

# Protein Denaturation During Model Storage Studies of Soybeans and Meals

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## ABSTRACT

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Protein denaturation of soybeans and meals as a function of storage time and conditions was studied. As storage time under improper conditions increased, protein extractability decreased rapidly. Extractability was influenced by high ionic strength and pH but was most affected by the presence of dithiothreitol. Extractabilities of 7S and 11S components decreased, indicating an even more rapid decrease of the 11S component. Hence, in the relative proportion of extractable protein from defatted

meals, 11S components decreased, and 2S components increased. Such changes in proteins were investigated by ultracentrifugation and electrophoresis. Whole beans were more resistant to deterioration during storage than meals, and full-fat meals deteriorated more rapidly than defatted meals. Quality seems to be more readily retained in the native state when the intracellular membranes are intact.

In our investigations of changes in soybean quality during model storage (Nakayama et al 1981, Saio et al 1980), we found rapid decreases of nitrogen solubility index (NSI), decreased extractability of solids and protein into soybean milk, increase of acid value of extracted crude oil, and marked decomposition of phospholipids as a result of storage at high relative humidity (rh) and high temperature. Degradation of lipids appeared to affect emulsification of the oil and proteins in the preparation of soybean milk and lowered quality of soybean foods such as edible oil and tofu.

This article explains changes in the proteins in whole beans and meals during long-term storage.

## MATERIALS AND METHODS

### Soybean Samples

Soybeans were from the 1977 and 1978 crops in Indiana, Ohio, and Michigan and were imported at the end of January and stored immediately in a refrigerator until the experiments were started a month later in 1978 and 1979, respectively. Full-fat meals were prepared with the same beans by conditioning to a suitable moisture level, cracking between corrugated rolls, flaking between smooth rolls, and grinding. Defatted meals were prepared from full-fat flakes by continuous extraction with *n*-hexane.

Samples were stored at 25 and 35°C at two levels of relative humidity: 65% (36% H<sub>2</sub>SO<sub>4</sub> at 25°C and 36.4% H<sub>2</sub>SO<sub>4</sub> at 35°C) and 85% (22.5% H<sub>2</sub>SO<sub>4</sub> at 25°C and 22.9% H<sub>2</sub>SO<sub>4</sub> at 35°C).

### Analysis of Samples

Moisture was measured by drying at 130°C for 3 hr. Protein content of beans and meals was determined by the micro-Kjeldahl method, using 6.25 as the protein to nitrogen conversion factor. Protein content in the extracts and protein isolates was estimated from optical density measurements at 560 nm by the biuret method (Gonall et al 1949), using bovine serum albumin as a standard. Oil content was determined by ethyl ether extraction. NSI values were obtained by measuring the dispersible nitrogen in extracts prepared by stirring 5 g of sample in 200 ml of water at 30°C for 120 min. Acid value is expressed as milliliters of 0.1M KOH needed to neutralize 1 g of the extracted crude oil.

### Preparation of Protein Isolates

Protein isolates used in these experiments were mainly prepared by the method of Otsuru et al (1976). A 3-g sample of flour from whole beans or meals was dispersed in 20 ml of water and kept in a

refrigerator overnight; 10 ml of 0.1M phosphate buffer (pH 7.6) containing 1.2M NaCl and 3 mM dithiothreitol (DTT) was then added. The mixture was stirred at room temperature for 60 min and centrifuged at 10,000 × *g* for 30 min. Chilled acetone (1.5-fold, -20°C) was added to the supernatant by drops in an ice bath with continuous stirring to precipitate the protein. The precipitate was separated by centrifugation at 10,000 × *g* for 30 min in the cold and washed with chilled acetone by suspending, centrifuging, and decanting twice. Residual acetone was removed in a vacuum desiccator.

### Determination of Protein Extractability

A 3-g sample of flour was dispersed in 20 ml of water and kept in a refrigerator overnight. Ten milliliters of one of the following solvents was then added: water (25 or 40°C); dilute NaOH (of concentration sufficient to adjust dispersion to pH 8.0); or 0.1M sodium phosphate buffer containing 1.2M NaCl plus 0, 1.5, 3, 4, 5, or 6 mM DTT. The dispersion was then stirred at room temperature for 60 min and centrifuged at 10,000 × *g* for 30 min. The protein in the supernatant was measured by the biuret method.

