TECHNICAL

Soymilk and Tofu Properties as Influenced by Soybean Storage Conditions

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Soybeans were stored at two temperatures, 20°C and 30°C, and two relative humidities, 65% and 85%. The amount of protein extracted into soymilk decreased by about 14% of the initial extractability in all cases after eight months of storage. The decline in protein extractability could not be explained by decreases in pH, nor by loss of solubility of certain protein components. Tofu made from beans that were stored at 85% relative humidity became less uniform in microstructure toward the end of the storage period. The volume of whey produced increased with bean storage time.

The major use of soybeans is in the production of soybean oil and meal. However, there is a smaller but still important market for direct conversion of soybeans to products such as soymilk, tofu, natto and tempeh. The beans usually preferred for this type of application are referred to as edible soybeans which in most cases are of the light hilum type (1). The traditional soybean-based foods are popular in the Orient but have found increasing acceptance in other parts of the world. Developments in processing technology have made it possible to prepare soy foods in which the beany off-flavor has been effectively eliminated.

Soymilk is a food product in its own right and also serves as the intermediate in the production of tofu. Soybeans grown in Ontario have found acceptance in the edible soybean market. A number of factors influence the quality of soybeans for the production of soymilk and tofu. It has been popular knowledge that prolonged storage of soybeans negatively affects the quality of edible soybeans. This study was undertaken to establish the effect of storage conditions on edible soybean quality, with special emphasis on protein extractability.

MATERIALS AND METHODS

Soybeans. The soybeans used were of the Corsoy 79 variety grown at the Malden, Ontario growing station of Agriculture Canada. Before starting the storage experiment the beans were kept at cool temperatures (5–15°C) and low humidity. The storage experiment was started in April following the harvest in the fall. The beans were placed in cotton bags for storage under the required conditions. The composition (d.b.) of the beans was protein, 42.4%; fat, 19.5%; Ca, .33%; Mg, .40%; P, .60%; K, 1.74%, and fiber, 5.6%. Average weight of 100 seeds was 17.6 g.

Storage conditions. Two levels of humidity (85% and 65%) and two temperatures (20°C and 30°C) were used for a total of four treatment combinations. The required humidity was maintained in desiccators containing dilute sulfuric acid, 36.0% for 65% RH and 20°C; 36.4% for 65% RH and 30°C; 22.5% for 85% RH and 20°C; 22.9% for 85% RH and 30°C. The desiccators were kept in temperature-controlled chambers. There were two replicates for each treatment combination and two subsamples within each replicate for all tests except for tofu, where six subsamples were used. The beans were analyzed at the beginning of the storage test and after 1, 2, 3, 4, 6 and 8 mo. The beans were held at refrigeration temperature for 3.5 mo before the start of the experiment.

Statistical analysis. The experimental design was a split plot with the time factor as the sub-plot factor and temperature and humidity as the main factors. The split plot design was simplified to a three-factor completely randomized design when the error between bags of beans did not exceed the error within bags.

Moisture content of beans. Beans were dried in a vacuum oven at 130°C for three hr (AOCS method Ac-41).

Soymilk preparation. One hundred and fifty g of beans were washed in distilled water and were soaked for 16 hr at 20°C in 500 ml of distilled water. The soaked beans were drained, weighed and placed in a Waring blender with 375 ml distilled water and ground at high speed for four min. To this slurry was added 400 ml of boiling water. Additional water was added if necessary to make up differences in water imbibed during soaking. The soymilk was filtered by means of a juicer (Golden Harvest Majestic; Natural Sales Co., Pittsburgh, Pennsylvania). This consisted of a basket centrifuge lined with a fine mesh nylon cloth. The insoluble residue remaining on the cloth is known as okara.

Protein determination. The protein content of whole soybeans, okara, soymilk and tofu were determined by the Kjeldahl method using the factor 6.25 to convert nitrogen to protein.

SDS-page. Stacking gel—10 ml consisting of 3% acrylamide, 0.08% bis-acrylamide, 0.125 M tris-HCl (pH 6.8) and 0.1% SDS. Separating gel—40 ml containing 11% acrylamide, 0.29% bis-acrylamide, 0.375 M tris-HCl (pH 8.8). Electrode buffer—the electrode buffer consisted of 0.025 M tris, 0.192 M glycine and 0.1% SDS. Samples were prepared by diluting the soymilk eightfold and filtering through a 0.45 μm filter. Two hundred μl of this solution was made up to one ml containing 0.0625 M tris-HCl buffer (pH 6.8), 1% SDS, 0.2 M mercapto-ethanol and 0.001% bromophenol blue. The samples were incubated at 50°C for 30 min before loading 20-μl aliquots into the sample wells. The slab gels were run at a current of 20 mA for five hr.

