Soybean Quality Changes During Model Storage Studies

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ABSTRACT

Soybean quality changes were investigated as a function of storage time under various conditions. The color of beans darkened; the acid values of extracted crude oil and the acidity of beans increased as deterioration progressed. The nitrogen solubility index, extractability of protein and of solids into soybean milk, decreased rapidly at high temperature and high relative humidity (rh) of storage. The color of soybean milk darkened and its pH decreased slightly. The hardness of tofulike gel from stored beans decreased. The levels of solids, nitrogenous constituents, sugar, and ash in the extracts obtained on water immersion definitely increased. Electron spin resonance spectra of stored beans suggested that the combination of phosphatidylcholine with the proteins in soybean milk was strengthened. Temperature and rh are both related to overall changes during storage, but rh seems to be more important.

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Soybeans, an important protein source and a familiar part of the Japanese diet, are processed by a wide variety of traditional or newly developed techniques. Because of uncertainties in the international trade of soybeans, the Ministry of Agricultue, Forestry, and Fishery is planning to set aside reserves of soybeans to avoid shortages. In response to this project, quality changes were investigated in two soybean varieties stored for one year at temperatures of 15, 25, and 35°C and relative humidity (rh) levels of 60, 70, and 80% (Saio and Ohta 1978).

This article presents a more detailed analysis of soybeans stored under conditions that cause marked changes in qualities important to food uses of soybeans.

MATERIALS AND METHODS

Soybeans were from the U.S. 1976 crop in Indiana, Ohio, and Michigan and contained 10.61% moisture, 41.83% protein, and 21.51% oil, expressed on dry basis. The beans, imported at the end of January, were stored immediately in a refrigerator, and the experiment was started a month later. The unsterilized soybeans were conditioned to their respective relative humidities with H_2SO_4 or $(NH_4)_2SO_4$. They were spread in thin layers in individual airtight vessels for each storage period and were kept in an incubator adjusted to 15, 25, or 35°C.

Samples were stored for two, four, and six months at each of two temperatures (25 and 35°C) with two levels of rh (60 and 90%) at each temperature. The control was a sample stored at 15°C and 60% rh for two months.

Analysis of Whole Beans

Color of whole beans was measured with a ND-K 6B colorimeter. The lightness of 2 g of soybeans placed in the cell was determined five times and averaged.

Moisture content of beans was measured by coarse grinding a 5-g sample and drying it at 130°C for 3 hr.

To determine the nitrogen solubility index (NSI), extractability of protein and solids into soybean milk, a 5-g ground sample was dispersed in 200 ml of water by stirring at 30°C for 120 min. The suspension was made up to 250 ml and centrifuged at $650 \times g$ for 10 min. The dispersible nitrogenous constituents in the supernatant were determined by a micro-Kjeldahl method. NSI was calculated as: (dispersible N/total N) × 100.

Acid value of the oil from stored beans was measured by grinding a 10-g sample and extracting with petroleum ether at 70°C for 8 hr in a Soxhlet extractor. The extracted oil was weighed after drying at 60°C for 1 hr in a vacuum. A solution of benzene/ethanol (1:1) was neutralized with KOH-ethanol and 30 ml of the solution was added to the crude oil; the mixture was titrated with 0.1 *N* standard KOH-ethanol and 10 ml of saturated NaCl and phenolphthalein.

Organic acids were measured by placing 5 g of ground sample into a 200-ml flask, adding 50 ml of 80% ethanol, extracting for 30 min while stirring, and filtering through filter paper. Water (25 ml) was added to 25 ml of the filtrate in a 100-ml beaker, and the mixture was titrated with 0.05 *N* NaOH by use of pH meter. Results are expressed in milligrams of citric acid per 100 g.

The samples for the determination of NSI, acid value, and organic acids were successively ground with a grinder (Kyoritsu-Riko, 10,000 rpm) for 1 min and with a coffee mill (Emide KM 6) for 1 min.

Preparation and Analysis of Soybean Milk

Soybeans (50 g) were immersed in 250 ml of water overnight. The swelled beans were homogenized with water (1:10, including water absored during immersion) in a home mixer (Sanyo SM 220) and clarified with a basket-type centrifuge (J-165, Fuji Electric Co.) to separate the soybean milk from the insoluble residue.

Lightness of soybean milk was measured with a ND-K 6B colorimeter.

The pH of the soymilk was measured with a pH meter immediately after preparation.

Solids content of the soymilk was determined by drying at 130°C for 2 hr. The nitrogen content of soymilk was determined by a micro-Kjeldahl method. Results were expressed as: (extractable solids or N/total weight or N) × 100%.

Preparation and Evaluation of Tofu

Tofulike gels were prepared by heating 10 ml of soybean milk in test tubes in a boiling water bath for 10 min and cooling immediately in an ice bath. Next, glucono-δ-lactone was added to 0.5% final concentration with stirring. Gelation occurred while the tubes stood in a water bath at 70°C for 60 min. After cooling with tap water, hardness of the gels was measured with a Tensipresser T-501 (Zenken Co.). A plunger, 50 mm in diameter, penetrated 38 mm into the gels.