### Ultracentrifugal Analysis

The protein isolates prepared from stored soybeans and meals were dissolved (about 10 mg/ml) in 0.1M phosphate buffer containing 0.4M NaCl and 10 mM mercaptoethanol and dialyzed against the same phosphate buffer overnight. Ultracentrifugation was performed at 51,200 rpm at 20°C with a Hitachi model UCA-1A. Protein components were measured by enlarging and tracing ultracentrifugal patterns at 45-min centrifugation on graph paper and then cutting out and weighing each peak.

### Electrophoretic Analysis

Protein isolates for electrophoresis were prepared from full-fat meals stored at 35°C (85% rh) by the method described, but the pH of the meal sample dispersed in phosphate buffer (pH 7.6) was adjusted during extraction because the pH decreased as low as 6, depending on the samples, as a result of marked deterioration. Protein isolates from defatted meals stored at 35°C (85% rh) were also submitted to electrophoretic analysis. The protein isolates from full-fat and defatted meals were dissolved in 0.1M phosphate buffer and applied to disk polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE according to the methods of Davis et al (1964) and Shapiro et al (1967). PAGE was performed in 7% gel at 2.5 mA per gel and SDS-PAGE in 10% gel at 8 mA per gel.

## RESULTS

### 1977 Crop

Soybeans and defatted meals from the 1977 crop contained 13.61 and 11.52% moisture, 47.84 and 56.14% protein, and 21.28 and 1.76% oil, respectively (expressed on a dry basis).

Before preparation of protein isolates, the effects of hexane extraction and acetone precipitation on the ultracentrifugation

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patterns were examined. Figure 1 shows that neither treatment significantly affected the patterns; hence, this method for preparation of protein isolates was used throughout the experiments.

Figure 2 shows changes of NSI during six-month storage of defatted meals and whole beans. We previously reported that improper storage caused a marked decrease in NSI of whole beans (Saio and Arisaka 1978, Saio et al 1980). The results in Fig. 2 confirm our earlier observation and reveal an even more rapid decrease of NSI of defatted meals with time of storage. NSI measures nitrogen extracted with water under defined conditions, but this solvent yields only limited information. Consequently, we used several other extraction solvents on defatted meals stored at 35°C, (85% rh) for four months (Fig. 3). Extractability was influenced by high ionic strength (NaCl) and pH but was most affected by DTT. Protein extractability of defatted meals and whole beans with phosphate buffer containing 0.4M NaCl and 1.0 mM DTT markedly decreased as a function of storage time (Fig. 4). The decrease in extractability of defatted meals was more rapid than that of whole beans. This was also the case with NSI measurements (Fig. 2). Changes in extractable proteins were not only quantitative but qualitative. Figure 5 shows the ultracentrifugation patterns of protein isolates prepared from defatted meals (after two to six months) and whole beans (after two and six months) stored at 35°C (85% rh). In isolates from defatted meals, the relative proportion of 11S component decreased, whereas that of 2S component increased.

Nash et al (1971) reported a decrease of extractability of protein from defatted meals (stored at 25°C for 200 days); however, ultracentrifugation patterns showed only slight decrease of 7S and

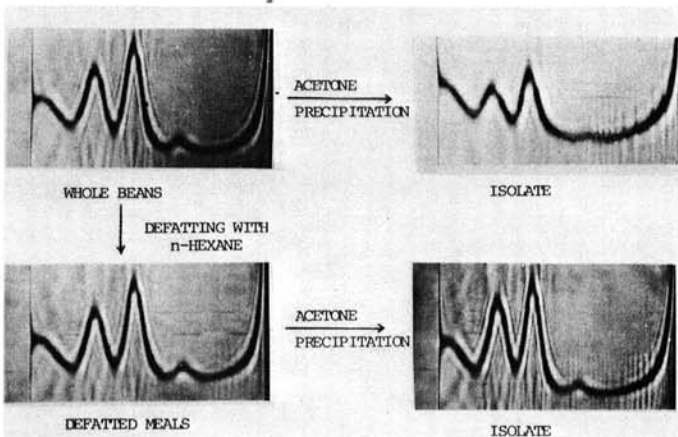


Fig. 1. Effects of experimental treatments on ultracentrifuge patterns of extracts and isolates of whole beans and defatted meal (1977 crop). Photographs were taken after 45 min at 51,200 rpm.

