Yield and Quality of Soft Tofu As Affected by Soybean Physical Damage and Storage†

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Damaged soybeans with 10% and 20% of splits and seedcoat cracks was stored in 85% relative humidity, 30 °C for up to 60 days. Samples were taken out at 15-day intervals for analysis and processing. Titratable acidity of soybeans increased significantly but protein extractability from soybean to soymilk decreased significantly with storage time. Soybeans with 20% split and 60 days of storage were not able to form tofu. Yield of tofu decreased significantly beyond 30 days of storage, and 20% split soybeans had a higher loss in yield than 10% split. The changes in color, hardness, and fracturability of tofu were significant ($p < 0.05$) upon prolonged storage of soybean. Relative amount of glycinin (11S protein) in soybeans increased as storage time increased for all treatments, but β-conglyciniin (7S) did not change significantly with storage time. Sensory evaluation showed significant off-flavor was produced in tofu made from soybeans stored for 45 days or longer at 85% RH and 30 °C.

Keywords: *Tofu; storage stability; soybean damage*

INTRODUCTION

After harvesting, soybeans are generally stored for a period of time before processing. However, prolonged storage of soybeans and undesirable environmental conditions during shipping may negatively affect the food quality. Various physical, chemical, and biochemical changes take place in soybeans during storage, depending on the storage conditions and storage time (Narayan et al., 1988a).

Yanagi et al. (1985) reported that acid values of soybeans increased from 0.13 mL to 0.90 mL (volume of 0.1 N KOH used to titrate 1 g of extracted oil) at 80% humidity and 30 °C after 10 months of storage, which resulted from the hydrolysis of neutral fat to form fatty acids. The nitrogen solubility index (NSI) decreased gradually with the prolonged storage at high humidity (Yanagi et al., 1985; Thomas et al., 1989; Murphy et al., 1997). Soybean storage conditions could influence the properties of soymilk and tofu. When soybean had been stored at 85% RH for 8 months, not only did protein extractability from soybean to soymilk decrease 14%, but also tofu made from stored soybeans became less uniform in microstructure (Thomas et al., 1989). The color of soymilk darkened, the pH of soymilk decreased slightly, and the hardness of tofu decreased at high temperature and high relative humidity of storage. Temperature and relative humidity cause deterioration of soybean during storage, but relative humidity seems to be more important. Low humidity can effectively preserve the original bean qualities, including some enzyme activities, even at high temperatures (Saio et al., 1980).

As storage time of soybeans progressed under adverse temperature and relative humidity (RH), glycinin (11S) and β-conglycinin (7S) components of soy protein, which are the major storage proteins of soybeans, became difficult to extract; however, the extractability of glycinin and β-conglycinin were variety-dependent (Saio et al., 1982; Murphy et al., 1997). Whole soybeans were more resistant to deterioration during storage than soy meal; furthermore, full-fat meal deteriorated more rapidly than defatted meal (Saio et al., 1982). When soybeans were stored at 80% RH and 30 °C, the sulfhydryl content of protein decreased rapidly as storage time increased from 0 to 5 months and remained constant beyond 5 months, but the disulfide bond content increased as storage time increased (Hong, 1994). The sensory qualities of the soymilk and tofu made from the stored soybean decreased with the increase in storage time from 1 to 9 years under ambient conditions; temperature ranged from 16 to 40 °C and relative humidity from 50 to 90% (Narayan et al., 1988b).

According to U.S. grading standards for soybeans, the maximum split percent of U.S. No.1 and No. 2 grade soybean is 10% and 20%, respectively. Split decreases the market values of soybeans. Soybean seedcoat cracking is a common problem in harvest, drying, handling, and storage of soybean. Seedcoat cracking may make soybeans more susceptible to microbial attack, and may reduce the food quality of soybeans. However, there are no literature reports on the effects of splitting and seedcoat cracking on the stability of soybean quality during storage. The objective of this study was to evaluate the effect of soybean storage with different ratios of split and seedcoat cracking on raw soybean composition and tofu quality.

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† Published with the approval of the Director of the North Dakota Agricultural Experiment Station.

10.1021/jf980037r CCC: $15.00
Published on Web 11/19/1998

MATERIALS AND METHODS

Materials. Soybeans of the Proto cultivar (1994) used in this study obtained from Sinner Brothers & Bresnahan Company (Casselton, ND). Soybean was harvested in late October of 1994. The growing conditions in 1994 were good-to-excellent. The Casselton soil type was silty clay loam with a medium soil texture suitable for soybean cultivation. The only fertilizer applied during cultivation was monomonium phosphate, which had an N:P of 11:52 (w/w). After harvest, soybeans were stored in cool ambient conditions for approximately two weeks. Soybean samples were stored in the freezer (−18 °C) after they were obtained from the bean company.

The antifoaming agent used in tofu processing, containing 89.5% glycerol fatty acid ester, 8% lecithin, 2% $MgCO_3$, and 0.5% silicon resin, was obtained from Koah Co. (Wakayama, Japan). The coagulant used was food-grade modified nigari (Ca^{2+} 14.73 g/100 g dry basis; Mg^{2+} 0.22 g/100 g dry basis), obtained from Taiwan Salt Workers (Tainan, Taiwan).

Mechanical Damage of Soybeans. Two levels of split soybean, 10% and 20%, and two levels of seedcoat cracking, 10% and 20%, were used as damage treatments of soybean. The untreated whole soybean, less than 1% split and 7% seedcoat cracking, was used as the control group of the damage treatment.