Analysis of Extract Obtained from Water Immersion

In the preparation of soybean milk, 50 g of soybeans were immersed in 250 ml of water overnight. The water was analyzed after the swollen beans were removed.

Color of the extract was measured with a spectrophotometer (Hitachi-181) as the absorbance at 360 nm.

Solids content was determined by weighing 25 ml of the extract after it was dried at 130°C for 2 hr. Nitrogenous constituents were determined by the micro-Kjeldahl method. They were expressed as: (extractable solid or N/total weight or N) × 100.

Total sugar and reducing constituents in the extract were determined by the phenol-H_2SO_4 method (Dubois et al 1956) and (1952), respectively.

Total ash was determined by burning the dried solids in an

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electric muffle at 550°C overnight, cooling, and weighing. The ash was dissolved with 10 ml of 6 *N* HCl, heated in a boiling bath to dryness, and diluted to 100 ml with water after 10 ml of 3 *N* HCl was added. The solution was analyzed for calcium, phosphorus, magnesium, iron, and potassium. Calcium was determined by the Kubo-Tsutsumi method (1951), phophorus by the method described by Nakamura (1950), iron by the *O*-phenanthroline colorimetric method (AOAC 1955); magnesium and potassium were determined with an atomic absorption spectrophotometer (model 303, Perkin-Elmer).

Electron Spin Resonance Analysis

Soybeans were immersed in water overnight. The swollen beans were homogenized with water (1:4). The homogenate was then heated for 3 min, after it was boiled to inactivate the enzymes, and was separated from the residue. The resulting soybean milk (containing about 20 mg of protein per milliliter) was sonicated with 250 μg of the spin-labeled phosphatidylcholine (Sigma Chemical Co.) at 15-sec intervals for 2 min in an ice bath with a sonicator (Kaijo Denki T-A-4201) at 20 kHz. The mixture was submitted to electron spin resonance (ESR) analysis at 20°C, by use of an X-band JES-ME-X ESR spectrophotometer.

RESULTS

Figure 1 shows changes in moisture content and color of the beans as a function of storage time under various conditions. Initial moisture content of the beans (10.61%) dropped to 9.7% after

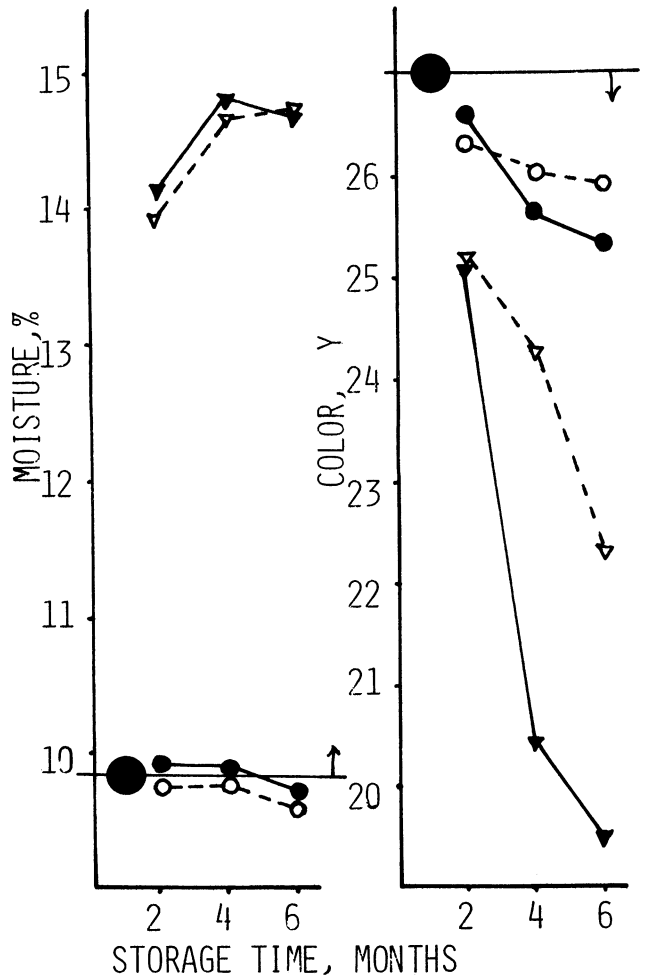

Fig. 1. Changes in moisture and color of whole soybeans as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o—o = beans stored at 25°C, 60% rh; Δ---Δ = beans stored at 25°C, 80% rh; •—• = beans stored at 35°C, 60% rh ▲—▲ = beans stored at 35°C, 80% rh.