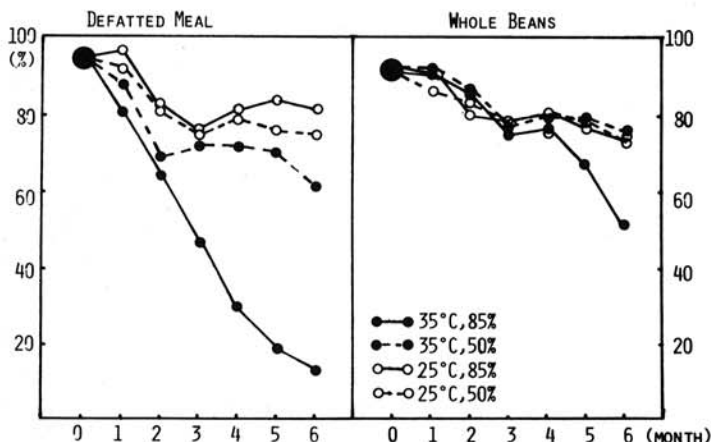


Fig. 2. Changes in nitrogen solubility index during six-month storage of defatted meal and whole beans (1977 crop).

11S and no change in 2S components.

Figure 6 shows total extractability of each protein component. Here, the 7S component decreased, the 11S component decreased even more rapidly after three months, and the 15S component declined with storage time. The decrease in extractability of the >15S fraction was less certain because the protein solution after dialysis was centrifuged at 10,000 rpm for 15 min before ultracentrifugation; loss of the >15S fraction may have occurred at this step. The 2S component seemed to be constant, although its values after five months were slightly elevated. Under such

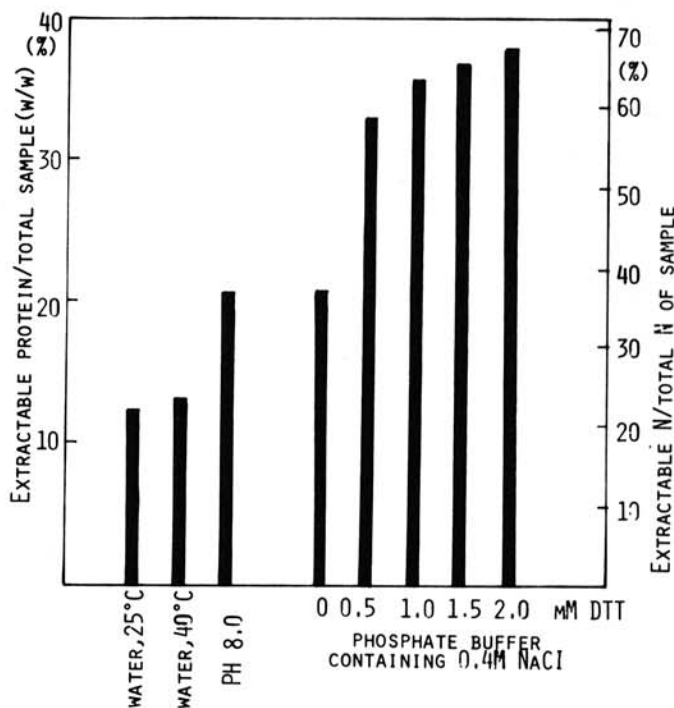


Fig. 3. Changes in amounts of protein extracted with different solvents using defatted meal stored at 35°C (85% rh) for four months (1977 crop). Results for water extracts and dilute alkali extract are expressed as extractable protein/sample weight, and buffer extracts are calculated as extractable nitrogen/total nitrogen of sample.