For preparing split soybean, whole soybean was treated with a grinder (Straub, Model 4E Grinding Mill, Straub Co., Philadelphia, PA) which can allow the distance between the two grinding plates to be adjusted and can be screened by 0.1378 in. meshes. The split soybean was mixed with the whole bean to adjust the ratio of split beans to 10% and 20%.

For preparing seedcoat-cracked soybean, whole soybean was treated in a soymilk extractor (Chang-Seng Mechanical Company, Taoyuan, Taiwan), in which the lower grinding stone was removed to cause damage in seedcoat, then screened by a special mesh (mesh size 4 mm × 19 mm) to select only the seedcoat-cracked samples. Whole soybean was mixed with seedcoat cracked soybean to adjust the ratios of seedcoat-cracked bean to 10% and 20%.

Soybean Storage Conditions. Before starting the storage experiment, bean samples were pretreated to increase the moisture content from the original 8.7% to 16% which is equivalent to 85% RH, 30 °C (Iglesias and Chirife, 1982). The samples, including damaged and control soybeans, were spread on perforated metal trays on a shelf in a chamber (Thermolyne, Model CN-A8005M, Sybron Co., Dubugue, IA) with water vapor at 25 °C for 24−36 h. The samples were weighed periodically. As the moisture content reached 16%, the beans were hermetically sealed in metal cans and stored at 4 °C for 2 weeks for equilibration. All samples including damaged and control soybeans were stored in an incubator at 30 °C for up to 60 days. Samples were taken out at 15-day intervals for various analyses and processed to produce soymilk and soft tofu.

Preparation of Soymilk and Soft Tofu. Soymilk and soft tofu were prepared according to Shih et al. (1997). Soybeans were soaked (8 h at 22 °C), ground in water (bean-to-water ratio about 1:6), and filtered to separate residues from soymilk. The solid content of soymilk was adjusted to 12 °Brix, using a refractometer (Auto Abbe Refractometer, Model 10500, Leica Company, Buffalo, NY). A 4.5-L sample of soymilk was placed in a stainless steel pot and heated to 95 °C with constant stirring and maintained for 5 min, then cooled to 87 °C. A coagulant suspension, containing 13.05 g (0.29% of soymilk volume) modified nigari in 130 mL of distilled water, was poured into soymilk while it was being stirred at speed 9 (equivalent to 285 rpm) of a stirrer (Model RZR1, Caframo LTD, Wiarton, Ontario, Canada) equipped with a paddle (7 cm × 7 cm) fixed at 4 cm from the bottom of the pot. A baffle (25 cm × 6 cm) was placed against the pot to increase turbulence of the flow. After 10 s, the mixture was poured immediately into a muslin cloth-lined wooden mold (25 × 25 × 7 cm), which was lined with a plastic film. After coagulation (10 min), the plastic film was removed and the cloth was folded over the top. The curd was pressed at 21.8 g/cm^2 for 10 min, increasing to 43.6 g/cm^2 for 10 min, and increasing to 65.4 g/cm^2 for another 30 min to separate whey from curd. The weight of freshly formed tofu was recorded. The tofu was cooled, stored at 4−5 °C for 24 h, and analyzed for textural properties. The tofu yield was expressed as grams of tofu/100 grams of raw soybeans.

Determination of Textural Properties of Tofu. The textural properties were measured using an Instron Universal Testing Machine (Model 1011, Instron Corporation, Canton, MA). Cylindrical samples (5-cm diameter × 1.5-cm height) were cut from the central portion of tofu cake with a stainless steel cylindrical cutter. Four samples were taken from the center of each tofu, and only the middle part of each cylindrical sample was used, because the middle part had a homogeneous texture. A cylindrical plunger with 5-cm diameter and a weight beam of 5 kg were used. The speed of the crosshead and the recording chart was set at 20 mm/min. The plunger traveled into the tofu sample 75% of its depth. Textural properties, including hardness and fracturability, were calculated from the curve according to Bourne (1978). Fracturability was defined as the force of the significant break on the first bite. Therefore, the higher the force required breaking the tofu; the lower fracturability was the tofu. Hardness was defined as the height of the force peak in the first compression cycle, which was the force necessary to attain a given deformation.

Proximate Chemical Analysis. Samples of soymilk and tofu were freeze-dried. The soybean and freeze-dried soymilk and tofu were ground with a Tekmar (A-10) Analytic Miller and passed through a 60-mesh sieve. Moisture content was measured by a vacuum oven method (AOAC 925.09, 1990). The crude protein content was determined by the Kjeldahl method (AOAC 955.04, 1990) using the factor of 6.25 to convert nitrogen to protein. Lipid content was determined by the Soxhlet extraction method (AOAC 945.39, 1990). Ash content was determined according to the AOAC method (AOAC 924.05, 1990).

Protein Extractability and Solid and Protein Recovery. Protein extractability was calculated from the amount of protein in soymilk dry matter divided by the amount of protein in soybean multiplied by 100%. Solid or protein recovery was expressed as the amount of solid or protein in tofu dry matter divided by solid or protein in soybean and multiplied by 100%.

