FEEDS

Analytical Evaluation of the Globulin Proteins of Cottonseed Meals

HENRY F. MARSHALL, JR[1] and EDITH J. CONKERTON
U.S. Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, PO Box 19687, New Orleans, LA 70179-0687

A method for the analytical evaluation of the globulin (salt-soluble) fraction of cottonseed meals has been developed. Cottonseed of 14% moisture content is stored for 6 and 9 weeks at 50°C. Meals obtained after seeds are defatted with pet ether are analyzed for nitrogen content, and then extracted with water. Residues are then extracted with 10% NaCl to isolate the globulin fraction. This fraction is characterized by liquid chromatography (LC) and gel electrophoresis (LDS-PAGE). Kjeldahl analyses showed no loss of nitrogen in meals from stored seed compared with those from unstored seeds. However, a large decrease in NaCl extractable nitrogen was noted in meals from stored cottonseed. LC and LDS-PAGE showed that an alteration of the globulin proteins occurred during storage, and the method demonstrated a progressive change in the 11S and 7S components of the globulin fraction.

Cotton is grown principally for its fiber, but the seed is an important by-product. In 1989, 5.8 million tons of cottonseed were produced in the United States, and 3.4 million tons were crushed to yield vegetable oil and meal, with economic values of $270 and $290 million, respectively (1). Although the latter is used principally as a feed for ruminant animals, it can serve as an important protein source in rations for nonruminants, and it has potential as a source of nutrients for human consumption (2). When freshly harvested cottonseeds are processed, both oil and meal are of high quality; however, most of the seed must be stored for weeks or months before it can be processed. During the storage period, biological processes in the seed are not dormant, and breakdown of seed structures occurs that causes deterioration (3). This deterioration could result in adverse changes in oil and meal quality. A recent research program at Southern Regional Research Center was directed toward preventing this deterioration. Decreasing oil quality can be measured easily by noting increases in the free fatty acid (FFA) content and/or refining loss of the oil. Meal quality measurement, however, is based only on protein content as determined by a modified Kjeldahl analysis (4) that measures total nitrogen (%N). Protein is estimated by multiplying %N by the factor 6.25. This method measures total nitrogen, i.e., protein and nonprotein nitrogen; it does not define changes in the protein quality of the meal that may occur as the seed deteriorates. Olcott and Fontaine (5) indicated that more than 80% of the nitrogen, designated by classical nomenclature as globulin proteins, was soluble in sodium chloride. They also determined that there was a correlation between the amount of these proteins and the nutritive quality of the meal. Later studies were directed toward the evaluation of nonstorage and storage proteins (water and alkali soluble nitrogen fractions) (6). The principal goal of these studies was the preparation of protein isolates having specific functional and nutritional properties for use in foods. The objective of the work reported here was to develop a precise, rapid method to evaluate changes in the protein quality of cottonseed meal that occurs during storage of the seed. Using the work of Olcott and Fontaine (5) as a guide, the meals were extracted with water, and then with sodium chloride. The globulin fraction was analyzed by liquid chromatography (LC) and gel electrophoresis (LDS-PAGE).

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[1] Present address: USDA, ARS, Rice Research, Route 7, Box 999, Beaumont, TX 77713.

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Experimental

Preparation of Cottonseed Meal Samples

Whole fuzzy cottonseed (200 g) was equilibrated to 14% moisture. Sample was divided into 3 equal portions. One portion was reserved for the control (0 week storage). The other 2 were placed in closed glass jars in a cabinet equipped with a forced air blower and thermostatically controlled to maintain a temperature of 50 ±2°C. One jar was removed for analysis after 6 weeks, and the other was removed after 9 weeks. If not processed immediately, samples were stored at 0°C until analyzed.

Preparation of Cottonseed Meal

Each sample of whole fuzzy cottonseed was frozen with liquid N_2 and placed in a blender. Contents were given a 2 s burst at full power to separate the fuzzy seed into linters, hulls, and kernels.

Cottonseed kernels were defatted with pet ether (40:1, solvent:kernels) by blending for 2 min in a Stomacher Lab Blender 400 (Tekmar Co., Cincinnati, OH 45222). Contents of the Stomacher bag were filtered to separate oil from meal. Residual meal was washed with 15 mL pet ether that was added to the filtrate. After evaporation of the solvent, oil was analyzed for FFA content. Meal was air dried overnight in the laboratory exhaust hood at ambient temperature (22°C).

Preparation of Protein Extracts

Cottonseed meal samples were ground with a Tekmar Mill, Model A-10 for 2 min. A consistency similar to that of a flour was obtained.