Calcium and magnesium determinations. Calcium and magnesium were determined in soymilk, okara and whole soybeans using atomic absorption spectrometry.

Inorganic phosphorus. A soybean sample was washed with distilled water, drained and soaked in 100 ml of water for two hr at 5°C. The beans and soak water were then ground at high speed in a Waring blender for five min with an additional 50 ml of water. The blender was rinsed with 50 ml of distilled water and the extracts mixed together and filtered. The residue was then washed with 100 ml of distilled water, and this was added to the rest of the extract.

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Five ml of 50% trichloroacetic acid were added to 20 ml of soybean extract. This was then placed on a shaking water bath for 15 min, centrifuged and the supernatant decanted.

The inorganic phosphorus content was then determined colorimetrically. The following were added to a test tube in the order listed: one ml sample, one ml 1% ammonium molybdate, three ml 0.2 N sodium acetate buffer pH 4.3, one ml 1% ascorbic acid. The absorbance at 660 nm was read after 20 min. Phosphorus concentration was derived from a series of potassium phosphate standards ranging from 0–4 μmol/ml of phosphorus.

Preparation of the curd. Two-hundred and twenty five ml of soymilk was heated to 100°C with continuous stirring. This milk and five ml of a calcium sulphate suspension, to produce a final coagulant concentration of 0.9%, were simultaneously poured into a container to achieve proper mixing. The mixture was then quickly stirred and left to coagulate and stored in the refrigerator overnight.

Texture analysis of curd. Curd texture was evaluated using a computerized Instron Universal Testing machine (2). A curd knife was used to penetrate the center of each tofu sample to a preset depth with a crosshead speed of one cm/min.

Microstructure of tofu. Microstructure of tofu was determined by scanning electron microscopy (SEM) (2). Fixation was carried out with osmium tetroxide. Samples were sputter-coated with 60:40 gold palladium and observed at 15KV in a model S570 Hitachi scanning electron microscope.

Volume of whey expelled. The tofu was placed in a cheese cloth and subjected to a pressure of 18.5 g/cm^2 for 10 min. The volume of whey expelled was measured.

Phytate analysis. Phytate extract of the soybeans was made by using the procedure of Harland and Oberleas (3). The beans were finely ground and extracted with 0.65N HCl. Phytate in the extract was determined colorimetrically (4).

Color of soymilk. The color of soymilk was measured using a Hunterlab color difference meter and expressed as L, a and b units (Hunterlab, Fairfax, Virginia).

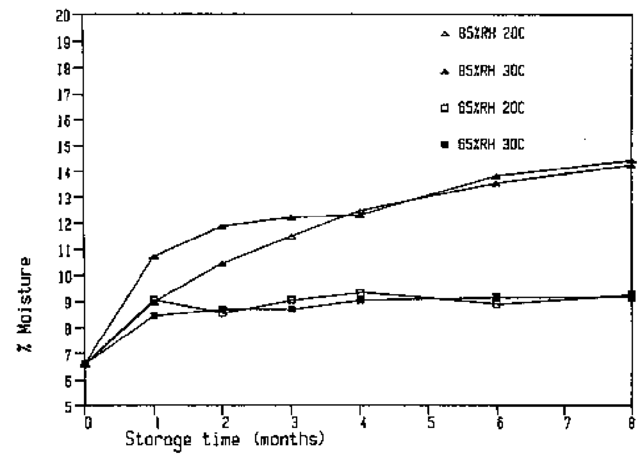

FIG. 1. Moisture content of soybeans during storage.