Titratable Acidity of Soybeans. The method of determining titratable acidity was modified from the AOAC method (AOAC 935.57, 1990). A finely ground sample (2 g) was mixed with 50 mL of CO_2-free water,

Table 1. Titratable Acidity of Soybeans with Split and Seedcoat-Cracked Stored at 85% RH, 30 °C for Up to 60 Days[a]

	treatment				
day	10% split	20% split	10% seedcoat crack	20% seedcoat crack	control
0	$0.95^{e,(C)}$ (0.01)	$0.97^{d,(BC)}$ (0)	$0.98^{c,(B)}$ (0.01)	$1.00^{b,(A)}$ (0)	$0.94^{c,(D)}$ (0.01)
15	$0.99^{d,(B)}$ (0.01)	$1.06^{c,(A)}$ (0.01)	$1.04^{b,(A)}$ (0.02)	$1.03^{b,(A)}$ (0.01)	$0.98^{b,(B)}$ (0.02)
30	$1.03^{c,(ABC)}$ (0)	$1.06^{c,(AB)}$ (0)	$1.07^{b,(A)}$ (0.01)	$1.02^{b,(BC)}$ (0.02)	$1.01^{b,(C)}$ (0.02)
45	$1.08^{b,(B)}$ (0.02)	$1.12^{b,(A)}$ (0)	$1.08^{b,(B)}$ (0.02)	$1.03^{b,(C)}$ (0.01)	$1.10^{a,(AB)}$ (0.03)
60	$1.17^{a,(B)}$ (0.01)	$1.23^{a,(A)}$ (0.01)	$1.12^{a,(C)}$ (0.02)	$1.14^{a,(C)}$ (0.01)	$1.13^{a,(C)}$ (0)

[a] Expressed as means (SD), data are means of two replicate on a dry weight basis. (A–D): Means with different superscript within the same row are significantly ($p < 0.05$) different. a–e: Means with different superscript within the same column are significantly ($p < 0.05$) different.

shaking to dissolve completely, and titrated with 0.1 N NaOH. The titratable acidity was calculated as citric acid.

Glycinin (11S) and β-Conglycinin (7S) Content. A freeze-dried sample (1 g) was extracted with 30 mL of acetone to defat the sample. After centrifuging to remove the supernatant, the content was dried under a hood. Protein was extracted by 15 mL of 1% sodium dodecyl sulfate (SDS) solution containing 50 mM 2-mercaptoethanol. The protein content was determined by the Bradford method (Bradford, 1976). The extract was diluted with doubly distilled water to get a protein concentration of 2 mg/mL. A 0.5-mL portion of this protein extract was combined with 0.5 mL of sodium dodecyl sulfate (SDS) sample buffer containing 1% of 2-mercaptorethonal to produce the final protein concentration of 1 mg/mL, and heated in a boiling water bath for 2 min.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the method of Laemmli (1970). The separating gel gradient was between 8% to 16% acrylamide, and 40 μL of sample solution was loaded. For 11S and 7S protein quantification, the electrophoresis gel was scanned with a densitometric scanner (Model GS300, Hoefer Scientific Instruments Co., San Francisco, CA) and an Integrator (Model HP3396A, Hewlett Packard Co., St. Paul, MN). The ratio of 11S and 7S protein was calculated from the sum of the area of their subunits (Nagano et al., 1992).

Sensory Evaluation. The tofu made from stored, damaged soybean was evaluated with a sensory method, using a trained panel. Tofu samples for sensory evaluation were stored for 24 h in water at 4–5 °C after being formed and warmed to room temperature before evaluation. Six Oriental panelists, who were familiar with tofu products, were selected and trained to evaluate off-flavor and smoothness. Fresh tofu without off-flavor, made from the original whole soybean that was kept at room temperature (20–21 °C), was used as a reference of no off-flavor in each sensory evaluation. Several commercial products with various degree of smoothness, Hinoichi tofu (House Foods American Corp. Los Angeles, CA) and Morinu tofu (Morinaga Nutritional Foods Inc., Torrance, CA), and a tofu with a coarse treatment produced in our lab were used as reference for low smoothness. There were four training sessions for panelists to learn the sensory characteristics of no off-flavor and smoothness by using the reference samples. All samples were numbered randomly and evaluated two times on different days. A new batch of tofu was produced for each sensory evaluation.

Statistical Analysis. At each storage time, two batches of soymilk and tofu were produced and all chemical analysis were conducted in duplicate. Data were evaluated using the Statistical Analysis System program (SAS, 1985). General linear regression (GLM) was conducted, and Duncan's Multiple Range Test analyzed differences among the group means. Statistical significance was established at $p \leq 0.05$. Pearson's correlationship analysis was used to determine the apparent correlations among measurements.

RESULTS AND DISCUSSION

Chemical Analysis of Soybean. The chemical composition of storage soybeans with split and seedcoat cracking did not shown significant changes after 60 days of storage. Protein content ranged from 42.17 to 43.35%, which did not change significantly and was relatively constant upon storage time. Lipid content ranged from 16.32 to 16.81% and was similar regardless of damaged bean ratio and storage time. The total ash content ranged from 5.53 to 5.72%.

Titratable acidity of soybeans increased significantly ($p < 0.01$) with storage time for all treatments, and was significantly different among treatments for all storage times (Table 1). Remarkable increases of titratable acidity may have resulted from the hydrolysis of neutral lipid to fatty acids and the oxidation of the fatty acids during storage (Saio et al., 1980). The 20% split soybean had the highest titratable acidity after 30 days of storage. There was no significant difference in titratable acidity between 10% and 20% seedcoat cracking.