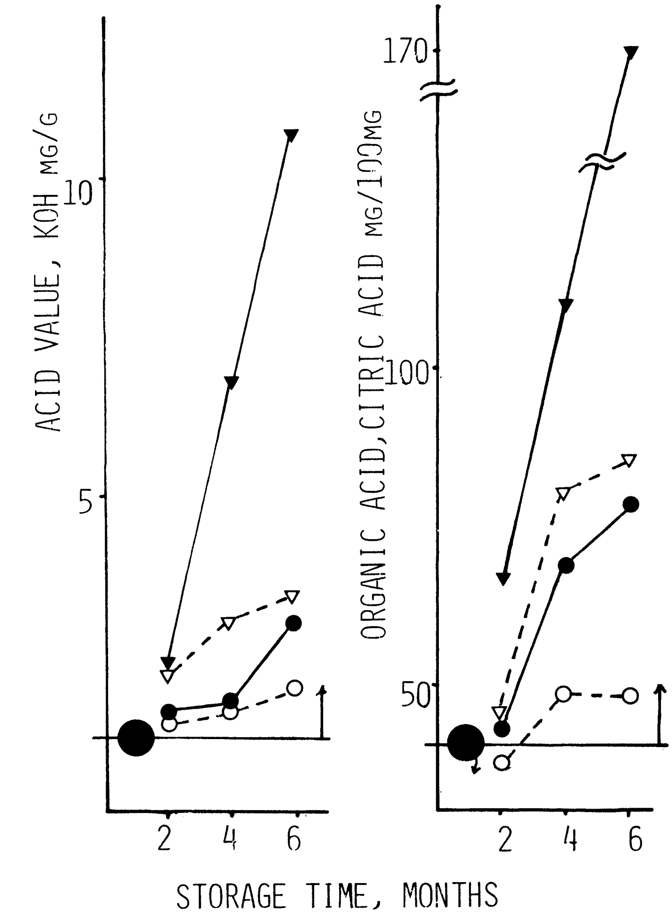

Fig. 2. Changes in acid value of extracted crude oil and organic acids titrated after extraction as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o---o = beans stored at 25°C, 60% rh; Δ---Δ = beans stored at 25°C, 80% rh; •—• = beans stored at 35°C, 60% rh; ▲—▲ = beans stored at 35°C, 80% rh.

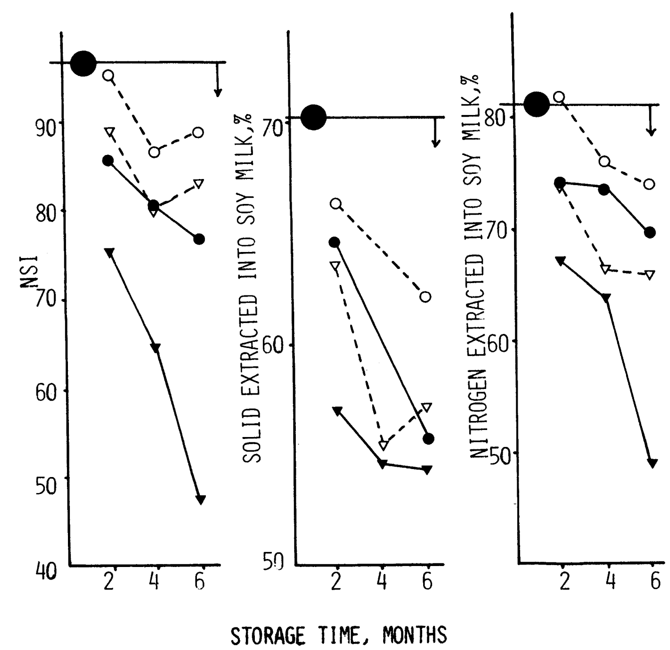

Fig. 3. Changes in NSI and in solid and nitrogenous constituents extracted into soybean milk as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o—o = beans stored at 25°C, 60% rh; Δ---Δ = beans stored at 25°C, 80% rh; •—• = beans stored at 35°C, 60% rh; ▲—▲ = beans stored at 35°C, 80% rh.

storage at 60% rh, whereas it rose to 14.8% after storage at 80% rh for six months. The color of the beans changed from pale yellow to brown depending on the temperature and rh during storage. The change in lightness was very pronounced at high rh, as shown in Fig. 1 (right) and Table I. After six-month storage, the beans could easily be placed in order by color.

Figure 2 shows changes in acid values and acidity. Acid values of extracted crude oil and the level of acidity titrated after the extraction both increased remarkably. Although acid values were increased under all storage conditions, values from beans stored at