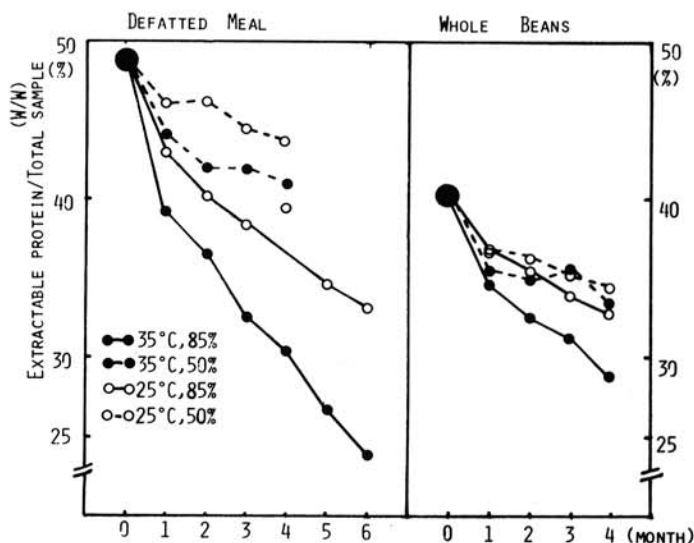


Fig. 4. Changes in extractable protein during six-month storage of defatted meal and whole beans (1977 crop). Protein was extracted with 0.1M phosphate buffer (pH 7.6) containing 0.4M NaCl and 1.0 mM DTT. Expressed as protein extractable with phosphate buffer/sample weight (whole beans and defatted meal contained 47.84 and 56.14% protein, respectively).

abnormal storage conditions, marked changes of extractability and relative proportion in each protein component occurred; hence, our results seem to coincide with those of Nash et al (1971).

The trend of changes in relative proportions of protein components was more clearly shown when extractions were made

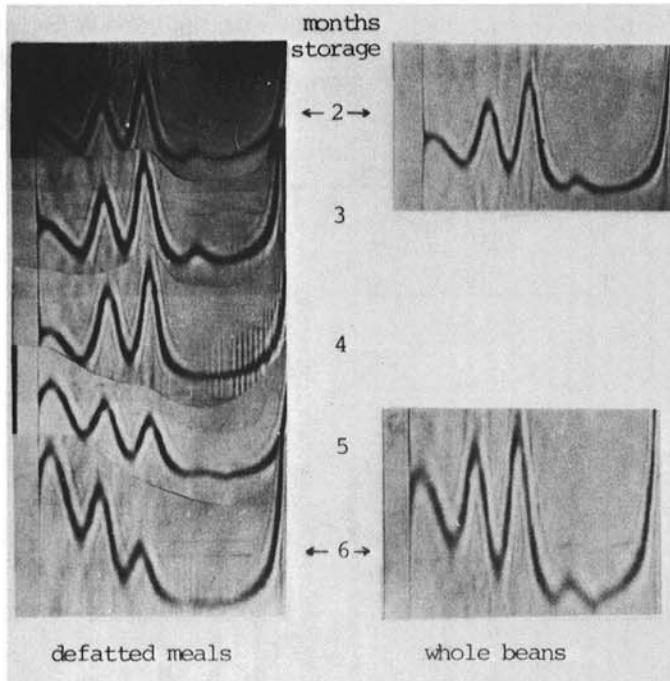


Fig. 5. Changes of relative proportion of protein components in isolates prepared from defatted meal and whole beans stored at 35°C, 85% rh (1977 crop). Photographs were taken after 45 min at 51,200 rpm.