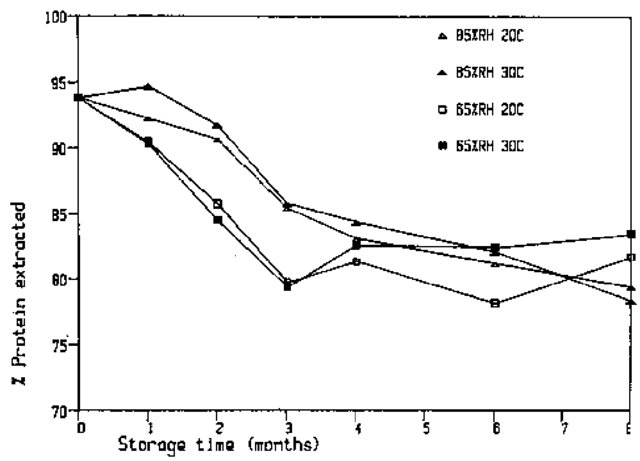

FIG. 2. Protein extractability from soybeans as influenced by storage.

RESULTS AND DISCUSSION

The change in moisture content of the soybeans stored under the four combinations of humidity and temperature is illustrated in Figure 1. Protein extractability decreased with storage time (Fig. 2). Relative humidity significantly influenced protein extractability ($P < 0.01$). The time × relative humidity interaction was also significant at the 1% level. Temperature had a significant ($P < 0.01$) effect on protein extractability. The magnitude of this effect at each humidity level was small but consistent. Temperature showed no interaction with any of the other factors. The beans stored at 65% RH suffered a more rapid decline in extractability during the initial months of storage. Beyond the third month the extractability remained constant. The beans stored at 85% RH suffered a slower but continuous decline in protein extractability. Other researchers (5–7) have also found that protein solubility in soybeans is influenced by temperature and relative humidity. Reported losses were higher than found in the present study. More than 50% loss was reported (5) after six mo storage at 35°C and 80% RH. The unextracted protein remains in the okara and tests were conducted to establish how tightly this protein was bound. For this purpose the okara remaining on the filter pad in the centrifuge basket was washed with four one-liter volumes of distilled water. The residue was then dried and weighed and the protein content determined (Table 1). The difference between the protein content of the beans and the combined protein content of soymilk and okara was taken as the amount of protein lost in the wash water. No correction was made for possible loss of nitrogenous substances in the soak water. The calculated percentages of protein removed by washing the okara are listed in Table 2. It was found that the decreased protein extraction into the soymilk during bean storage could be attributed virtually entirely to reduced solubility.

It has been suggested (5) that the decrease in protein solubility during storage of soybeans is caused by pH changes. The results obtained in the present study do not support this hypothesis. The largest pH change for any of the storage treatments was a decrease of 0.15 units (from 6.55 to 6.40) at 30°C and 85% RH (Fig. 3). The pattern of pH change did not reflect the trend of reduced

TABLE 1

Quantity and Protein Content of Okara Recovered from Soymilk Made from Stored Soybeans

	85% RH	20°C	85% RH	30°C	65% RH	20°C	65% RH	30°C
Month	g[a]	%[b]	g	%	g	%	g	%
1	20.66	2.16	20.54	1.89	20.65	2.17	20.75	2.52
2	20.53	2.04	20.91	2.35	21.34	2.20	21.63	2.24
3	21.02	2.23	20.99	2.36	23.13	2.64	22.74	2.60
4	21.82	2.42	22.72	2.72	21.82	2.86	23.14	2.90
6	22.94	2.86	23.53	2.92	24.07	3.05	24.36	2.96
8	24.52	2.93	24.46	3.14	24.48	3.05	24.73	3.08

[a]Weight of okara

[b]Protein content of okara as percentage of total protein of soybean.

TABLE 2

Protein Removed from Okara by Repeated Washing with Water, Expressed as Percentage of Total Protein in the Soybeans

	85% RH 20°C	85% RH 30°C	65% RH 20°C	65% RH 30°C
Month	%	%	%	%
1	5.58	3.38	7.35	6.47
2	7.70	5.87	12.04	13.25
3	12.70	12.04	17.65	18.07
4	14.57	12.90	18.10	15.99
6	16.05	14.71	18.91	14.73
8	17.77	18.51	15.32	13.51