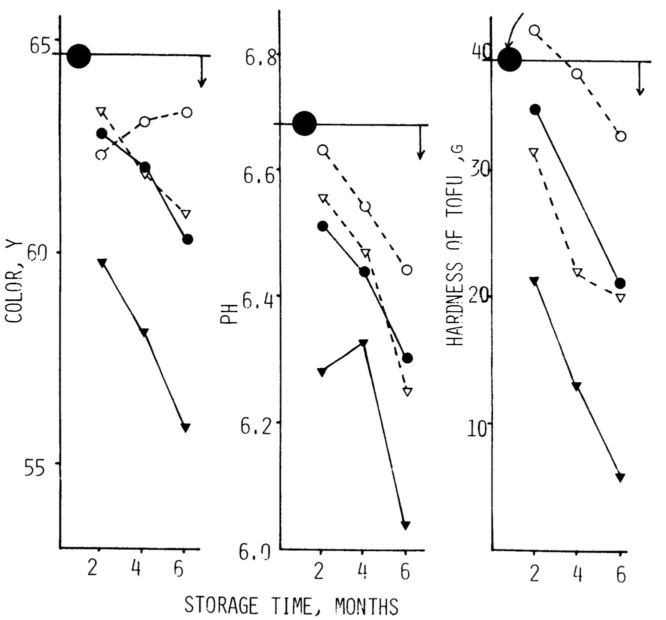

Fig. 4. Changes in color and pH of soybean milk and in hardness of tofulike gel as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o—o = beans stored at 25°C, 60% rh; Δ---Δ = beans stored at 25°C, 80% rh; •—• = beans stored at 35°C, 60% rh; ▲—▲ = beans stored at 35°C, 80% rh.

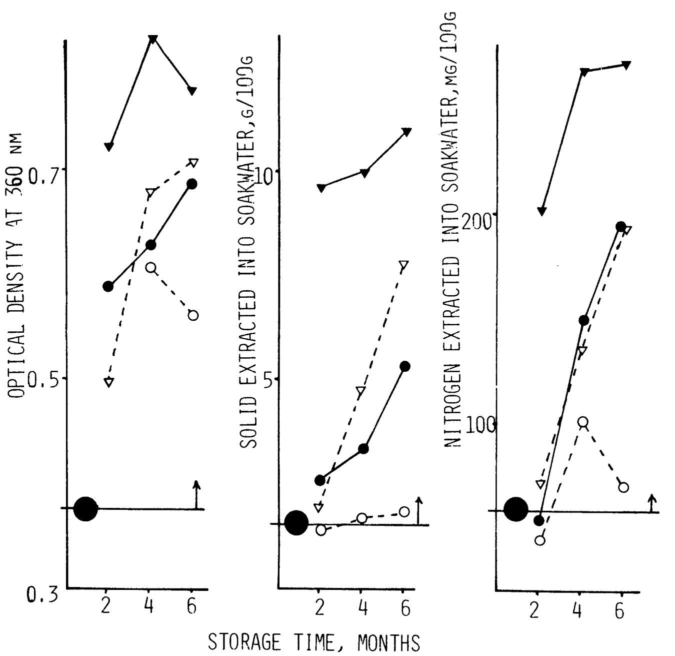

Fig. 5. Changes in color of soakwater and in solids and nitrogenous constituents extracted as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o---o = beans stored at 25°C, 60% rh; Δ—Δ = beans stored at 25°C, 80% ri • = beans stored at 35°C, 60% rh; ▲—▲ = beans stored at 35°C, 80% ı

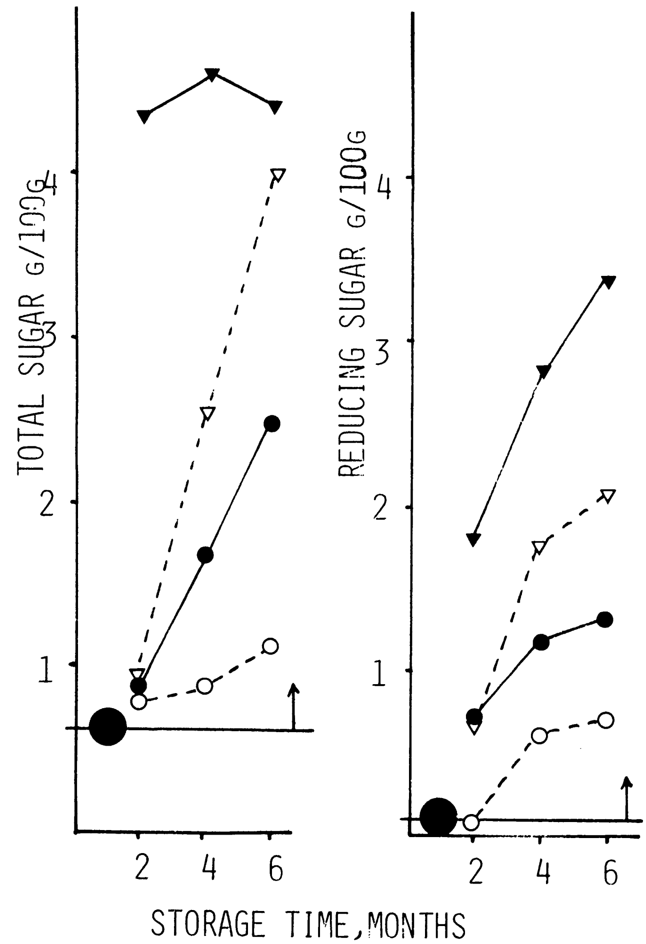

Fig. 6. Changes in total and reducing sugars extracted into the soaking water as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o---o = beans stored at 25°C, 60% rh; Δ---Δ = beans stored at 25°C, 80% rh; •—• = beans stored at 35°C, 60% rh; ▲—▲ = beans stored at 35°C, 80% rh.