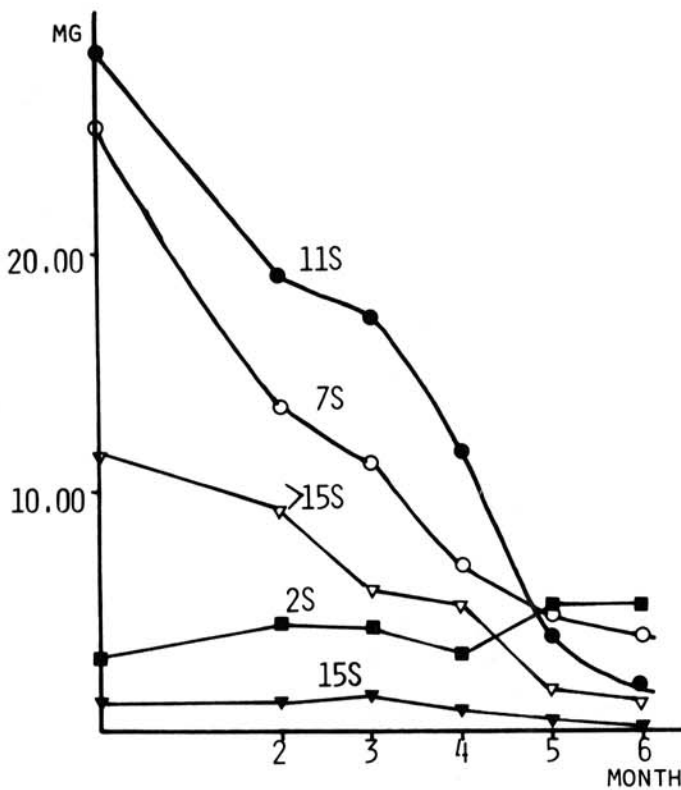


Fig. 6. Changes in amounts of protein components extracted from defatted meals as a function of storage time at 35°C, 85% rh. Total amount of extractable protein components are arbitrary units calculated by ultracentrifugal area of each protein component (mg)/used protein amount for ultracentrifugation (mg/ml) × total amount (mg) of extractable protein from meals with different storage times.

with solvents such as water. Figure 7 shows ultracentrifugation patterns of protein components in protein isolates prepared by extraction with different solvents, using defatted meals stored at 35°C (85% rh) for four months (Fig. 3). When the isolates were extracted with phosphate buffer containing 0.4M NaCl and 1.0 mM DTT, the pattern was almost identical to the initial one, whereas when phosphate buffer containing 0.4M NaCl, dilute alkaline solution, and especially water were used as extracting

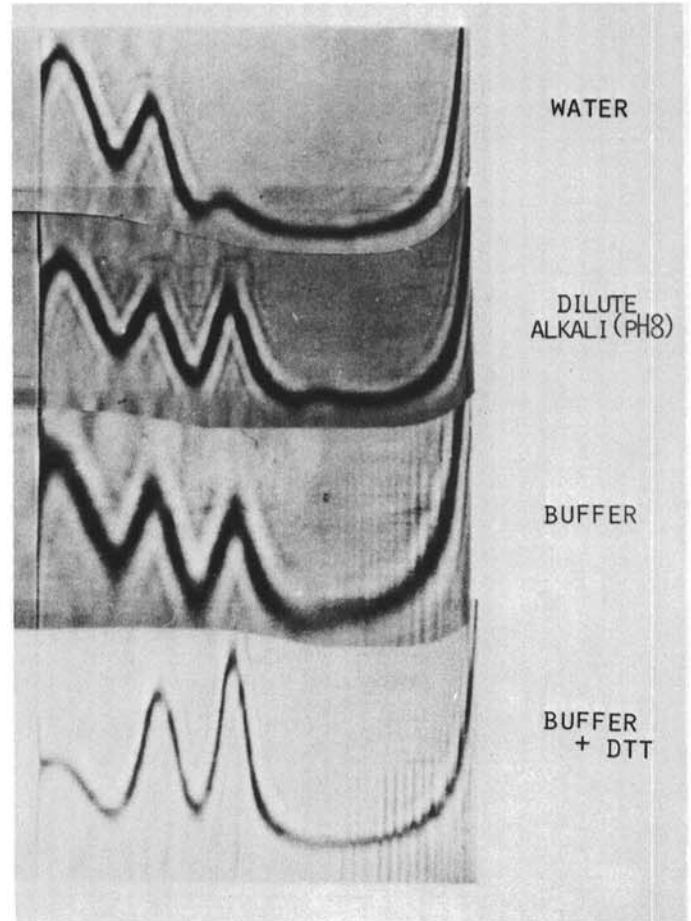


Fig. 7. Changes of relative proportion of protein components in isolates prepared from defatted meal stored at 35°C (85% rh) by extraction with different solvents. Photographs were taken after 45 min at 51,200 rpm.

