mg/g)	Soluble oxalate (mg/g)
2003	Proto	20	75	9	0.787	0.178
2003	Vinton 81	20	32	9	1.134	-0.082
2003	IA2032 LS	20	32	9	1.310	0.064
2003	Proto	20	32	9	0.656	0.272
2003	Vinton 81	-9	25	9	1.172	0.137
2003	IA2032 LS	-9	25	9	1.332	0.512
2003	Proto	-9	25	9	0.857	0.253
2003	Vinton 81	40	75	12	2.075	0.890
2003	IA2032 LS	40	75	12	1.604	0.263
2003	Proto	40	75	12	0.811	0.261
2003	Vinton 81	40	32	12	1.241	0.457
2003	IA2032 LS	40	32	12	1.480	0.156
2003	Proto	40	32	12	1.098	0.616
2003	Vinton 81	30	75	12	1.090	0.079
2003	IA2032 LS	30	75	12	1.556	0.220
2003	Proto	30	75	12	0.708	0.010
2003	Vinton 81	30	32	12	1.422	0.262
2003	IA2032 LS	30	32	12	1.551	0.214
2003	Proto	30	32	12	1.082	0.481
2003	Vinton 81	20	75	12	1.639	0.390
2003	IA2032 LS	20	75	12	1.470	0.184
2003	Proto	20	75	12	0.871	0.112
2003	Vinton 81	20	32	12	1.202	-0.147
2003	IA2032 LS	20	32	12	1.537	0.956
2003	Proto	20	32	12	0.713	0.131
2003	Vinton 81	-9	25	12	1.741	0.452
2003	IA2032 LS	-9	25	12	1.695	0.483
2003	Proto	-9	25	12	1.037	0.428

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Soymilk yield (%)	Tofu yield (%)	°Brix	Hardness (Force, N)	Springiness (Distance, mm)	Cohesiveness	Chewiness (Nmm)
2002	Vinton 81	0	0	0	973.21	176.67	6.9	908.22	0.88	0.54	429.41
2002	IA2032 LS	0	0	0	932.34	163.33	7.1	1270.13	0.88	0.55	617.06
2002	Proto	0	0	0	963.68	140.00	7.2	1017.50	0.89	0.44	401.00
2002	Vinton 81	40	75	3	996.67	128.33	6.0	1144.10	0.88	0.44	443.13
2002	IA2032 LS	40	75	3	861.67	140.00	6.4	955.18	0.89	0.44	387.31
2002	Proto	40	75	3	913.33	108.33	6.3	2211.00	0.89	0.44	865.57
2002	Vinton 81	40	32	3	955.00	166.67	6.8	874.62	0.88	0.48	367.26
2002	IA2032 LS	40	32	3	890.00	170.00	6.6	959.52	0.89	0.49	417.71
2002	Proto	40	32	3	803.33	165.00	7.1	791.49	0.85	0.48	330.49
2002	Vinton 81	30	75	3	843.33	140.00	7.6	1320.98	0.88	0.50	579.39
2002	IA2032 LS	30	75	3	860.00	186.67	7.8	753.71	0.90	0.50	342.01
2002	Proto	30	75	3	886.67	205.00	7.4	580.72	0.88	0.44	232.08
2002	Vinton 81	30	32	3	830.00	170.00	7.4	907.36	0.88	0.46	372.64
2002	IA2032 LS	30	32	3	898.33	223.33	7.0	459.62	0.89	0.49	201.40
2002	Proto	30	32	3	965.00	195.00	8.0	836.54	0.88	0.49	361.61
2002	Vinton 81	20	75	3	831.67	225.00	8.2	408.47	0.87	0.38	134.77
2002	IA2032 LS	20	75	3	860.00	160.00	7.5	1060.36	0.89	0.52	491.26
2002	Proto	20	75	3	945.00	203.33	6.8	713.41	0.88	0.50	311.98
2002	Vinton 81	20	32	3	973.33	235.00	8.5	595.70	0.91	0.51	277.42
2002	IA2032 LS	20	32	3	966.67	245.00	8.3	545.06	0.85	0.48	233.88
2002	Proto	20	32	3	970.00	210.00	7.3	632.06	0.89	0.43	242.08