Protein Extractability of Soybean. Protein extractability of soybean stored at 85% RH and 30 °C decreased significantly with storage time regardless of the damaged ratio (Table 2). After storing for 45 days, the 20% split soybean had the lowest protein extractability. Soybean samples with higher damaged ratios had lower extractability of protein, especially for longer storage times. The titratable acidity of soymilk (average value from 1.06 to 1.22) was similar to that of the raw soybean and increased remarkably with storage time for all treatments. A negative correlation existed between protein extractability and titratable acidity ($r = -0.91$). Saio et al. (1980) reported that a decrease in pH caused a decrease in protein extractability during storage of soybean. However, Thomas et al. (1989) reported that the decreased protein extraction into the soymilk during bean storage could be attributed to reduced solubility, and the pattern of pH change did not reflect the trend of reduced protein extractability because the largest pH change was only 0.15 units (from 6.55 to 6.40). The results obtained in this study are consistent with the finding of Saio et al. (1980). The significant increase in titratable acidity suggested that the hydrolysis of neutral lipid and the oxidation of fatty acids might be increased with storage time. Protein and phosphates in beans served as pH buffers. That is why the decrease in pH was not significant (pH of soymilk

Table 2. Protein Extractability of Soymilk Made from Split and Seedcoat-Cracked Soybeans Stored at 85% RH, 30 °C for up to 60 Days[a]

	treatment				
day	10% split	20% split	10% seedcoat crack	20% seedcoat crack	control
0	80.9^{a} (2.3)	80.0^{a} (0.4)	79.1^{a} (0.1)	80.8^{a} (0.1)	81.4^{a} (2.1)
15	78.6^{a} (0.2)	76.8^{ab} (4.2)	79.6^{a} (1.6)	78.5^{ab} (1.2)	77.1^{ab} (0.2)
30	$78.2^{a,(A)}$ (0.5)	$76.5^{ab,(AB)}$ (0.3)	$74.8^{b,(B)}$ (1.4)	$75.0^{bc,(B)}$ (0.6)	$75.4^{ab,(B)}$ (1.0)
45	74.4^{b} (0.7)	72.8^{bc} (1.8)	73.8^{b} (1.5)	74.3^{c} (1.4)	74.9^{b} (3.0)
60	$73.3^{b,(A)}$ (0.1)	$68.0^{c,(B)}$ (0.1)	$71.7^{b,(A)}$ (0)	$71.7^{c,(A)}$ (1.4)	$71.6^{b,(A)}$ (0.6)

[a] Expressed as means (SD), data are means of two replicate on a dry weight basis. (A−B): Means with different superscript within the same row are significantly ($p < 0.05$) different. a−c: Means with different superscript within the same column are significantly ($p < 0.05$) different.

Table 3. Yield of Soft Tofu Made from Split and Seedcoat-Cracked Soybeans Stored at 85% RH, 30 °C for up to 60 days[a]

	treatment				
day	10% split	20% split	10% seedcoat crack	20% seedcoat crack	control
0	536.5^{a} (2.1)	536.5^{a} (3.5)	536.0^{a} (2.8)	536.0^{a} (2.8)	536.5^{a} (2.1)
15	538.0^{a} (7.0)	524.0^{ab} (8.5)	543.0^{a} (7.1)	533.5^{a} (0.7)	530.5^{a} (3.5)
30	517.5^{ab} (4.9)	510.0^{b} (2.8)	508.0^{b} (2.8)	507.5^{b} (2.1)	509.5^{b} (6.4)
45	$506.0^{b,(A)}$ (1.4)	$480.5^{c,(B)}$ (7.8)	$487.5^{c,(B)}$ (4.9)	$487.0^{c,(B)}$ (2.8)	$500.0^{b,(A)}$ (0)
60	435.0^{c} (17.0)	*b*	452.5^{d} (12.0)	458.5^{d} (3.5)	458.5^{c} (3.5)

[a] Expressed as means (SD), data are means of two replicate on a dry weight basis. (A−B): Means with different superscript within the same row are significantly ($p < 0.05$) different. a−d: Means with different superscript within the same column are significantly ($p < 0.05$) different. [b] No tofu produced due to poor coagulation property.

Table 4. Protein Recovery in Tofu Made from Split and Seedcoat-Cracked Soybeans Stored at 85% RH, 30 °C for up to 60 Days[a]

	treatment				
day	10% split	20% split	10% seedcoat crack	20% seedcoat crack	control
0	83.3^{a} (0.8)	81.7^{a} (1.4)	84.3^{a} (0.6)	81.4^{a} (0.5)	83.8^{a} (1.7)
15	$79.5^{ab,(A)}$ (1.8)	$76.0^{b,(B)}$ (0.6)	$76.1^{b,(B)}$ (0.5)	$78.4^{b,(AB)}$ (0.5)	$80.6^{b,(A)}$ (0.7)
30	$79.3^{ab,(A)}$(2.7)	$73.3^{c,(B)}$ (0.3)	$76.7^{b,(AB)}$ (1.3)	$76.2^{c,(A)}$ (0.4)	$78.6^{b,(A)}$ (0.4)
45	$78.4^{b,(A)}$ (0.8)	$74.2^{bc,(B)}$ (0.2)	$76.1^{b,(AB)}$ (0.8)	$76.7^{c,(AB)}$ (0.4)	$78.5^{b,(A)}$ (1.8)
60	$71.5^{c,(B)}$ (1.9)	*b*	$71.0^{c,(B)}$ (2.2)	$69.9^{d,(B)}$ (0.8)	$75.0^{c,(A)}$ (0.2)

[a] Expressed as means (SD), data are means of two replicate on a dry weight basis. (A−B): Means with different superscript within the same row are significantly ($p < 0.05$) different. a−d: Means with different superscript within the same column are significantly ($p < 0.05$) different. [b] No tofu produced due to poor coagulation property.

made from 20% split dropped from 6.55 to 6.28), but the increase in titratable acidity was significant.