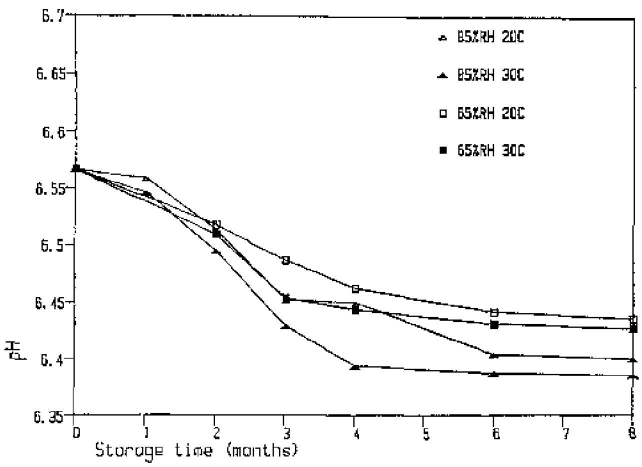

FIG. 3. The pH values of soymilk made from stored soybeans.

protein extractability. Between pH 6.2–6.4 at low temperatures the solubility of the 7S globulin is very high whereas the solubility of the 11S globulin is low (8). The insolubility of glycinin in this pH range is highly dependent on ionic strength. A change in ionic strength of the medium from 0.06 to 0.1 is accompanied by a marked increase in solubility of the glycinin fraction. Iwabuchi and Yamauchi (9) estimated the ionic strength of soybeans extracted with 5–10 volumes of water using immunoelectrophoretic techniques to be between .2 and .1. In this work beans were extracted with six volumes of water; consequently, the ionic strength of the soymilk would be in the upper regions of the range and a decrease in the solubility of glycinin would not be expected.

The polyacrylamide gels were run in dissociating media and as such demonstrate the presence of subunits rather than proteins with quaternary structure. Nomenclature and fractionation of soy proteins have been described in detail (10). There was a general but slight decrease in the density of protein bands with increasing storage time (Fig. 4). This is in agreement with the fact that the overall protein extractability was shown to decrease with increasing storage time (Fig. 2). The relative abundance of the subunits did not appear to change.

Another possible cause for the decreased solubility may be divalent cations combining with the soy proteins. An increase in the cation:anion ratio would indicate increased levels of cations capable of interacting with the proteins. Total extractable divalent cations did not change during storage (Table 3). The calcium content of the soymilk, however, decreased during bean storage at 85% RH (Table 4). The inorganic phosphorus content of the beans increased with storage time, especially at high relative humidity (Fig. 5). Analysis of variance showed the interaction to be significant. Increased inorganic phosphorus must be the result of breakdown of other forms of phosphorus. Phosphorus in soybeans is present as phytate P (75–80%), phospholipids (12%), and inorganic P (5%), as proposed by Smith and Circle (11). Lolas et al. (12) reported that phytic acid P was 53% of

TABLE 3

Total Calcium and Magnesium in Soymilk and Washwater, Expressed as Percentage of Total Calcium and Magnesium Content of Soybeans

	85% RH 20°C		85% RH 30°C		65% RH 20°C		65% RH 30°C	
Month	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
1	65.3	47.8	64.2	45.2	67.1	43.5	62.4	43.9
2	64.7	43.9	61.7	47.7	67.5	45.7	68.1	47.6
3	65.1	51.2	63.8	43.0	63.2	48.3	64.3	44.3
4	63.9	48.1	64.0	45.9	65.4	44.9	63.9	50.1
6	64.9	47.2	65.3	48.3	66.1	42.7	66.8	44.7
8	65.2	47.6	64.5	46.1	64.9	45.8	65.9	43.6

TABLE 4

Calcium and Magnesium Content of Soymilk, Expressed as Percentage of the Calcium and Magnesium Content of the Soybeans

	85% RH 20°C		85% RH 30°C		65% RH 20°C		65% RH 30°C	
Month	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
1	64.8	48.4	64.9	44.9	65.5	44.3	63.9	43.6
2	63.9	46.9	65.1	46.3	63.4	47.6	65.0	44.3
3	63.7	47.2	63.0	44.3	64.1	44.9	64.2	44.5
4	60.2	49.2	56.7	48.3	62.0	46.8	62.1	46.2
6	57.2	45.2	49.9	45.9	63.7	45.9	64.7	47.1
8	54.3	47.8	46.8	46.4	61.8	44.4	62.8	44.0