2002	Vinton 81	-9	25	3	1013.33	133.33	6.9	2075.02	0.89	0.48	875.02
2002	IA2032 LS	-9	25	3	1058.33	158.33	6.1	1104.79	0.88	0.48	466.88
2002	Proto	-9	25	3	1010.00	130.00	7.0	2240.33	0.88	0.48	945.84
2002	Vinton 81	40	75	6	756.67	N/A	2.1	N/A	N/A	N/A	N/A
2002	IA2032 LS	40	75	6	713.33	N/A	2.8	N/A	N/A	N/A	N/A
2002	Proto	40	75	6	791.67	N/A	2.6	N/A	N/A	N/A	N/A
2002	Vinton 81	40	32	6	913.33	193.33	6.3	642.63	0.89	0.43	251.94
2002	IA2032 LS	40	32	6	903.33	168.33	6.2	1338.98	0.88	0.49	581.57
2002	Proto	40	32	6	820.00	166.67	6.5	602.13	0.89	0.42	225.03
2002	Vinton 81	30	75	6	961.67	165.00	6.4	991.09	0.87	0.49	425.30
2002	IA2032 LS	30	75	6	943.33	163.33	7.2	1467.60	0.87	0.54	689.27
2002	Proto	30	75	6	915.00	146.67	7.1	1051.44	0.88	0.45	417.53
2002	Vinton 81	30	32	6	870.00	175.00	8.0	1154.96	0.88	0.52	530.54
2002	IA2032 LS	30	32	6	826.67	163.33	7.7	770.77	0.90	0.53	367.71
2002	Proto	30	32	6	798.33	163.33	8.2	996.92	0.88	0.52	453.64
2002	Vinton 81	20	75	6	936.67	173.33	7.4	1050.11	0.88	0.53	487.78
2002	IA2032 LS	20	75	6	888.33	185.00	7.6	683.02	0.88	0.52	316.18
2002	Proto	20	75	6	805.00	168.33	7.8	602.36	0.87	0.41	214.52
2002	Vinton 81	20	32	6	880.00	185.00	7.9	736.38	0.88	0.55	352.77
2002	IA2032 LS	20	32	6	895.00	170.00	7.6	1186.53	0.88	0.54	562.56
2002	Proto	20	32	6	773.33	175.00	8.8	813.38	0.87	0.50	353.54
2002	Vinton 81	-9	25	6	845.00	186.67	8.7	577.53	0.86	0.54	267.12
2002	IA2032 LS	-9	25	6	900.00	183.33	7.7	743.51	0.84	0.49	308.10
2002	Proto	-9	25	6	900.00	180.00	7.8	797.25	0.88	0.54	380.82

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Soymilk yield (%)	Tofu yield (%)	°Brix	Hardness (Force, N)	Springiness (Distance, mm)	Cohesiveness	Chewiness (Nmm)
2003	Vinton 81	0	0	0	828.33	176.67	8.6	1224.98	0.88	0.49	526.61
2003	IA2032 LS	0	0	0	800.00	165.00	8.0	1120.54	0.89	0.51	505.34
2003	Proto	0	0	0	796.67	171.67	8.3	935.02	0.86	0.52	423.33
2003	Vinton 81	40	75	3	823.33	105.00	6.9	2819.30	0.88	0.54	1355.27
2003	IA2032 LS	40	75	3	783.33	171.67	7.3	670.86	0.87	0.53	310.28
2003	Proto	40	75	3	731.67	140.00	7.7	926.29	0.89	0.50	413.42
2003	Vinton 81	40	32	3	873.33	211.67	7.2	676.36	0.90	0.52	317.02
2003	IA2032 LS	40	32	3	835.00	206.67	7.6	708.57	0.89	0.52	325.46
2003	Proto	40	32	3	825.00	171.67	7.3	1029.15	0.87	0.54	480.36
2003	Vinton 81	30	75	3	768.33	200.00	8.4	788.33	0.89	0.54	380.25
2003	IA2032 LS	30	75	3	680.00	188.33	8.9	435.60	0.86	0.46	171.11
2003	Proto	30	75	3	813.33	176.67	8.0	938.87	0.87	0.53	426.14
2003	Vinton 81	30	32	3	801.67	193.33	8.0	781.62	0.88	0.53	366.47