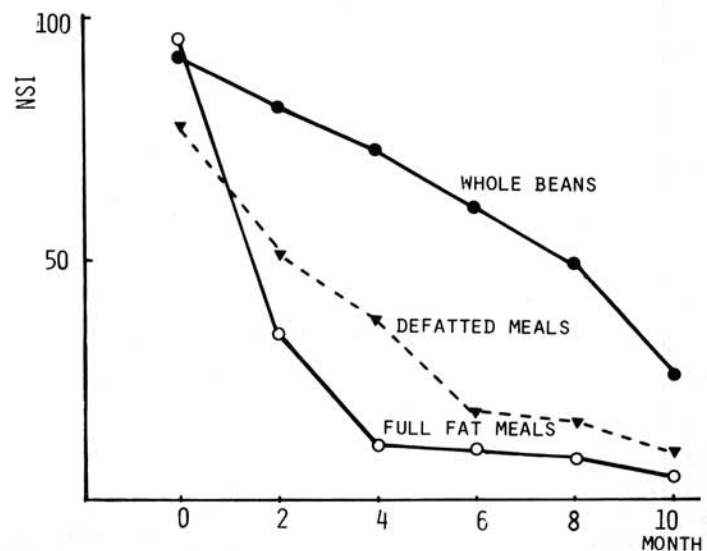


Fig. 8. Changes of nitrogen solubility index of whole beans, defatted meal, and full-fat meal stored at 35°C, 85% rh (1978 crop).

solvents, relative amounts of IIS component were low, and those of 2S components were high.

### 1978 Crop

Soybeans, defatted meals, and full-fat meals of the 1978 crop contained 13.00, 13.91, and 12.87% moisture; 41.82, 56.19, and 42.25% protein; and 22.66, 1.70, and 22.50% oil, respectively (expressed on a dry basis).

Figure 8 shows a change in NSI of whole beans, full-fat meals, and defatted meals stored for 10 months. The decrease in NSI occurred in decreasing order in the following samples: full-fat meals, defatted meals, and whole beans. Figure 9 shows changes in acid value and oil content extracted with ethyl ether of whole beans and full-fat meals, indicating that extractable oil from full-fat meals decreased to 16.67% after 10 months of storage.

The results of ultracentrifugal analysis of protein isolates from defatted meals of 1978 crops were almost the same as those for the 1977 crop; ie, the IIS component decreased, and 2S components increased relatively as storage time progressed. Changes in full-fat meals were extremely rapid. Most of the IIS component and part of the 7S component disappeared from the patterns for protein isolates prepared from full-fat meals stored at 35°C (85% rh) for more than four months, as shown in Fig. 10.

Decreased extractability of 7S and IIS proteins was also confirmed in PAGE patterns run without SDS or mercaptoethanol. The bands corresponding to 7S and IIS components were in the patterns of full-fat meals at storage times as long as two to four months, but after five to six months of storage, only fast-migrating bands were found. Figure 11A shows SDS-PAGE patterns of protein isolates prepared from full-fat meals stored at 35°C, (85% rh) for one, two, four, six, and eight months (Fig. 10). For reference, SDS-PAGE patterns of isolates prepared by extracting defatted meals stored at 35°C (85% rh) for four months with different solvents and those of isolates prepared from defatted meals stored at 35°C (85% rh) for zero, three, and five months are shown in Fig. 11B and C. Changes in relative proportions of protein components were not as clear in SDS-PAGE patterns as in the ultracentrifugation patterns; however, 7S and IIS components disappeared, and fast-migrating components (Fig. 11A-C, peaks 7 and 8) increased in full-fat meals. Increases of fast-migrating components also were found in defatted meals extracted with water and in those stored for five months.