Yield of Soft Tofu. Because the soymilk made from soybeans with a 20% split and 60 days of storage became highly viscous and colloidal after heating and cooling to 87 °C, partial coagulation occurred before mixing with the coagulant. Therefore, tofu gel was not able to be produced from 60 days stored, 20% split soybean. This phenomenon may be related to the significant increase in the titratable acidity of soymilk, which increased from 0.92 to 1.34. The acid may have contributed to the pregelation of soymilk prior to adding the coagulant.

For all damage treatments, yield did not change during the first 15 days of storage, but decreased significantly beyond 30 days. The effect of the damaged ratio of soybean on yield was not significant at first three storage periods, but it was significant after 45 days (Table 3). The weight of each fresh tofu ranged from 3731 to 3973 g and did not decrease significantly over the storage period, but the amount of the soybean used for producing 4.5 L of 12 °Brix soymilk for making tofu increased significantly with storage time (from 735 to 874 g). This result also indicated the decrease in soluble solid extractability of soybeans. Seedcoat-cracked groups also had a higher loss in yield as soybean was stored for 45 days at 85% RH and 30 °C. The control bean that was stored at 85% RH and 30 °C for 60 days had similar reduction in tofu yield as the damaged beans except that the 20% split did not form tofu gel. The loss of tofu yield might be due to the reduction in solid and protein extractability from soybean to soymilk. High positive correlation coefficient ($r = 0.90$) existed between yield of tofu and protein extractability of soymilk. Yield was significantly negatively correlated with titratability acidity of soybeans ($r = -0.87$).

Solid recovery from soybeans to tofu decreased from 65.5% to 57.7% with storage time up to 60 days regardless damaged ratio of soybeans. Protein recovery from soybean to tofu decreased progressively with the increase of storage time (Table 4). With the exception of the 10% split, the effect of damaged ratio on protein recovery was significantly beyond 15 days of storage. There was no statistical difference on protein recovery between the control and the 10% split group and between the 10% and 20% seedcoat cracking as storage time increased from 15 to 45 days. Soybean with a 20% split had the lowest protein recovery for the 15 to 45 days storage and was significantly different from the control and 10% split group. At the end of 60 days of storage, the soybeans without damage had the highest protein recovery and were significantly different from other groups. There were significant relationships between yield and protein recovery ($r = 0.81$) and between yield and solid recovery ($r = 0.90$). Narayan et al. (1988b) reported that the recovery of total solids and protein decreased 25.2% and 36.6%, respectively, in soymilk with the increase in the time of storage at ambient temperature, and the decreases were probably due to degradation of cellular membrane in soybean by

Table 5. Color Analysis of Soft Tofu Made from Split and Seedcoat-Cracked Soybeans Stored at 85% RH, 30 °C for up to 60 days^{a}

day	treatment				
	10% split	20% split	10% seedcoat crack	20% seedcoat crack	control
			Hunter *L* value		
0	87.46^{a} (0.08)	87.47^{a} (0.12)	87.50^{a} (0.28)	87.65^{a} (0.49)	87.49^{a} (0.01)
15	87.34^{a} (0.25)	87.54^{a} (0.02)	87.35^{a} (0.05)	87.51^{a} (0.28)	87.58^{a} (0.11)
30	87.13^{ab} (0.30)	86.76^{b} (0.14)	86.37^{b} (0.11)	87.01^{a} (0.20)	86.90^{b} (0.09)
45	86.78$^{b,(A)}$ (0.25)	87.00$^{b,(A)}$ (0.14)	85.86$^{bc,(B)}$ (0.29)	85.90$^{b,(B)}$ (0.55)	86.36$^{c,(AB)}$ (0.06)
60	84.91^{c} (0.02)	*b*	85.35^{c} (0.72)	85.99^{b} (0.99)	85.41^{d} (0.36)
			Hunter *a* value		
0	−0.27$^{b,(A)}$ (0.07)	−0.19$^{b,(A)}$ (0.01)	−0.71$^{c,(B)}$ (0.16)	−0.75$^{d,(B)}$ (0.08)	−0.36$^{c,(A)}$ (0.01)
15	−0.27^{b} (0.13)	−0.18^{b} (0.02)	−0.10^{b} (0.01)	−0.05^{c} (0.04)	−0.22^{c} (0.04)
30	−0.17$^{b,(B)}$ (0.07)	0.21$^{a,(A)}$ (0.07)	0.28$^{a,(A)}$ (0.05)	−0.09$^{c,(B)}$ (0.09)	0.17$^{b,(A)}$ (0.01)
45	0.26^{a} (0.06)	0.23^{a} (0.06)	0.24^{ab} (0.02)	0.17^{b} (0)	0.30^{b} (0.03)
60	0.52^{a} (0.15)	*b*	0.53^{a} (0.25)	0.45^{a} (0.12)	0.65^{a} (0.21)
			Hunter *b* value		
0	14.69^{b} (0.01)	14.74^{a} (0.10)	14.72^{a} (0.35)	14.66 (0.35)	14.69 (0.01)
15	14.21^{b} (0.09)	14.21^{b} (0.09)	14.01^{b} (0.12)	14.17 (0.08)	14.29 (0.01)
30	14.35$^{b,(B)}$ (0.01)	14.29$^{b,(B)}$ (0.08)	14.23$^{ab,(B)}$ (0.01)	14.56$^{(A)}$ (0.05)	14.33$^{(B)}$ (0.04)
45	14.23^{b} (0.09)	14.30^{b} (0.01)	14.59^{a} (0.13)	14.41 (0.18)	14.17 (0.19)
60	14.87^{a} (0.24)	*b*	14.60^{a} (0.12)	14.49 (0.11)	14.44 (0.35)