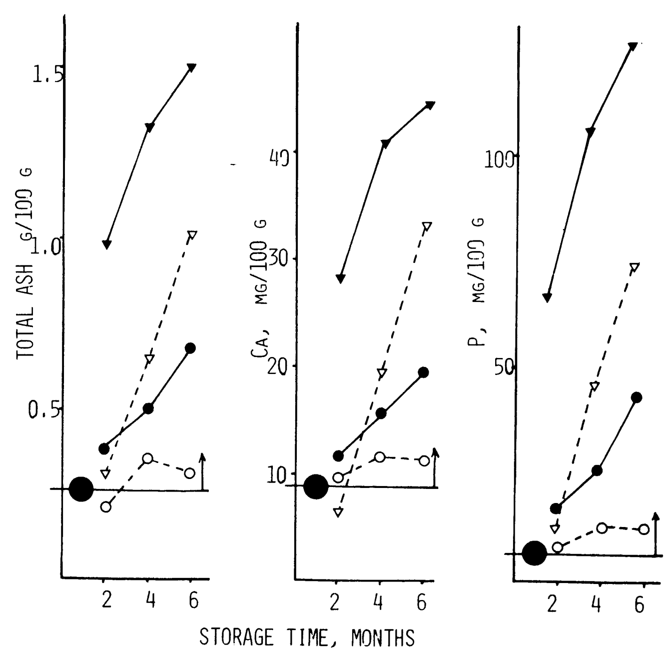

Fig. 7. Changes in total ash, calcium, and phosporus extracted into sakwater as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o—o = beans stored at 25°C, 60% rh; Δ---Δ = beans stored at 25°C, 80% rh; •—• = beans stored at 35°C, 60% rh; ▲—▲ = beans stored at 35°C, 80% rh.

35°C and 80% rh increased more than tenfold. By comparison, the acid value of new crop soybeans is less than 1.

Figure 3 shows changes in the extractability of protein and solids from soybean tissues. The NSI value decreased rapidly at 35°C and 80% rh. Extractabilities of nitrogenous constituents and solids into soybean milk likewise decreased rapidly at high storage temperature and rh.

As shown in Fig. 4, the color of soybean milk darkened remarkably, and the pH of the milk changed from about 6.7 to 6.1 after six-month storage of soybeans at 35°C and 80% rh. The hardness of tofulike gels coagulated with glucono-δ-lactone from the milk of stored beans decreased as deterioration progressed. The acceptable hardness of tofu measured in this way is a minimum of 30 g.

In the preparation of many traditional soybean foods, whole beans are immersed in water to absorb water and swell. Figure 5 shows changes in color, solids, and nitrogenous constituents of the extracts obtained from water immersion. The spectrum of the extract has a main peak at 360 nm and a small shoulder at 500 nm. The latter was recognized only in the beans stored under high temperature and humidity. Absorbances at 360 nm increased as shown in Fig. 5. The amount of solids and of nitrogenous constituents in the extract, which are losses of nutrients from the beans, increased remarkably.

Figure 6 shows that storage caused considerable increases of sugar and reducing constituents in the extract from water immersion.

Figures 7 and 8 demonstrate rapid increases of total ash and of various minerals in the water extractives as a consequence of storing soybeans at high temperature and high rh.

Figure 9 shows ESR spectra of stored beans. The spin at 20.5 *g* (A) from the central main peak shows resonance resulting from the

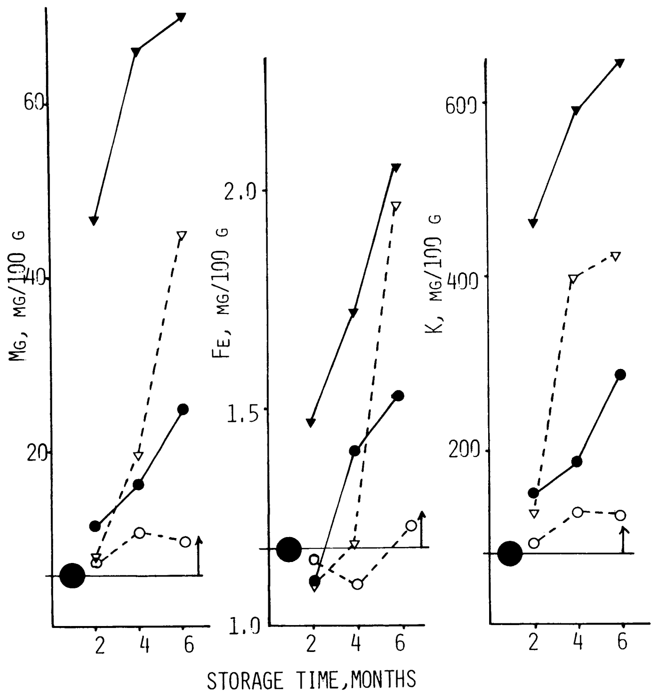

Fig. 8. Changes in magnesium, iron, and potassium extracted into soakwater as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o---o = beans stored at 25°C, 60% rh; Δ---Δ = beans stored at 25°C, 80% rh; •—• = beans stored at 35°C, 60% rh; ▲—▲ = beans stored at 35°C, 80% rh.