### DISCUSSION

As storage time progressed under adverse temperature and humidity, 7S and IIS components became difficult to extract.

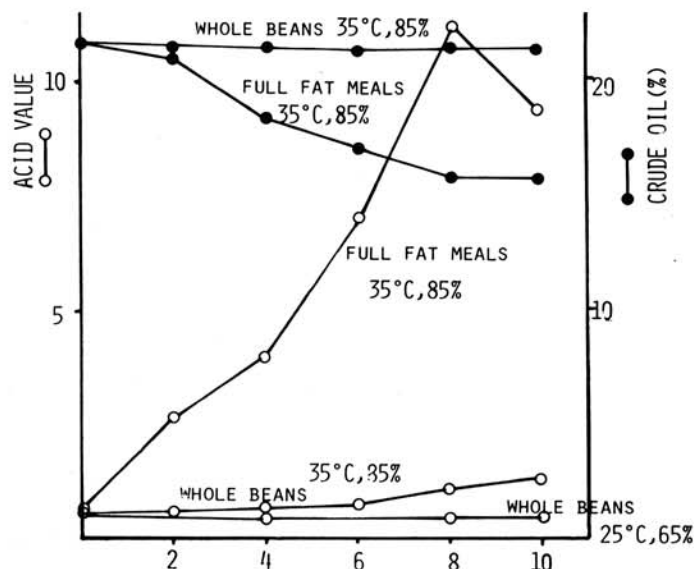


Fig. 9. Changes of acid value and extracted crude oil in whole beans and full-fat meal stored at 35°C, 85% rh, except for lowest acid value curve, which is for whole beans stored at 25°C (65% rh) as control (1978 crop).

Extractability of the IIS component, in particular, decreased to very low levels (Fig. 6). Consequently, the relative proportion of protein components in protein isolates prepared from the extracts with phosphate buffer showed a marked decrease of the IIS component and an increase of the 2S component in the ultracentrifugation patterns (Figs. 5 and 10). Fast-migrating components in electrophoretic patterns correspond to the 2S component in protein isolates. When our extractability results with different solvents were examined, a part of the IIS component seemed to be aggregated into an insoluble state involving S-S binding. Formation of disulfide polymers was reversed by treatment with DTT.

Whole beans were more resistant to deterioration during storage than meals, and full-fat meals deteriorated more rapidly than defatted meals. Quality seems to be more readily retained in the native state with the intracellular membranes intact. Nakayama et al (1981) reported that, during storage, activity of phospholipase D increased, and its inhibitor decreased with decomposition of the