Samples were extracted with distilled water (50:1, solvent:meal) in 50 mL centrifuge tubes by continuous stirring for 30 min at ambient temperature. Contents were centrifuged for 30 min at 12 000 × *g* at 20°C. Supernatant was discarded. Pellets were extracted in the centrifuge tubes with 10% NaCl (25:1, solvent:meal) by continuous stirring for 30

Table 1. Effect of storage of high moisture content cottonseed at 50°C on nitrogen content (%) of defatted meal

	Storage, weeks		
	0	6	9
Total	8.63	8.20	8.58
Soluble[a]	51.88	10.93	3.49

[a] Percent of total nitrogen soluble in 10% NaCl.

min at ambient temperature. Contents were centrifuged for 30 min at 12 000 × *g* at 20°C. Residue was discarded. Supernatants were filtered immediately, using 0.45 and 0.22 μm Millipore syringe filters arranged in sequence. Portions of 100 μL of each extract were diluted with LDS-PAGE sample buffer (1:3 dilution for the 0 week sample, 1:2 for the 6 and 9 week sample), placed in a boiling water bath for 2 min, cooled, stoppered, and frozen until analyzed by LDS-PAGE. Remaining portion of each extract was frozen until analyzed by LC.

Analyses

Free fatty acids.—FFA content of oil was determined according to Official American Oil Chemists Society (AOCS) Method Ca 5a-40 (7).

Kjeldahl analyses.—Kjeldahl analyses were performed on meals and protein extracts according to AOCS Modified Kjeldahl Method Ba 4b-87 (7).

Electrophoresis (LDS-PAGE).—Gel electrophoresis was a modification of Laemmli (8) with lithium dodecyl sulfate (LDS) substituted for sodium dodecyl sulfate (SDS). Electrophoresis was performed in a Hoeffer SE 600 series vertical slab unit with power supplied by a PS 2500 DC Power Supply (Hoeffer Scientific Instruments, San Francisco, CA 94107). Conditions for electrophoresis were constant 20 mA per slab with a running time of 7 h. Gel formulation was 10% acrylamide with crosslinker concentration of 2.7% *bis*-acrylamide. Gels were stained with a 0.25% Coomassie Brilliant Blue R, 40% methanol, and 7% acetic acid solution.

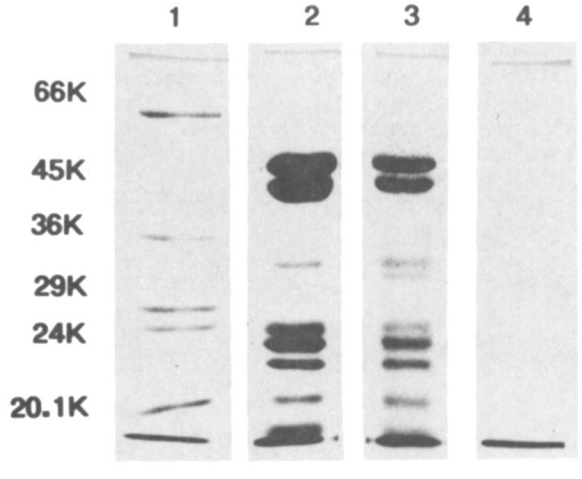

Figure 1. Comparison of electrophoretic patterns of salt-soluble globulin proteins extracted from meals prepared from unstored cottonseed and cottonseed stored at 50°C. (1) Molecular weight standard, (2) 0 weeks storage, (3) 6 weeks storage, and (4) 9 weeks storage.

Liquid chromatography.—Aliquots of the extracts (100 μL) were analyzed, with no further dilution, using a Pharmacia Superose 12 column, 30 cm × 10 mm id (Pharmacia LKB Biotechnology, Piscataway, NJ 08854). Eluant was 10% NaCl, pH 7.0, with a 0.5 mL/min flow rate. System was monitored at 280 nm using a Kratos 770R variable wavelength detector (Applied Biosystems, Inc., Foster City, CA 94404). A 286 PC programmed with Lab Calc software was used for data acquisition and processing (Galactic Industries Corp., Salem, NH 03079).

Results and Discussion

Oils extracted from unstored cottonseed and seed stored for 6 and 9 weeks had FFA contents of 0.8, 2.7, and 3.1%, respectively. This increase in FFA indicated that seed had undergone deterioration during storage.

The total nitrogen content of the 3 cottonseed meals measured by the Kjeldahl method showed no differences (Table 1). Conversely, after cottonseeds were stored for 6 and 9 weeks, there was a dramatic decrease in the amount of nitrogen extracted from the meal with 10% NaCl.