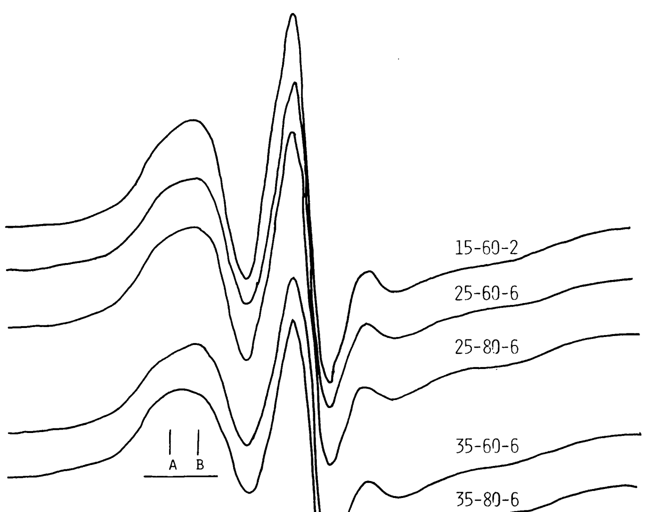

Fig. 9. Electron spin resonance spectra of stored beans. **A,** phosphatidylcholine with protein, 20.5 *g* from central peak; **B,** phosphatidycholine in the phospholipid lamellae, 16 *g* from central peak; 15, 25, 35 = storage temperature; 60, 80 = storage rh; 2, 6 = months of storage.

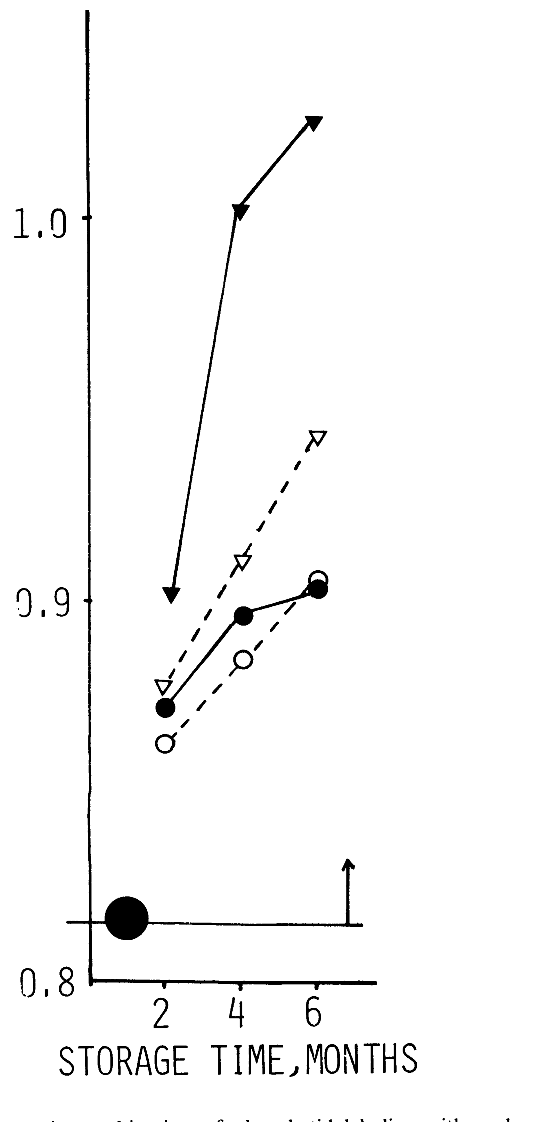

Fig. 10. Changes in combination of phosphatidylcholine with soybean proteins as a function of storage time. ● = beans stored at 15°C, 60% rh for two months (control); o—o = beans stored at 25°C, 60% rh; Δ---Δ = beans stored at 25°C, 80% rh; •—• = beans stored at 35°C, 60% rh; ▲—▲ = beans stored at 35°C, 80% rh.

combination of the spin-labeled phosphatidylcholine with the protein, whereas the spin at 16 g (B) from the main peak represents resonance of the phosphatidylcholine included in the phospholipid lamellae (Otsuru et al 1976). As storage deterioration progresses, A/B increased markedly as shown in Fig. 10, indicating that the combination of phosphatidylcholine with the proteins strengthened in soybean milk.

Tables I–III show the means for levels of storage time, temperature, and relative humidity. The corresponding F ratios and significance of these main effects are indicated. In some cases the factors interacted, particularly relative humidity and temperature.

DISCUSSION

Classical studies on storage-damaged soybeans (Milner and Geddes 1945, 1946) have dealt with biological processes such as respiration or mold growth. Practical investigations of soybeans in storage have concerned the effects of moisture and temperature in bins, insect or fungal damage, and aeration systems (Holman and Carter 1952). These earlier studies clearly established that moisture content of the seed is the most critical of all factors influencing the deterioration of stored beans.

Our investigation focused on physical and chemical changes that occur during storage and that are important in the Japanese food uses of soybeans, such as soy milk and tofu.