^{a} Expressed as means (SD), data are means of two replicate on a wet weight basis. (A−B): Means with different superscript within the same row are significantly ($p < 0.05$) different. a−d: Means with different superscript within the same column are significantly ($p < 0.05$) different. ^{b} No tofu produced due to poor coagulation property.

Table 6. Textural Properties of Soft Tofu Made from Split and Seedcoat-Cracked Soybeans Stored at 85% RH, 30 °C for up to 60 days^{a}

day	treatment				
	10% split	20% split	10% seedcoat crack	20% seedcoat crack	control
			Fracturability (g)		
0	1016.0^{b} (8.5)	1040.0^{a} (11.3)	1069.5 (27.6)	1058.0 (67.9)	1009.0^{ab} (65.1)
15	1104.5$^{a,(A)}$ (16.3)	1050.0$^{a,(AB)}$ (19.8)	1031.0$^{(B)}$ (19.8)	1015.0$^{(B)}$ (29.7)	1100.0$^{a,(A)}$ (18.4)
30	1021.5^{ab} (61.5)	1037.5^{a} (24.7)	1048.5 (60.1)	1044.0 (97.6)	1085.0^{a} (24.0)
45	1011.5^{b} (61.5)	980.0^{b} (2.8)	963.0 (32.5)	984.0 (31.1)	957.0^{bc} (28.3)
60	732.0$^{c,(B)}$ (24.0)	*b*	868.5$^{(AB)}$ (91.2)	972.0$^{(A)}$ (0)	887.0$^{c,(A)}$ (53.7)
			Hardness (g)		
0	683.5^{bc} (38.9)	604.5^{c} (0.7)	593.0^{c} (9.2)	635.5^{c} (36.1)	641.5^{c} (7.8)
15	647.5^{bc} (64.3)	702.5^{b} (20.5)	659.0^{bc} (17.0)	642.5^{bc} (16.3)	639.5^{c} (21.9)
30	620.0^{c} (24.0)	690.5^{b} (14.8)	718.0^{bc} (7.1)	702.0^{abc} (59.4)	680.0^{c} (8.5)
45	756.0^{b} (53.7)	807.0^{a} (17.0)	757.5^{ab} (51.6)	743.0^{ab} (46.7)	817.5^{b} (50.2)
60	1073.5$^{a,(A)}$ (33.2)	*b*	882.0$^{a,(B)}$ (111.7)	788.0$^{a,(B)}$ (15.6)	888.0$^{a,(B)}$ (1.4)

^{a} Expressed as means (SD), data are means of two replicate on a wet weight basis. (A−B): Means with different superscript within the same row are significantly ($p < 0.05$) different. a−c: Means with different superscript within the same column are significantly ($p < 0.05$) different. ^{b} No tofu produced due to poor coagulation property.

various phospholipases that caused leaching out of constituents into the soaking water.

Color Analysis of Tofu. Tofu made from each treatment showed significant changes ($p < 0.05$) in Hunter *L* and *a* values with respect to storage time (Table 5). The color of tofu did not change significantly in the first 15 days of storage. The results showed a decreasing tendency in lightness (*L* value) and an increase in the redness (*a* value) in tofu as storage time progressed for all treatments. There were no significant differences in color among treatments for most storage periods. Tofu color changed from cream yellow at the beginning to slightly brownish toward 60 days of storage. The phenomenon of color change was similar to that reported by Saio et al. (1980), Narayan et al. (1988a), and Thomas et al. (1989).

Narayan et al. (1988a) reported that the change in color of stored soybeans with increase in storage period was attributed to the occurrence of enzymatic and nonenzymatic browning reaction. Enzymatic browning may result from the reaction between oxygen and phenolic substrates. Nonenzymatic browning involved the Maillard reaction, interactions between proteins and reducing sugars, was hypothesized to be the main contributing factor to the browning reaction of soybean (Friedlander and Navarro, 1972). The changes in color of tofu were more remarkable than in color of soybean as heating enhances the Maillard reaction.

Textural Properties of Tofu. There were no significant differences among damage treatments in textural properties for most storage periods (Table 6). Fracturability of the tofu remained constant for the first 30 days of storage, but decreased significantly beyond 45 days of storage (Table 6). The soybeans stored at 85% RH and 30 °C for more than 30 days led to an increase in tofu fragility. In contrast to fracturability, hardness increased significantly beyond 45 days of storage. Saio et al. (1980) and Murphy et al. (1997) also observed the decreasing tendency in fracturability of the tofu made from stored soybean. The changes in hardness of the tofu from stored soybean of this study were similar to Thomas et al. (1989) who reported that the peak force of the tofu made from soybean stored at 85% RH increased significantly as storage time increased from 1 to 8 months; this increase was caused by the nonuniformity of the curd which could not hold the imbibed water. The results, however, were contrary to Saio et al. (1980), Hong (1994), and Murphy et al. (1997).