2003	IA2032 LS	30	32	3	865.00	211.67	7.5	636.56	0.87	0.51	281.38
2003	Proto	30	32	3	820.00	195.00	7.8	673.91	0.87	0.51	296.25
2003	Vinton 81	20	75	3	878.33	188.33	7.8	804.77	0.87	0.54	377.56
2003	IA2032 LS	20	75	3	773.33	226.67	8.5	319.22	0.83	0.38	101.85
2003	Proto	20	75	3	883.33	178.33	7.2	971.85	0.87	0.53	446.05
2003	Vinton 81	20	32	3	803.33	168.33	8.4	1061.55	0.86	0.48	437.08
2003	IA2032 LS	20	32	3	738.33	173.33	8.6	502.33	0.87	0.46	204.02
2003	Proto	20	32	3	791.67	153.33	7.8	869.63	0.86	0.47	345.29
2003	Vinton 81	-9	25	3	831.67	165.00	8.3	428.89	0.87	0.47	172.86
2003	IA2032 LS	-9	25	3	871.67	151.67	7.8	694.48	0.87	0.49	291.70
2003	Proto	-9	25	3	855.00	148.33	7.6	1306.04	0.88	0.53	602.74
2003	Vinton 81	40	75	6	863.33	150.00	6.4	1020.59	0.88	0.45	403.54
2003	IA2032 LS	40	75	6	810.00	136.67	6.7	1224.80	0.90	0.48	523.21
2003	Proto	40	75	6	790.00	130.00	6.7	1430.64	0.87	0.49	610.94
2003	Vinton 81	40	32	6	830.00	146.67	7.3	1274.19	0.88	0.50	564.05
2003	IA2032 LS	40	32	6	806.67	166.67	8.4	1343.38	0.88	0.53	626.89
2003	Proto	40	32	6	790.00	156.67	7.2	1886.39	0.89	0.55	923.03
2003	Vinton 81	30	75	6	826.67	143.33	7.6	1362.41	0.87	0.53	624.43
2003	IA2032 LS	30	75	6	795.00	180.00	7.8	776.12	0.90	0.49	340.16
2003	Proto	30	75	6	905.00	166.67	6.8	987.39	0.89	0.41	359.88
2003	Vinton 81	30	32	6	806.67	180.00	8.0	962.07	0.85	0.46	375.11
2003	IA2032 LS	30	32	6	780.00	168.33	8.0	1011.10	0.87	0.47	411.64
2003	Proto	30	32	6	763.33	126.67	8.0	1676.37	0.87	0.43	635.19
2003	Vinton 81	20	75	6	865.00	160.00	7.4	1192.56	0.88	0.48	501.84
2003	IA2032 LS	20	75	6	810.00	180.00	7.5	982.21	0.89	0.54	467.18
2003	Proto	20	75	6	808.33	163.33	8.0	1113.53	0.88	0.46	444.08
2003	Vinton 81	20	32	6	763.33	155.00	8.5	1291.85	0.89	0.48	552.58
2003	IA2032 LS	20	32	6	771.67	163.33	8.1	1032.52	0.87	0.51	450.23
2003	Proto	20	32	6	801.67	148.33	7.9	1688.04	0.88	0.54	805.61
2003	Vinton 81	-9	25	6	811.67	175.00	8.1	1124.90	0.87	0.54	524.60
2003	IA2032 LS	-9	25	6	885.00	158.33	7.3	1155.61	0.87	0.49	495.03
2003	Proto	-9	25	6	916.67	160.00	7.1	1578.29	0.88	0.53	735.43
2003	Vinton 81	40	75	9	855.00	103.33	5.8	616.25	0.86	0.31	162.06
2003	IA2032 LS	40	75	9	780.00	88.33	5.2	502.92	0.83	0.29	120.47
2003	Proto	40	75	9	835.00	116.67	5.2	1118.40	0.85	0.42	399.79
2003	Vinton 81	40	32	9	793.33	148.33	8.0	2069.05	0.88	0.54	992.81
2003	IA2032 LS	40	32	9	911.67	153.33	7.3	1773.38	0.88	0.51	790.93
2003	Proto	40	32	9	851.67	151.67	7.4	1914.50	0.87	0.53	893.43
2003	Vinton 81	30	75	9	821.67	143.33	7.1	1582.15	0.89	0.50	706.38