The beans stored at 35°C and 80% rh showed gradual infestation of fungi after two-month storage, and those at 25°C and 80% rh were infested after six-month storage. Decoloration of beans was clearly recognized under high humidity conditions (Fig. 1). Remarkable increases in acid value (Fig. 2) suggest that the hydrolysis of lipids to fatty acids and the increase of organic acids may have resulted from oxidation of fatty acids or other active biological processes during storage. They contributed to the drop in pH value of soybean milk. Such deterioration of lipids may cause changes in the form by which lipids are bound to proteins.

Decreases in NSI and in extractability of solids and nitrogenous constituents into soybean milk were remarkable under improper

TABLE I
Analysis of Whole Beans Stored Under Various Conditions

	Moisture (%)	Color (Y)	Acid Value (KOH mg/g)	Acidity[a] (mg/100g)	NSI[b] (%)
Total	12.15	24.38	3.28	73.61	79.25
Time, months					
2	11.95	25.80	1.83	48.31	86.31
4	12.30	24.08	3.26	77.01	77.29
6	12.20	23.28	4.75	95.51	74.16
F ratio	31.21[c]	11.06	3.52	15.26	6.46
Temperature, °C					
25	12.10	25.00	2.21	57.56	86.79
35	12.21	23.77	4.35	89.66	71.71
F ratio	9.32	7.57	5.68	20.84[c]	27.66[d]
Relative humidity, %					
60	9.78	25.95	1.83	54.14	85.18
80	14.52	22.82	4.73	93.08	73.32
F ratio	16,258.73[c]	48.88[c]	10.42	30.68[c]	17.14

[a] Citric acid.
[b] NSI = Nitrogen Solubility Index.
[c] $P = 0.05$.
[d] $P = 0.01$.

TABLE II
Analysis of Soybean Milk from Beans Stored Under Various Conditions

	Solids (g/100 g)	N in Milk (mg/100g)	Color of Milk (Y)	pH of Milk	Hardness of Gel (g)
Total	60.85	68.58	61.14	6.40	26.75
Time, months					
2	62.73	74.03	62.08	6.49	32.24
4	62.37	68.78	61.25	6.45	28.18
6	57.43	62.93	60.10	6.27	19.83
F ratio	7.13	15.81	15.69	54.43[a]	26.81[a]
Temperature, °C					
25	62.19	72.85	62.47	6.49	30.80
35	59.50	64.31	59.82	6.32	22.69
F ratio	4.39	28.10[a]	83.99[a]	83.54[a]	33.01[a]
Relative humidity, %					
60	64.60	73.07	62.32	6.48	34.58
80	57.07	64.09	59.97	6.33	18.91
F ratio	34.82[a]	31.04[a]	66.05[a]	65.20[a]	123.25[b]

[a] $P = 0.05$.
[b] $P = 0.01$.

TABLE III
Analysis of Extract from Water Immersion of Soybeans

	Color of Extract[a]	pH	Solid (g/100g)	N in Extract (mg/100g)	Total Sugar (mg/g)	Reducing Substances (mg/g)	Total Ash (%)	Minerals (mg/100g) Ca	P	Mg	Fe	K	Protein-Lipid (A/B)[b]
Total	0.671	6.04	5.09	145.8	24.1	14.7	0.676	20.9	45.1	28.0	1.38	293.4	0.914
Time, months													
2	0.640	6.10	3.82	92.0	17.4	9.2	0.468	14.0	24.9	18.1	1.20	203.8	0.877
4	0.690	6.03	4.95	163.6	24.4	16.1	0.693	22.1	47.8	28.2	1.38	317.7	0.921
6	0.684	5.99	6.50	181.7	30.4	18.9	0.868	26.5	62.6	37.6	1.55	358.8	0.943
F ratio	0.23	0.19	2.39	4.18	1.43	187.93[c]	13.37	12.27	22.04[d]	5.79	1.40	10.01	11.87
Temperature, °C													
25	0.635	5.96	3.23	101.8	17.2	10.6	0.467	14.7	27.0	16.7	1.28	210.3	0.895
35	0.707	6.12	6.96	189.7	30.9	18.8	0.885	27.0	63.2	39.2	1.47	376.6	0.932
F ratio	1.24	1.07	13.77	10.78	4.68	386.17[c]	43.63[d]	34.48[d]	59.98[d]	23.25[d]	1.30	32.17[d]	10.68
Relative humidity, %													
60	0.638	5.94	2.70	101.4	13.3	8.4	0.397	13.1	19.0	13.1	1.17	155.9	0.885
80	0.704	6.15	7.49	190.2	34.9	21.0	0.955	28.6	71.3	42.8	1.58	431.0	0.942
F ratio	1.02	1.92	22.75[d]	11.01	11.74	904.91[c]	77.72[d]	54.68[d]	125.43[c]	40.59[d]	5.58	88.10[d]	25.58[d]

[a] Absorbance at 360 nm.
[b] A/B = Ratio of spin at 20.5 g from peak to spin at 16 g from peak.
[c] $P = 0.01$.
[d] $P = 0.05$.