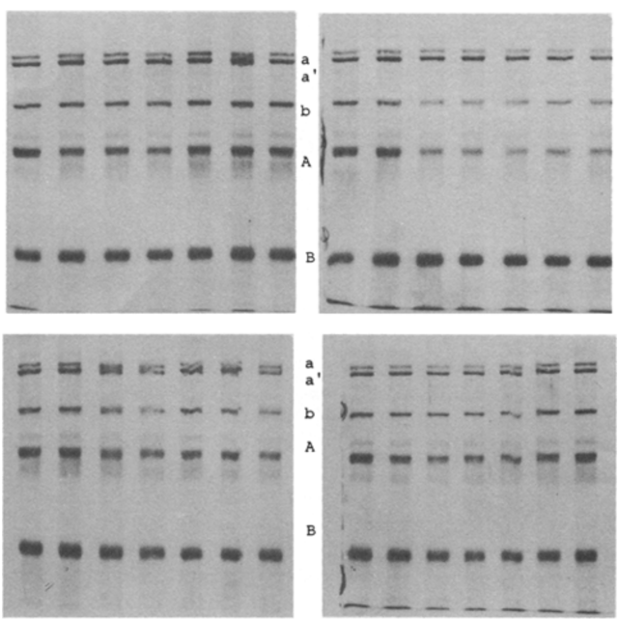

FIG. 4. Gel electrophoresis patterns of soymilk proteins made from stored soybeans (0, 1, 2, 3, 4, 6 and 8 mo). Upper left, 85% RH—20°C; upper right, 85% RH—30°C; lower left, 65% RH—20°C, lower right, 65% RH—30°C.

total phosphorus, based on the average of 15 samples. Phytic acid content was 1.14% of the dry weight. The increase in inorganic P was not likely to be caused by breakdown of phytate (Fig. 6). The variability of the

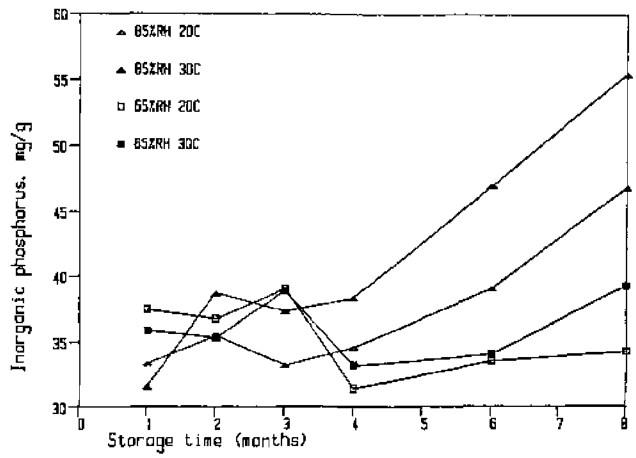

FIG. 5. Inorganic phosphorus content of soybeans during storage.

phytate data obscure any possible relationship between the two. A recent study (13) has demonstrated a loss of phosphorus in the form of phospholipids in crude oil extracted from soybeans stored at high temperature and humidity. Phospholipid content was not determined in this study.

Calcium may react with the increasingly available phosphate to form insoluble calcium phosphate. This would explain the decreased calcium content in the soymilk at 85% RH (Table 4). It does not explain the decrease in protein extractability unless calcium interacted with the protein instead.

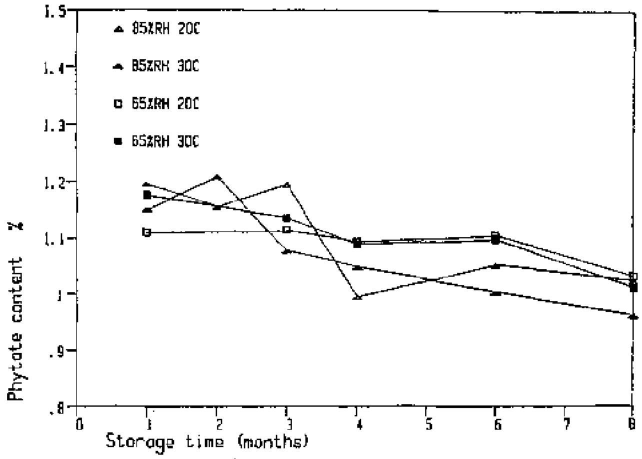

FIG. 6. Change in phytate content of soybeans during storage.

Krol (14) has demonstrated that calcium ion binding takes place in the pH region where side chain carboxyl and imidazole groups dissociate. Up to pH 6 the binding sites associated with the carboxyl groups are aspartic and glutamic acid residues. From pH 6–7 the imidazole groups act as calcium binding sites. Gel electrophoresis did not demonstrate major changes in acidic or basic subunits (Fig. 4).