2003	IA2032 LS	30	75	9	813.33	155.00	8.0	1253.21	0.88	0.50	555.71

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Soymilk yield (%)	Tofu yield (%)	°Brix	Hardness (Force, N)	Springiness (Distance, mm)	Cohesiveness	Chewiness (Nmm)
2003	Proto	30	75	9	833.33	140.00	7.8	1650.42	0.87	0.54	771.42
2003	Vinton 81	30	32	9	835.00	165.00	8.4	1646.46	0.88	0.49	718.03
2003	IA2032 LS	30	32	9	795.00	173.33	8.9	1099.43	0.85	0.43	408.87
2003	Proto	30	32	9	880.00	145.00	8.2	2056.71	0.87	0.51	915.98
2003	Vinton 81	20	75	9	843.33	171.67	8.5	1117.75	0.89	0.47	474.14
2003	IA2032 LS	20	75	9	768.33	165.00	8.5	1041.36	0.85	0.54	480.88
2003	Proto	20	75	9	933.33	143.33	7.0	1902.14	0.87	0.50	822.42
2003	Vinton 81	20	32	9	858.33	156.67	7.7	1527.91	0.87	0.54	723.29
2003	IA2032 LS	20	32	9	850.00	165.00	8.2	817.43	0.87	0.49	344.49
2003	Proto	20	32	9	793.33	168.33	8.0	1210.96	0.85	0.43	447.90
2003	Vinton 81	-9	25	9	805.00	173.33	8.3	1128.41	0.86	0.51	499.74
2003	IA2032 LS	-9	25	9	865.00	186.67	8.0	587.83	0.87	0.45	230.72
2003	Proto	-9	25	9	865.00	170.00	7.9	1538.41	0.86	0.52	692.85
2003	Vinton 81	40	75	12	773.33	N/A	4.8	469.42	0.76	0.29	104.24
2003	IA2032 LS	40	75	12	750.00	N/A	4.7	523.71	0.80	0.30	123.72
2003	Proto	40	75	12	890.00	N/A	5.0	701.94	0.85	0.33	196.68
2003	Vinton 81	40	32	12	970.00	163.33	6.8	1821.57	0.89	0.49	787.02
2003	IA2032 LS	40	32	12	873.33	153.33	6.9	1560.42	0.89	0.53	735.98
2003	Proto	40	32	12	800.00	140.00	7.1	1836.51	0.88	0.44	716.11
2003	Vinton 81	30	75	12	886.67	126.67	6.8	2220.17	0.90	0.52	1044.54
2003	IA2032 LS	30	75	12	816.67	133.33	7.5	1906.21	0.88	0.52	871.60
2003	Proto	30	75	12	836.67	133.33	7.0	1203.98	0.87	0.46	478.35
2003	Vinton 81	30	32	12	923.33	173.33	7.3	1538.44	0.89	0.50	684.35
2003	IA2032 LS	30	32	12	843.33	176.67	7.6	1091.97	0.89	0.47	453.55
2003	Proto	30	32	12	880.00	146.67	7.3	2170.99	0.87	0.52	978.73
2003	Vinton 81	20	75	12	896.67	173.33	7.6	1317.04	0.89	0.49	579.19
2003	IA2032 LS	20	75	12	886.67	160.00	7.5	1529.77	0.88	0.48	646.83
2003	Proto	20	75	12	800.00	136.67	7.8	2246.35	0.88	0.52	1034.92
2003	Vinton 81	20	32	12	856.67	170.00	8.5	2060.43	0.88	0.51	926.03
2003	IA2032 LS	20	32	12	863.33	163.33	7.8	1329.35	0.86	0.51	584.34
2003	Proto	20	32	12	836.67	153.33	7.8	1925.97	0.88	0.50	850.80
2003	Vinton 81	-9	25	12	873.33	186.67	7.9	933.43	0.85	0.50	398.17
2003	IA2032 LS	-9	25	12	846.67	170.00	7.6	1214.88	0.86	0.47	491.43
2003	Proto	-9	25	12	826.67	166.67	8.2	1485.20	0.86	0.51	655.48

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Soybean			Soymilk			Tofu		