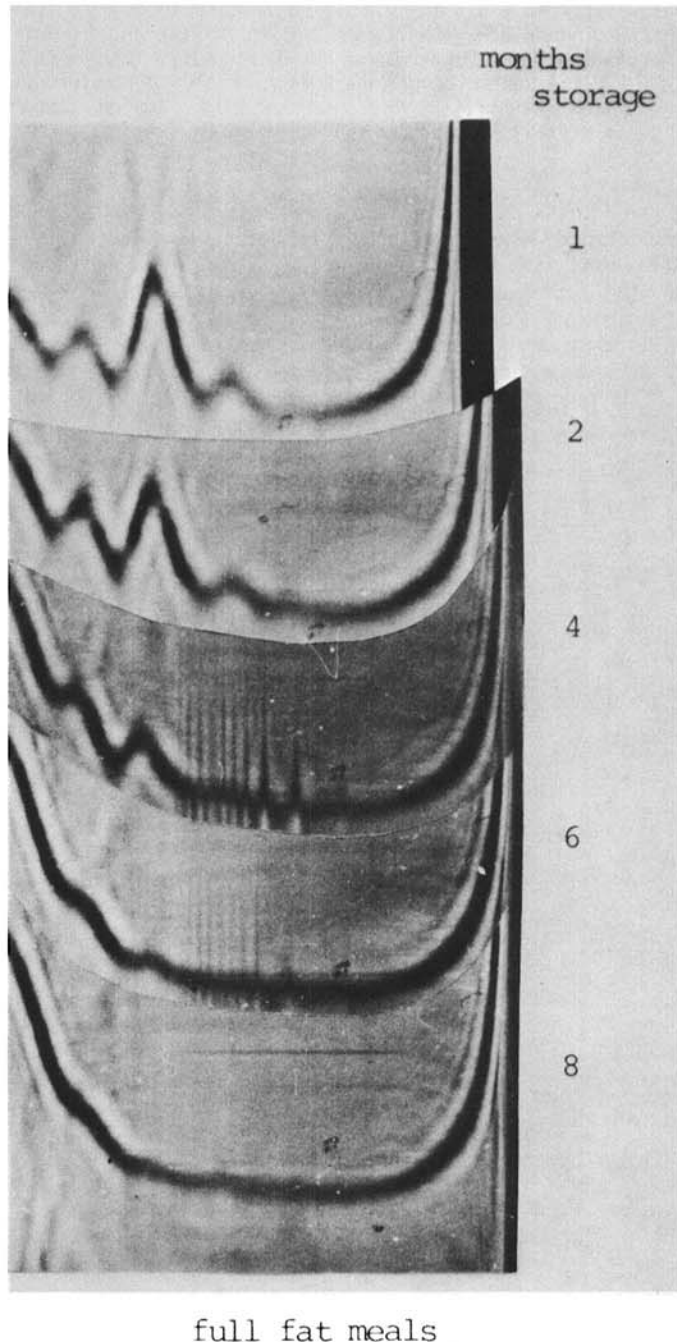
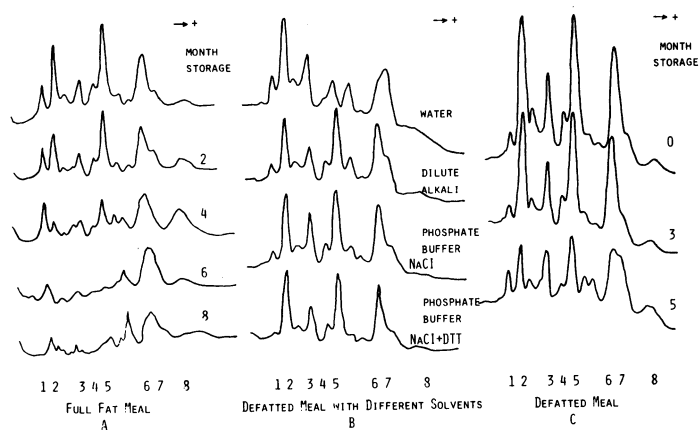


Fig. 10. Changes of relative proportion of protein components in isolates prepared from full-fat meal stored at 35°C, 85% rh. Photographs were taken after 45 min at 51,200 rpm.



**Fig. 11.** SDS-polyacrylamide gel electrophoresis patterns of full-fat meal and defatted meal. **A**, SDS-PAGE patterns of full-fat meals stored at 35°C (85% rh) for one, two, four, six, and eight months (1978 crop); **B**, SDS-PAGE patterns of defatted meals stored at 35°C (85% rh) for four months. Samples were prepared by extraction with different solvents (1977 crop); **C**, SDS-PAGE patterns of defatted meals stored at 35°C (85% rh) for zero, three, and five months (1977 crops). Peaks 1, 2, and 3 = 7S subunits; peaks 4, 5, and 6 = 11S subunits; peaks 7 and 8 = unknown.

phospholipids, which are important components of cellular membranes. Destruction of cellular membranes seems to accelerate the mutual reactions between proteins, fat, and other constituents. Studies with a transmission electron microscope have shown deformation of lipid bodies during improper storage of defatted and full-fat meals (unpublished). These changes may be the result of membrane alteration by phospholipase D.

High temperature and humidity decrease solubility of soybean

proteins, increase color and acid value of the oil, and alter other properties (Saio et al 1980). Our studies also indicate that storage of whole beans is preferable to storage of defatted or full-fat meals under adverse conditions if high extractabilities of proteins or oil are desired.

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