Earlier work has shown that proteins from cottonseed meals consist of 3 components, 11S, 7S, and 2S, as determined by sedimentation coefficients (9). Of these, the 11S and 7S components represent the primary globulin protein fraction (10). Previous investigations with SDS-PAGE showed 3 principal protein subunits of the 11S component. These have molecular weights of 22, 20, and 15K (11, 12). The 7S component has 2 principal subunits of 52 and 46K (11–13).

Results of LDS-PAGE of the globulin fractions extracted from meal prepared from seed stored for 0, 6, and 9 weeks are presented in Figure 1. Using the method of Laemmli (8), electrophoresis results in denaturation and reduction of proteins to their individual polypeptide subunits. Therefore, for the electrophoresis of cottonseed globulins, protein bands in the range of 52 and 46K should appear for the 7S component, and bands in the range of 22, 20, and 15K should be present for the 11S component. Allowing for the possible inherent error (±5%) in LDS-PAGE, these bands are readily apparent in the extract of meal from cottonseed stored for 0 weeks (Lane 2). Lane 3, the 6 week storage sample, shows some disappearance of the 7S component and considerable loss of the 11S component. The sample stored for 9 weeks (Lane 4) shows a complete absence of protein bands. However, the Coomassie Blue used for staining may not detect low levels of protein present in the latter sample.

LC (13–15) has been used for isolation and fractionation of the principal globulins of cottonseed. The elution profile obtained for the 0 week sample in the experiments reported here (Figure 2) does not differ significantly from those previously documented (13–15). Peak I corresponds to an aggregate of large molecular weight proteins, with Peaks II and III corresponding to the 11S and 7S components of the globulins, respectively. Kjeldahl analyses of the NaCl extracts indicate that during storage the amount of extractable globulins decreased dramatically: 1.78 mg N/mL, 0 weeks; 0.36 mg N/mL, 6 weeks; and 0.12 mg N/mL, 9 weeks. There was also a marked decrease in the 11S and 7S components from the samples stored for 6 and 9 weeks.

Table 2. Effect of storage of high moisture cottonseed at 50°C on nitrogen content (%) in water- and salt-soluble and insoluble fractions of cottonseed meals

	Storage, weeks		
Fraction	0	6	9
Water extract	26	9	8
10% NaCl extract	52	11	4
Residue	5	55	71

Because there was no change in total nitrogen content of the meals prepared from unstored cottonseed compared with meals from stored cottonseed, but there was a significant reduction in the globulin fraction of these meals, an attempt was made to determine how the solubility of the globulin fraction had been changed during storage. A second series of samples was extracted, as described in *Experimental*, but the supernatant from the water extraction and residues from the salt extraction were collected rather than discarded. Nitrogen analyses were performed on all fractions. Because of the manipulations and transfers involved, complete recovery of the nitrogen was not expected. However, 73% or more of the total nitrogen was accounted for in each of the samples (Table 2). These data also indicate a significant change in the solubility of the proteins from unstored cottonseed compared with that from stored seed. Initially, 78% of the protein was soluble; however, after storage for 6 and 9 weeks, the total soluble protein was 20 and 12%, respectively. Apparently, as the seed deteriorates, the proteins aggregate together to form nitrogenous compounds insoluble in water and salt rather than being hydrolyzed into small peptides or amino acids.

The reason for the reduced salt solubility of the globular proteins of meals from deteriorated cottonseed and potential effects on the actual nutritive quality of the meal cannot be determined from these data, and this information is beyond the scope of this study. The results indicate, however, that a simple determination of the salt soluble nitrogen content of cottonseed meal could be used to indicate deterioration of the proteins of cottonseed during storage.

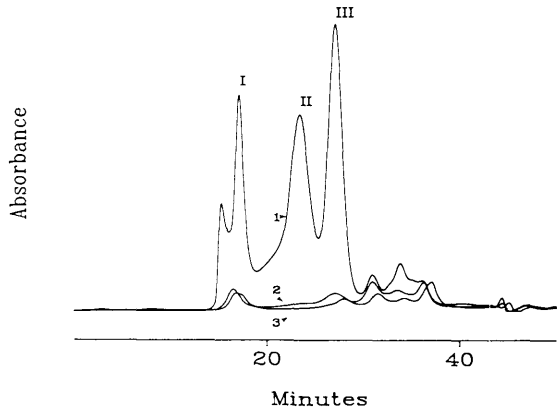

Figure 2. Comparison of LC patterns of salt-soluble globulin proteins extracted from meals prepared from unstored cottonseed and cottonseed stored at 50°C. (1) 0 weeks storage, (2) 6 weeks storage, (3) 9 weeks storage, (I) aggregate of large molecular weight proteins, (II) 11S component, and (III) 7S component.

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