					L	*a*	*b*	*L*	*a*	*b*	*L*	*a*	*b*
2002	Vinton 81	0	0	0	54.42	4.90	17.33	73.75	-2.89	9.29	86.84	0.13	15.21
2002	IA2032 LS	0	0	0	51.52	6.80	18.60	75.75	-2.66	9.41	86.80	0.54	15.27
2002	Proto	0	0	0	50.45	5.97	19.07	72.53	-2.06	8.79	86.27	0.57	14.28
2002	Vinton 81	40	75	3	49.17	7.87	17.49	78.24	-0.09	12.96	81.35	1.92	14.13
2002	IA2032 LS	40	75	3	47.94	7.89	16.20	75.92	-0.09	12.88	83.08	1.70	15.01
2002	Proto	40	75	3	45.30	8.29	17.20	72.92	0.03	11.12	80.45	1.98	13.84
2002	Vinton 81	40	32	3	48.07	7.89	16.89	76.11	-0.45	12.88	83.95	1.19	14.74
2002	IA2032 LS	40	32	3	50.32	7.35	16.86	76.31	-0.74	12.29	84.30	0.80	13.99
2002	Proto	40	32	3	47.52	7.91	17.72	76.63	0.12	11.95	80.66	1.70	13.91
2002	Vinton 81	30	75	3	53.14	7.08	18.56	77.25	-1.91	12.79	84.99	0.68	16.20
2002	IA2032 LS	30	75	3	52.86	6.33	17.14	83.62	-1.68	14.03	86.95	0.03	14.47
2002	Proto	30	75	3	49.66	7.42	18.58	80.75	-1.07	12.69	85.11	0.80	13.83
2002	Vinton 81	30	32	3	53.03	7.02	18.79	77.69	-1.84	12.72	85.56	-0.07	15.06
2002	IA2032 LS	30	32	3	52.26	6.73	17.56	78.36	-1.84	11.94	86.63	0.10	14.17
2002	Proto	30	32	3	52.95	6.98	18.59	78.74	-1.24	11.79	85.98	0.66	13.94
2002	Vinton 81	20	75	3	52.84	6.53	18.71	82.45	-1.83	15.07	87.20	-0.43	15.07
2002	IA2032 LS	20	75	3	53.51	6.38	17.99	79.13	-1.99	12.26	87.25	0.13	14.22
2002	Proto	20	75	3	52.16	6.70	18.73	79.18	-1.84	11.76	86.78	0.41	14.03
2002	Vinton 81	20	32	3	53.38	6.10	18.46	80.96	-1.38	11.07	87.33	-0.23	14.88
2002	IA2032 LS	20	32	3	54.65	6.15	18.26	80.01	-1.80	13.19	87.65	-0.04	14.27
2002	Proto	20	32	3	52.61	6.36	18.57	80.09	-1.48	11.76	86.59	0.29	13.74
2002	Vinton 81	-9	25	3	55.30	5.18	19.54	78.18	-2.41	12.15	86.19	0.29	15.78
2002	IA2032 LS	-9	25	3	51.12	6.90	19.87	79.49	-2.14	11.50	87.26	0.42	14.50
2002	Proto	-9	25	3	52.16	6.24	18.62	79.41	-1.76	11.18	85.81	0.84	14.13
2002	Vinton 81	40	75	6	41.95	7.43	13.61	52.43	-1.30	4.43	N/A	N/A	N/A
2002	IA2032 LS	40	75	6	42.31	7.66	14.34	53.33	-1.39	4.90	N/A	N/A	N/A
2002	Proto	40	75	6	42.25	7.77	14.65	53.88	-1.40	3.61	N/A	N/A	N/A
2002	Vinton 81	40	32	6	48.31	7.69	16.50	73.39	-1.26	9.83	81.44	1.36	13.65
2002	IA2032 LS	40	32	6	47.76	8.13	17.08	66.79	-1.28	8.13	83.62	1.30	13.75
2002	Proto	40	32	6	48.52	8.35	18.27	71.19	-0.98	9.06	78.83	0.78	11.51
2002	Vinton 81	30	75	6	50.76	7.81	18.88	73.65	-2.27	8.61	83.64	0.72	14.10
2002	IA2032 LS	30	75	6	49.07	7.58	17.18	76.71	-2.38	9.54	85.65	0.65	14.01
2002	Proto	30	75	6	50.83	7.18	18.10	73.64	-1.79	8.24	83.12	0.89	12.88
2002	Vinton 81	30	32	6	51.38	7.72	17.74	75.29	-2.44	9.47	83.33	-0.11	13.14