In a study (1) of the composition and properties of soymilk and tofu made from light-hilum soybeans, the content of phosphorus, magnesium and potassium in soymilk solids was higher than in the soybeans. However, the calcium content was lower in the soymilk solids than in the beans, indicating that the extraction of calcium from soybeans is relatively more difficult.

The texture of tofu made from the stored beans is reported in Table 5 as peak force required for penetration with the curd knife. The beans stored at 85% RH and 20°C and 30°C showed significantly increased peak force as storage time increased. This increase was caused by the nonuniformity of the curd. In the case of beans stored at high humidity, the curd had a tendency to settle at the bottom of the container. The penetration curve showed a gradual increase indicating that the deeper the penetration the firmer was the curd. In the case of beans stored at 65% RH there was an initial increase of force at about 1/5 of the depth of penetration; then there was a plateau force which did not increase upon further penetration. The curd in the latter case was more uniform and was able to hold the imbibed water. This is also indicated by the volumes of whey that were expelled from the different tofu curds. More whey was expelled by curds made from beans stored at 85% RH (Table 6). The decreased water holding capacity of the gels can be explained by the decrease in protein content of the soymilk. There was a high negative correlation (−.92 to −.99) in each case between the protein content of the milk and the volume of whey expelled.

Scanning electron microscopy verified the nonuniformity of the top portion of the tofu made from 85% RH stored beans (Fig. 7). SEMmicrographs of the bottom

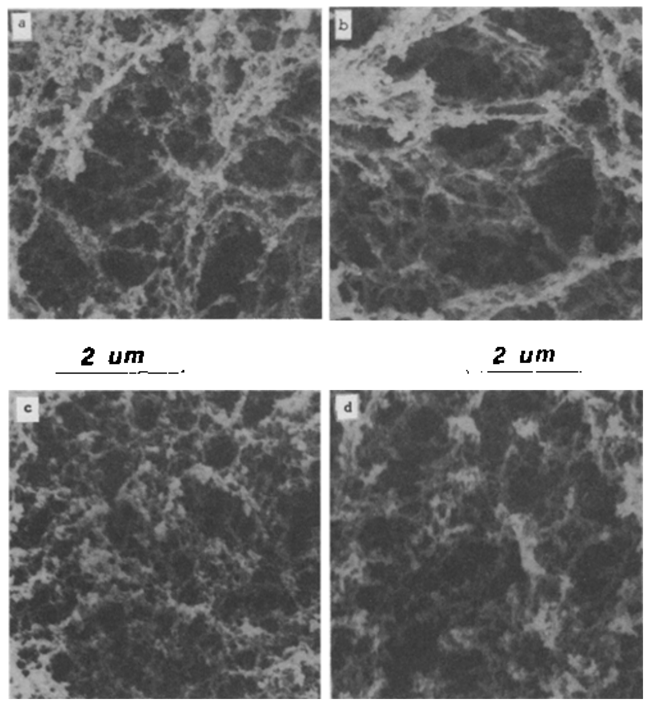

FIG. 7. Microstructure of the top portion of tofu gel made from soybeans stored for 8 mo. a, 85% RH and 20°C; b, 85% RH and 30°C; c, 65% RH and 20°C; d, 65% RH and 30°C.

TABLE 5

Texture of Tofu (Peak Force in N) Made From Stored Soybeans

	85% RH	20°C	85% RH	30°C	65% RH	20°C	65% RH	30°C
Month	F[a]	c.v.[b]	F	c.v.	F	c.v.	F	c.v.
1	4.5	20.9	4.9	21.7	4.8	15.5	6.1	24.9
2	4.9	10.7	5.5	18.3	4.5	14.6	6.0	20.5
3	3.7	9.6	5.2	10.8	4.0	18.1	4.1	19.3
4	4.9	5.2	5.4	4.1	5.1	6.7	5.9	3.3
6	6.1	8.3	7.0	9.0	4.8	10.1	6.2	14.1
8	7.1	8.4	7.3	9.1	5.7	3.0	6.2	5.5

[a]F, force.

[b]c.v., Coefficient of variability.