storage (Fig. 3). Decrease in protein extractability from the seed tissues seemed to be caused primarily by lowering of the pH of the whole water extract or soybean milk. The effects of lower pH involved predominantly the 11S rather than the 7S component, as reported previously (Saio and Arisaka 1978). The finding that the 11S protein is sensitive to lowering of pH agrees with the finding that its solubility decreases more rapidly than that of the 7S globulin below pH 6.5 (Thanh and Shibazaki 1976, Wolf and Sly 1967). On the other hand, interactions of proteins with lipids are also suggested, especially in beans stored at high temperature and high rh, in which severe quality changes occurred. The changes in the ERS spectra during storage may mean that phosphatidylcholine, namely lecithin, combines with hydrophobic regions in denatured proteins so that their emulsification role in soybean milk is reduced. We noted that soybean milk prepared from damaged beans separated easily into water and oil phases. Our continuing studies also indicate that extractability of total lipids decreases markedly in these beans. Shimada and Matsushita, using a model system, recently reported (1978), that deteriorated lipid accelerates the aggregation of soybean proteins. Interaction of proteins with carbohydrates is another possibility because reducing sugars increase markedly after water immersion. Previous work on milk and egg provides a wide variety of examples of interactions between carbohydrates and proteins during processing and storage by the Maillard reaction. Lower extractability thus would seriously influence the yield and quality of conventional or new soybean foods that require extraction processes in their preparation. Moreover, deterioration of lipids would surely influence the yield and quality of oil prepared from soybeans stored under adverse conditions.

Losses from soybeans during water immersion after poor storage were notable. The solids lost during water immersion, consisting of about 50% of the carbohydrate, 20% ash, 10% nitrogenous constituents, and other compounds (Saio and Arisaka 1978), reached about 10% in beans stored at 35°C, rh 80% for six months. Increases of sugar and ash losses were also considerable. During storage at 35°C and 80% rh for six months, soakwater losses from soybeans changed from 5 to 30% in total ash, 3 to 16% in calcium, 3 to 30% in magnesium, 1 to 25% in phosphorus, 20 to 30% in iron, and 4 to 32% in potassium. These losses mean not only a lowering of the usual levels of these nutrients but also greater waste disposal problems and a conspicuous shortage of nutrients such as sugars and minerals that are required for the preparation of fermented foods.

Hardness of tofu gel made from poorly stored soybeans decreased markedly, and beans stored at 35°C and 85% rh for six months nearly reached a noncoagulative state. The decrease in hardness depends mainly on the decrease in concentration of soybean milk solids because of decreased extractability into soybean milk (Saio and Arisaka 1978). Although our experiments measured only hardness, changes in quality such as loss in cohesion, increased fragility, off-flavor, and darkening in color of gels were recognized.

As mentioned, the deterioration depends on temperature and moisture content, and the latter seems to be predominant, as previous work had suggested. Consequently, maintaining a low moisture content of soybeans during storage is very important, especially in Japan during the summer, when temperature and humidity are high.

LITERATURE CITED

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1955. Official Methods of Analysis (8th ed.) p. 101. The Association: Washington, DC.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., ROBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

HOLMAN, L. E., and CARTER, D. G. 1952. Soybean storage in farm type bins. Ill. Agric. Stn. Bull. 553:45.

KUBO, S., and TSUTSUMI, T. 1951. Method for determination of calcium in foods. Rep. Food Res. Inst. (Tokyo) 5:171.

MILNER, M., and GEDDES, W. F. 1945. Grain storage studies. II. The effect of aeration, temperature, and time of the respiration of soybeans containing excessive moisture. Cereal Chem. 22:484.

MILNER, M., and GEDDES W. F. 1946. Grain storage studies. III. The relation between moisture content, mold growth, and respiration of soybeans. Cereal Chem. 23:225.

NAKAMURA, M. 1950. Colorimetric determination of phosphorus. Nippon Nogei Kagaku Kaishi 24:1.

OTSURU, M. KITO, M., TAKEUCHI, Y., and OHNISHI, S. 1976. Association of phosphatidylcholine with soybean protein. Agric. Biol. Chem. 40:2261.

SHIMADA, K., and MATSUSHITA, Y. 1978. Polimerization of soybean 11S globulin due to reactions with peroxidizing linoleic acid. Agric. Biol. Chem. 42:781.

SAIO, K., and ARISAKA, M. 1978. Deterioration of soybean during storage under high moisture and temperature. Nippon Nogei Kagaku Kaishi 25:451.

SAIO, K., and OHTA, T. 1978. An emergency report on soybean storage for food uses. Res. Rep. Minist. Agric. For. Fish. (Jpn).

SOMOGYI, M. 1952. Notes on sugar determination. J. Biol. Chem. 195:19.

THANH, V. H., and SHIBAZAKI, K. 1976. Major proteins of soybean seeds. A straightforward fractionation and their characterization. Agric. Food Chem. 24:1117.

WOLF, W. J., and SLY, D. A. 1967. Cryoprecipitation of soybean 11S protein. Cereal Chem. 44:453.

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