2002	IA2032 LS	30	32	6	49.58	6.41	16.02	75.87	-2.39	9.38	85.37	0.30	13.26
2002	Proto	30	32	6	49.55	7.66	18.44	71.65	-2.00	7.99	83.01	0.22	12.80
2002	Vinton 81	20	75	6	53.12	6.18	17.98	72.50	-3.03	8.90	84.47	-0.32	14.28
2002	IA2032 LS	20	75	6	50.51	6.84	17.14	72.89	-2.64	8.51	82.61	-0.43	12.79
2002	Proto	20	75	6	52.31	6.68	18.64	73.54	-2.40	8.31	84.27	-0.24	12.70
2002	Vinton 81	20	32	6	55.16	6.20	19.03	63.90	-2.35	7.54	83.51	-0.38	13.99
2002	IA2032 LS	20	32	6	55.29	5.62	17.74	65.28	-2.27	7.22	84.54	-0.31	13.75
2002	Proto	20	32	6	51.69	6.68	18.59	72.14	-2.27	8.16	84.70	-0.21	13.14
2002	Vinton 81	-9	25	6	56.11	5.97	19.67	71.18	-2.93	8.96	81.15	-1.19	12.71
2002	IA2032 LS	-9	25	6	54.93	6.10	18.18	74.66	-2.74	9.00	84.78	0.01	13.70
2002	Proto	-9	25	6	51.46	6.69	19.41	68.31	-2.31	7.08	83.30	-0.30	12.92

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Soybean			Soymilk			Tofu		
					L	*a*	*b*	*L*	*a*	*b*	*L*	*a*	*b*
2003	Vinton 81	0	0	0	52.49	5.40	17.11	72.58	-2.85	9.79	85.63	-0.21	15.72
2003	IA2032 LS	0	0	0	54.79	6.32	19.88	75.91	-2.65	10.80	86.20	0.35	16.36
2003	Proto	0	0	0	54.42	6.01	18.20	78.55	-2.25	9.56	84.91	0.47	13.30
2003	Vinton 81	40	75	3	54.27	6.86	17.41	70.19	-1.85	9.87	82.10	1.44	16.86
2003	IA2032 LS	40	75	3	47.07	7.18	16.90	76.11	-1.59	12.57	81.39	0.85	15.53
2003	Proto	40	75	3	47.57	8.34	17.92	72.67	-1.42	9.03	82.97	1.40	14.11
2003	Vinton 81	40	32	3	48.39	7.82	17.41	78.54	-2.37	12.20	83.83	0.20	15.05
2003	IA2032 LS	40	32	3	54.50	5.84	18.08	73.67	-2.03	10.84	84.65	0.85	16.43
2003	Proto	40	32	3	48.90	8.34	18.15	65.89	-1.66	7.24	85.27	0.57	13.04
2003	Vinton 81	30	75	3	52.84	6.09	18.29	78.46	-2.86	12.26	87.73	-0.51	15.83
2003	IA2032 LS	30	75	3	53.00	6.14	18.27	69.51	-2.00	10.13	81.65	0.18	15.37
2003	Proto	30	75	3	55.94	7.21	19.07	65.63	-1.84	7.17	85.90	0.63	13.31
2003	Vinton 81	30	32	3	53.90	5.38	17.44	73.69	-2.76	10.39	84.64	-0.28	15.13
2003	IA2032 LS	30	32	3	54.40	6.12	19.17	58.02	-1.64	8.56	85.81	0.25	15.98
2003	Proto	30	32	3	53.51	6.84	18.96	65.04	-1.87	6.81	85.61	0.69	13.05
2003	Vinton 81	20	75	3	53.68	4.90	16.72	67.10	-2.68	8.71	86.78	0.22	16.79
2003	IA2032 LS	20	75	3	53.73	5.15	17.86	70.76	-2.40	10.00	83.88	-0.26	15.28
2003	Proto	20	75	3	48.48	6.48	17.69	70.71	-2.29	7.22	84.51	0.14	13.30
2003	Vinton 81	20	32	3	51.17	4.54	18.32	78.28	-3.02	11.51	86.33	-0.22	16.44
2003	IA2032 LS	20	32	3	55.90	5.48	18.84	73.61	-2.59	10.39	84.88	-0.45	15.35
2003	Proto	20	32	3	46.59	7.14	17.21	76.70	-2.27	8.98	84.01	0.88	14.76
2003	Vinton 81	-9	25	3	53.30	5.33	18.72	71.43	-2.87	9.66	86.72	-0.30	15.49