TABLE 6

Volume (ml) of Whey Expelled From Tofu Made From Stored Soybeans

Month	85% RH 20°C	85% RH 30°C	65% RH 20°C	65% RH 30°C
1	46.8	44.5	49.0	52.7
2	49.8	57.4	60.1	62.3
3	72.0	69.5	77.1	75.2
4	82.1	80.4	73.5	76.5
6	89.2	91.3	75.3	72.5
8	89.8	91.5	81.6	74.8

TABLE 7

Hunterlab Color Values of Soymilk Made From Stored Soybeans

Month 6	Color values			Month 8	Color values		
Hum/temp	L	a	b	Hum/temp	L	a	b
65/30	83.08	0.97	12.79	65/30	77.88	3.53	16.37
65/20	82.69	−1.41	12.00	65/20	80.45	−0.79	13.86
				85/30	81.20	−0.85	13.95
				85/20	81.43	−0.81	13.94

portion of the curd were very similar. Because tofu is sold by weight it is important that water take-up is at its maximum capacity without the loss of firm texture. After eight mo of storage of the beans, polyacrylamide gel electrophoresis showed that the protein composition of the whey was the same regardless of storage conditions.

The amount of solids lost in the soakwater was 0.3% based on dry weight of the beans for all treatments for the first four mo of storage. The solids lost in the soakwater of the beans stored at 30°C and 85% RH increased to 2% after eight mo. The beans stored at the other conditions showed only a slight increase in soaking loss. Increased solids lost would indicate cell breakdown.

By the fourth month of storage at 65% RH and 30°C a pink discoloration of the beans became evident. Microscopic examination of the beans excluded mold growth. No such discoloration was observed under the other conditions of storage. The discoloration was located in the outer regions of the cotyledons and was visible through the seed coat. The pigment produced in the beans was extracted into the soymilk as was demonstrated with measurement of the Hunter L, a and b values (Table 7). The increase in redness (a value) of soymilk made from beans stored at 65% RH and 30°C was significant. Attempts to isolate and identify this pigment so far have been unsuccessful. Although this pink discoloration is known in the trade there is little information in the literature about this phenomenon. Saio (6) has reported darkening of soybeans stored at high humidity.

The work reported in this paper indicates that proper storage conditions are important for maintaining the quality of soybeans used for making soymilk and tofu. In addition to changes in protein extractability, textural and microstructural changes of tofu and discoloration may occur, as well as deterioration of the oil, as shown by Frankel et al. (9).

More research is needed into the mechanism of formation of tofu gels. In addition, more information is needed on the composition of the solids that are lost during soaking of the beans, destabilization of the proteins and changes in the inorganic ions during soybean storage.

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REFERENCES

1. deMan, L., J.M. deMan and R.I. Buzzell, *Can. Inst. Food Sci. Technol. J. 20*:363 (1987).
2. deMan, J.M., L. deMan and S. Gupta, *Food Microstructure 5*:83 (1986).
3. Harland, B.F., and D. Oberleas, *Cereal Chem. 54*:827 (1977).
4. Latta, M., and M. Eskin, *Food Chem. 28*:1313 (1980).
5. Saio, K., I. Nikkuni, Y. Ando, M. Otsuru, Y. Takeuchi and M. Kito, *Cereal Chem. 57*:77 (1980).
6. Saio, K., K. Kobayakawa and M. Kito, *Ibid. 59*:408 (1982).
7. Yanagi, S.D., M.A.M. Galeazzi and K. Saio, *Agric. Biol. Chem. 49*:525 (1985).
8. Thanh, V.H., and K. Shibasaki, *J. Agric. Food Chem. 24*:1117 (1976).
9. Iwabuchi, S., and F. Yamauchi, *J. Food Sci. 49*:1289 (1984).
10. Brooks, J.R., and C.V. Morr, *J. Am. Oil Chem. Soc. 62*:1357 (1985).
11. Smith, A.K., and S.J. Circle, in *Soybean: Chemistry and Technology*, AVI Publishing Co., Westport, Conn., 1972, p. 61.
12. Lolas, G.M., N. Palamidis and P. Markakis, *Cereal Chem. 53*:867 (1976).
13. Frankel, E.N., A.M. Nash and J.M. Snyder, *J. Am. Oil Chem. Soc. 64*:987 (1987).
14. Krol, R.D., *Cereal Chem. 61*:490 (1984).

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