Table 7. Protein Components Analysis of Soybeans with Split and Cracked Seedcoat and Stored at 85% RH, 30 °C for up to 60 days[a]

	treatment				
day	10% split	20% split	10% seedcoat crack	20% seedcoat crack	control
			Glycinin (11S) (%)[b]		
0	37.77^{b} (0.89)	36.19^{b} (0.59)	39.41^{bc} (1.37)	37.88 (2.48)	35.44^{c} (0.95)
15	40.78^{a} (0.18)	36.81^{b} (0.28)	38.20^{c} (0.18)	38.11 (1.94)	37.12^{c} (1.36)
30	$38.64^{b,(BC)}$ (1.22)	$38.57^{b,(BC)}$ (0.37)	$40.29^{ab,(AB)}$ (0.64)	$40.87^{(A)}$ (0.77)	$37.87^{bc,(C)}$ (0.69)
45	40.85^{a} (0.98)	41.26^{a} (1.37)	41.86^{a} (0.39)	42.41 (0.18)	40.46^{ab} (1.45)
60	40.96^{a} (0.29)	41.00^{a} (1.33)	41.62^{a} (0.07)	41.67 (1.87)	42.19^{a} (0.20)
			β-Conglycinin (7S) (%)[b]		
0	20.80 (2.19)	19.95 (0.64)	21.34 (1.82)	20.90 (1.69)	19.15 (0.23)
15	20.45 (0.38)	19.81 (0.56)	19.72 (0.10)	19.95 (0.41)	19.04 (0.07)
30	$19.62^{(B)}$ (1.17)	$19.11^{(B)}$ (0.30)	$21.80^{(A)}$ (0.10)	$22.61^{(A)}$ (0.69)	$19.28^{(B)}$ (0.11)
45	19.85 (1.20)	20.05 (0.66)	21.06 (2.00)	19.93 (0.15)	21.38 (1.78)
60	19.41 (1.75)	21.03 (1.31)	20.35 (1.14)	20.04 (1.26)	19.95 (0.65)
			Ratio of 11S/7S		
0	1.83 (0.15)	1.82 (0.09)	1.86 (0.22)	1.83 (0.26)	1.85 (0.07)
15	2.00 (0.05)	1.86 (0.04)	1.94 (0.02)	1.92 (0.13)	1.95 (0.06)
30	1.98 (0.18)	2.02 (0.01)	1.85 (0.02)	1.81 (0.08)	1.97 (0.02)
45	2.06 (0.07)	2.06 (0)	2.00 (0.18)	2.13 (0.03)	1.90 (0.23)
60	2.12 (0.21)	1.96 (0.18)	2.05 (0.11)	2.09 (0.22)	2.12 (0.08)

[a] Expressed as means (SD), data are means of two replicate on a dry weight basis. (A−C): Means with different superscript within the same row are significantly ($p < 0.05$) different. a−c: Means with different superscript within the same column are significantly ($p < 0.05$) different. [b] Percent of total protein on a dry basis.

Table 8. Sensory Evaluation of Soft Tofu Made from Soybeans with Split and Cracked Seedcoat Stored at 85% RH, 30 °C for up to 60 days[a]

	treatment				
day	10% split	20% split	10% seedcoat crack	20% seedcoat crack	control
			Off-flavor[c]		
0	1.2^{a} (0.4)	1.4^{a} (0.5)	1.2^{a} (0.4)	1.4^{a} (0.5)	1.2^{a} (0.4)
15	1.4^{a} (0.5)	1.4^{a} (0.5)	1.4^{a} (0.5)	1.4^{a} (0.5)	1.2^{a} (0.4)
30	1.6^{a} (0.9)	1.8^{a} (1.1)	1.8^{a} (0.8)	2.2^{a} (1.3)	2.2^{a} (1.3)
45	3.2^{a} (0.4)	3.4^{a} (0.5)	2.8^{a} (1.1)	3.0^{a} (1.0)	2.8^{a} (0.8)
60	5.6^{b} (0.9)	*b*	5.2^{b} (0.8)	5.6^{b} (1.1)	5.4^{b} (0.5)
			Smoothness[d]		
0	6.8^{a} (0.4)	6.6^{a} (0.9)	6.8^{a} (0.4)	6.8^{a} (0.8)	6.8^{a} (0.9)
15	6.0^{a} (0.7)	5.3^{a} (1.0)	5.4^{a} (1.1)	5.9^{a} (0.9)	6.0^{a} (0.7)
30	5.6^{a} (1.3)	4.2^{a} (0.8)	5.0^{a} (1.6)	4.4^{a} (1.5)	5.2^{a} (0.8)
45	5.4^{a} (1.5)	5.0^{a} (1.4)	5.4^{a} (1.1)	4.6^{a} (0.9)	5.2^{a} (1.3)
60	3.8^{b} (0.8)	*b*	4.6^{b} (0.5)	4.6^{b} (1.1)	3.8^{b} (0.8)

[a] Expressed as means (SD), data are means of six panelists. a−c: Means with different superscript within the same column are significantly ($p < 0.05$) different. [b] No tofu produced due to poor coagulation property. [c] Off-flavor (1 = least and 7 = most). [d] Smoothness (1 = least and 7 = most).