2003	IA2032 LS	-9	25	3	59.17	4.91	19.48	78.92	-2.91	11.69	83.74	-0.14	15.27
2003	Proto	-9	25	3	53.78	5.68	17.89	73.65	-2.47	8.19	85.49	0.75	13.87
2003	Vinton 81	40	75	6	38.53	7.08	14.24	56.81	-0.47	8.20	80.87	1.73	14.44
2003	IA2032 LS	40	75	6	46.35	7.31	16.15	70.05	-0.66	11.19	80.60	2.13	15.91
2003	Proto	40	75	6	46.70	7.59	16.92	66.90	-0.59	8.53	79.29	2.26	13.45
2003	Vinton 81	40	32	6	53.07	6.70	18.26	65.68	-1.69	8.79	81.42	0.71	14.73
2003	IA2032 LS	40	32	6	52.77	7.42	18.73	70.57	-1.60	10.28	84.18	0.74	15.09
2003	Proto	40	32	6	48.02	8.39	18.31	64.64	-1.28	7.10	82.65	1.31	13.15
2003	Vinton 81	30	75	6	51.60	7.04	17.96	67.45	-2.14	9.47	85.20	0.37	15.51
2003	IA2032 LS	30	75	6	52.80	7.29	19.03	66.87	-1.78	9.73	83.60	0.44	15.33
2003	Proto	30	75	6	50.11	6.35	17.36	64.34	-1.80	6.60	85.29	0.68	12.83
2003	Vinton 81	30	32	6	53.80	5.71	17.66	74.11	-2.62	10.13	84.13	0.30	16.44
2003	IA2032 LS	30	32	6	54.85	5.95	18.32	66.21	-1.91	9.21	81.94	0.32	15.87
2003	Proto	30	32	6	52.76	6.94	18.65	63.13	-1.73	6.83	80.36	0.15	11.50
2003	Vinton 81	20	75	6	53.67	4.71	17.20	69.11	-2.61	9.16	84.15	-0.20	15.04
2003	IA2032 LS	20	75	6	54.50	5.25	17.52	66.31	-2.06	9.21	85.44	0.53	16.15
2003	Proto	20	75	6	53.46	5.49	17.38	70.72	-2.27	7.69	85.18	0.28	13.49
2003	Vinton 81	20	32	6	54.62	5.05	18.44	76.97	-2.89	10.93	86.06	-0.14	15.17
2003	IA2032 LS	20	32	6	54.28	4.98	17.74	75.38	-2.58	10.80	85.53	0.27	15.75
2003	Proto	20	32	6	52.62	6.22	18.33	72.98	-2.28	7.74	86.05	0.59	12.98
2003	Vinton 81	-9	25	6	59.89	4.25	17.78	76.71	-2.98	10.87	85.97	-0.35	15.34
2003	IA2032 LS	-9	25	6	55.37	5.93	18.62	74.38	-2.76	10.05	84.31	0.27	16.04
2003	Proto	-9	25	6	49.71	6.94	18.56	68.16	-2.36	6.89	85.98	0.60	13.51
2003	Vinton 81	40	75	9	45.48	6.38	14.77	64.35	-0.52	8.75	76.66	2.68	15.23
2003	IA2032 LS	40	75	9	38.84	7.49	14.34	66.39	-0.27	10.16	74.48	2.70	15.50
2003	Proto	40	75	9	43.99	8.36	15.94	63.17	-0.32	7.88	75.69	2.60	13.29
2003	Vinton 81	40	32	9	52.57	6.43	18.03	73.90	-2.10	9.95	83.06	0.62	14.71
2003	IA2032 LS	40	32	9	52.43	7.26	18.80	70.75	-1.94	9.40	82.17	0.71	14.51
2003	Proto	40	32	9	48.19	8.49	18.19	72.33	-1.86	7.51	83.08	1.06	12.80
2003	Vinton 81	30	75	9	52.05	6.48	17.62	71.01	-2.02	9.35	82.50	1.06	15.01
2003	IA2032 LS	30	75	9	52.05	6.80	18.06	65.82	-1.55	9.39	81.91	1.11	15.85
2003	Proto	30	75	9	53.46	7.10	18.45	72.63	-1.73	8.13	81.07	0.48	11.29
2003	Vinton 81	30	32	9	50.79	5.86	17.07	72.92	-2.54	9.72	84.11	0.20	14.88

Crop year	Soybean cultivar	Temperature (°C)	Relative humidity (%RH)	Storage time (months)	Soybean			Soymilk			Tofu		
					L	*a*	*b*	*L*	*a*	*b*	*L*	*a*	*b*
2003	IA2032 LS	30	32	9	56.57	5.99	18.73	76.98	-2.42	11.29	83.25	0.41	15.17
2003	Proto	30	32	9	47.17	7.60	17.83	72.81	-2.29	7.35	84.22	0.40	11.60
2003	Vinton 81	20	75	9	54.61	5.64	17.97	78.55	-2.97	11.95	85.73	0.40	16.94
2003	IA2032 LS	20	75	9	56.14	4.48	17.28	75.46	-2.52	11.02	84.77	0.32	15.70
2003	Proto	20	75	9	47.69	6.58	17.36	75.76	-2.35	7.75	84.59	1.02	12.60
2003	Vinton 81	20	32	9	53.71	5.20	17.82	75.90	-2.88	10.09	84.91	-0.40	14.48
2003	IA2032 LS	20	32	9	55.18	5.77	18.62	76.16	-2.80	10.09	82.45	-0.23	14.20
2003	Proto	20	32	9	54.85	5.94	17.62	77.66	-2.31	8.96	81.18	0.11	12.72
2003	Vinton 81	-9	25	9	53.13	4.69	16.97	78.06	-2.95	11.28	83.50	-0.77	14.09
2003	IA2032 LS	-9	25	9	54.18	5.88	18.40	78.38	-2.80	11.55	83.96	-0.23	15.55
2003	Proto	-9	25	9	53.65	6.34	18.30	74.77	-2.49	8.65	85.65	0.42	13.54
2003	Vinton 81	40	75	12	41.95	8.10	15.13	66.51	-0.37	9.72	75.17	3.30	16.50
2003	IA2032 LS	40	75	12	45.81	7.46	16.47	68.06	-0.43	11.12	73.68	2.69	15.86
2003	Proto	40	75	12	41.00	8.53	15.56	66.00	-0.55	8.34	76.40	2.98	14.08
2003	Vinton 81	40	32	12	35.55	7.17	13.84	75.06	-2.16	9.86	84.83	0.95	14.33
2003	IA2032 LS	40	32	12	50.73	7.92	18.46	72.38	-1.77	10.04	81.85	1.41	15.76
2003	Proto	40	32	12	44.20	8.02	17.02	73.56	-1.51	8.49	83.56	1.51	13.06
2003	Vinton 81	30	75	12	48.83	5.76	15.19	70.50	-1.74	8.94	82.55	1.16	14.40
2003	IA2032 LS	30	75	12	50.05	6.84	17.35	70.87	-1.54	10.54	82.02	1.71	16.61
2003	Proto	30	75	12	46.20	7.50	16.77	70.91	-1.49	7.87	81.64	1.47	13.41
2003	Vinton 81	30	32	12	53.23	7.54	19.40	75.80	-2.75	9.79	84.83	0.40	15.50
2003	IA2032 LS	30	32	12	54.80	6.59	18.42	74.41	-2.48	9.89	83.81	0.21	15.45
2003	Proto	30	32	12	50.65	6.78	17.99	77.16	-2.37	8.59	82.65	0.41	12.39
2003	Vinton 81	20	75	12	52.11	6.72	19.09	74.24	-3.02	10.93	86.44	0.26	17.28
2003	IA2032 LS	20	75	12	52.18	6.51	18.67	73.54	-2.51	10.52	85.59	0.58	15.98
2003	Proto	20	75	12	54.85	5.54	17.43	73.37	-2.21	7.72	84.72	0.84	13.58
2003	Vinton 81	20	32	12	49.50	6.06	17.74	75.96	-2.80	10.01	87.13	0.19	15.75
2003	IA2032 LS	20	32	12	56.29	5.43	18.99	75.25	-2.68	10.03	86.33	0.61	16.18
2003	Proto	20	32	12	50.97	6.80	17.90	76.87	-2.41	8.63	86.21	0.66	14.25
2003	Vinton 81	-9	25	12	57.00	4.55	17.92	78.65	-3.10	11.10	86.48	-0.19	16.75
2003	IA2032 LS	-9	25	12	53.10	5.77	17.59	76.40	-2.78	10.72	85.58	0.14	16.46
2003	Proto	-9	25	12	52.04	5.80	16.56	71.84	-2.29	8.05	86.62	0.62	13.97

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