Saio et al. (1980) reported that many changes of textural characteristics occurred in tofu made from stored soybean, such as decrease in hardness, loss in cohesion, increased fragility, off-flavor, and darkening in color of gel, and the decrease in hardness depended mainly on the decrease in the solid concentration of soymilk because of the decreased extractability into soymilk. In this study, all tofus were made from soymilk that was adjusted to 12 °Brix of solids. Changes in hardness of tofu could not be due to the solid concentration of soymilk, but would be due to the decrease of water-holding capacity of protein extracted from stored soybean. The interactions of proteins with lipids occurred in soybean, especially in soybean stored at high temperatures and high relative humidity, causing severe quality changes (Saio et al., 1980). The protein−lipid interactions may weaken the soy protein three-dimension network by decreasing the probability of protein−protein interactions, which was another possible factor causing the changes in tofu texture. The discrepancies in textural changes of tofu made from stored soybean also can be partly attributed to the methodologies used for making tofu. Different methodologies, used by different researchers, result in different tofu structures.

Composition of Soy Proteins. Many intra- and intermolecular binding forces are involved in the stabilization of the three-dimensional network gel structures. These forces include hydrogen bonding, hydrophobic associations, ionic interaction, and disulfide linkage (Utsumi and Kinsella, 1985). Ionic interactions and disulfide bonds were involved in the formation of 11S globulin gel; however, hydrogen bonding was important in 7S globulin gel formation. Fukushima (1991) reported that the 11S soy protein (glycinin) has 2 −SH groups and 20 S−S bonds per molecule; 7S protein (β-conglycinin) does not have −SH groups and has 2 S−S bonds per molecule.

There was a tendency toward an increase in relative amount of glycinin as storage time increased for all treatments (Table 7). No significant difference existed among treatments in relative amounts of glycinin for most of storage periods. The relative amount of β-conglycinin did not vary significantly as storage time increased for all treatments. The ratio of 11S/7S (ranged from 1.81 to 2.12) did not change significantly ($p > 0.05$)

regardless of treatments and storage time. The relative amount of 11S protein significantly correlated negatively with yield ($r = -0.74$), and positively with hardness of tofu ($r = 0.63$).

The relationships between 11S protein and hardness of tofu agreed with Saio et al. (1969) who reported that 11S gel was harder than 7S gel, and 11S protein greatly affected the hardness of tofu gels. Saio and Watanabe (1978) reported that the 11S gel had a higher water-holding capacity and higher hardness than 7S gel. There was no significant correlation between 7S protein and the texture. Saio et al. (1969) reported 11S/7S ratio in soymilk significantly related to textural properties of tofu made from six soybean varieties. Kang et al. (1991) also reported that the 11S/7S ratio related to texture of the tofu gel, especially on fracturability. Murphy et al. (1997) reported that the ratio of 11S/7S was significantly correlated with hardness and fracturability of tofu, but the relationship was not uniform for 7 soybean varieties. However, Taira (1990) summarized that the ratio of protein to lipid affected the hardness of tofu. No significant change was observed in the 11S/7S ratio in soybean cultivars. From that, the ratio did not correlate with the yield and hardness of tofu.

Sensory Analysis of Tofu. Sensory scoring showed no significant differences among treatments (Table 8). The effect of storage time on sensory quality was more important than that of the damaged soybean ratio. The analysis of variance showed the effect of storage time on both sensory qualities of off-flavor and smoothness were significant ($p < 0.001$), but the effect of damage treatments were not significant ($p > 0.05$). As soybean storage time increased to 60 days, the tofu made from stored soybeans became less smooth ($p < 0.05$). The off-flavor of tofu developed as soybean storage time increased. Soybean lipoxygenases causing oxidation of polyunsaturated lipids, lipid autooxidation, and volatile materials derived from Maillard reactions during bean storage might have played an important role in producing off-flavor in tofu. Clark and Snyder (1991) reported hydroperoxides, products of lipid oxidation during storage, broken down to form second volatile oxidation products, can result in the off-flavor formation in soy products. Chen (1993) evaluated seven soybean varieties which were stored at 84% RH and 35 °C for up to 110 day, and found that the overall sensory quality of tofu made from all varieties decreased with increase in storage time.

CONCLUSION

Yield of tofu decreased significantly beyond 30 days of storage; higher damage ratios caused greater losses in yield as soybean was stored at 85% RH and 30 °C. Tofu color changed from cream yellow at the beginning to slightly brownish toward 60 days of storage. Textural properties of tofu changed significantly upon prolonged storage of soybean. The relative amount of 11S protein in soybean increased as storage time increased for all treatments.

As soybean storage time increased to 60 days, tofu became darker in color, coarser in appearance, and harder in texture. The flavor of tofu made from damaged and whole soybeans deteriorated as soybean stored up to 45 days at 85% RH and 30 °C. Both U.S. No. 1 and No. 2 soybeans should not be stored at 30 °C and 85% RH for more than 30 days. Bean quality may be negatively affected during shipping and handling prior to tofu processing, particularly, if they are shipped to a country where the climate is hot and humid.

ACKNOWLEDGMENT

The technical assistance of Dr. M. C. Shih is acknowledged.

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Received for review January 15, 1998. Revised manuscript received September 28, 1998. Accepted October 8, 1998. This research was supported in part by the USDA-CSREES Great Plains International Trade Research Program, and by the Alternate Crops research program.

JF980037R