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# Extractability, Solubility, and Molecular Size Distribution of Nitrogenous Constituents in Coastal Bermuda Grass

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Coastal Bermuda grass was extracted with buffer (pH 8.14) containing 0, 0.2, 0.5, or 1% sodium dodecylsulfate (SDS). The Bermuda grass was fractionated into insoluble residue ($R_I$), chloroplasts ($R_{II}$); and soluble fractions, high molecular weight (cut 1), polypeptides (cut 2), and low molecular weight (cut 3). At 0% SDS, total nitrogen ($N_T$) was cut 1 = 10.7 and 28.6% solubilized. Increasing percent SDS decreased percent $N_T$ in the insolubles, and increased $N_T$ in cut 1. At 1% SDS, the $N_T$ was cut 1 = 35.6 and 55.8% solubilized. At 0% SDS, the protein of cut 1 was predominantly cytoplasmic, but at higher percent SDS, it was predominantly chloroplastic with some cytoplasmic. It had a broad molecular weight range (1 million to 2500). The gel chromatography average molecular weight for cut 1 ranged from 21 000 to 5000 and depended on the wavelength of detection (i.e., 206 or 254 nm) and on percent SDS. The protein extracted with SDS had more aromatic impurities than that extracted at 0% SDS and, when lyophilized first, was insoluble in 0.8 N NaCl. The two-stage extraction suggested would maximize the yield of protein suitable for humans and give two other fractions suitable for monogastric and ruminant animals, respectively.

Geometric growth in world population has increased interest in new sources of high-quality proteins for supplementing animal and human diets. In the last decade, several plants have been investigated, but alfalfa received the most attention (Pirie, 1971). The proteins in alfalfa have been partially characterized by various methods. For example, nitrogen has been determined in isolated fractions by the Kjeldahl method and by trichloracetic acid precipitation (Betschart and Kinsella, 1973), amino acids, by ion-exchange chromatography (Byers, 1971), and component proteins by gel chromatography (Sarkar et al. 1975; Knuckles et al., 1975). Grasses, particularly, those that fix $CO_2$ via the phosphoglyceric acid pathway (i.e., C-3 grasses), also have been investigated as sources of extractable protein. In contrast, little work has been done on the extractability, solubility, and molecular size dis-

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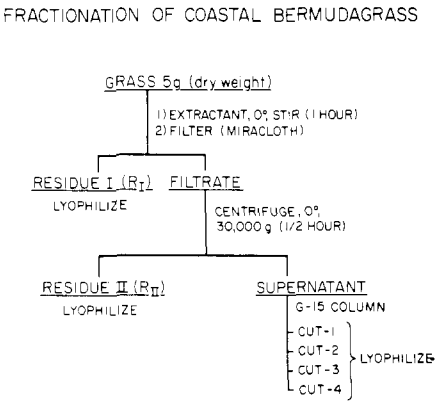


**Figure 1.** Flow diagram for the preparation of fractions from Coastal Bermuda grass.

tribution of the nitrogenous constituents in grasses that fix $CO_2$ via the dicarboxylic acid pathway (i.e., C-4 grasses). Due to decreased photorespiration, these grasses have high photosynthetic efficiency (Zelitch, 1975) which results in high productivity. One such grass, Coastal Bermuda grass (Chen et al., 1971), is grown on an estimated 10 million acres in the South. When immature it can contain 13–18% crude protein of good quality as indicated by amino acid analysis (Wilkinson, 1968).

We fractionated the nitrogenous constituents of Coastal Bermuda grass according to extractability, solubility, and the molecular size distribution of detergent-soluble constituents. Each fraction was analyzed for nitrogen content, molecular size distribution, resolubility in 0.8 N NaCl, and changes in aromatic content. We chose conditions of extraction and fractionation that would minimize destruction of the primary protein structure and maximize yield. We believe that the system of fractionation and analysis are important laboratory tools for evaluating the potential of Coastal as a source of protein concentrate and that the approach could be a model for evaluating other grasses. Also, the methodology characterizes more completely than other methods the nitrogenous constituents in feedstuffs intended for ruminants. Recently, Burroughs pointed out the need for such characterization of protein in feedstuffs (Burroughs et al., 1974a,b).

## EXPERIMENTAL SECTION

**Coastal Bermuda Grass.** Coastal Bermuda grass (*Cynodon dactylon* (L.) Pers) was obtained from Coastal Farms, Inc., Estill, S.C. It was harvested in late July, 30 days after the third cutting from a well-established sod that had been fertilized with 200 lb of N/acre at the beginning of the growing season and with 50 lb of N/acre after each cutting. The Coastal was frozen in the field immediately after harvest and stored at −20 °C. Later, the material was freeze-dried, ground to pass a 420 μm screen, ground with a ball mill for 96 h at 5 °C, and stored at 5 °C until extraction. Earlier work indicated no difference in the amount of extractable protein for fresh Coastal compared to freeze dried (Evans et al., 1974). Micro-Kjeldahl analysis (McKenzie and Wallace, 1954) revealed that the forage contained 13.75 ± 0.18% crude protein (N × 6.25) on a dry weight basis.

**Composition of Extractant.** The ground lyophilized forage (5 g dry weight) was extracted with 100 mL of buffer containing 0.1 M boric acid–sodium borate buffer adjusted to pH 8.14 at 20 °C and 5 mmol of sodium metabisulfite. In addition 0, 0.2, 0.5, or 1.0% w/v of sodium dodecylsulfate (SDS) was added to the extractant.

**Fractions.** Figure 1 is a flow sheet for the fractionation procedure. Triplicate samples were run with each extractant composition. The supernatant was fractionated into four cuts on a 2.5 × 85 cm column packed with G-15 Sephadex. Typically, 75–80 mL was pumped onto the column with a peristaltic pump (Buchler Inst., Fort Lee, N.J.) at ca. 6 mL/min, then eluted through the gel with distilled water at 3 mL/min under the action of a piston pump (Waters Associates, Framingham, Mass.) which had been switched into the system with a six port valve (LDC, Riviera Beach, Fla.). The effluent from the column passed through a UV flow through monitor (254 nm) attached to a potentiometric recorder, just prior to collection of the fractions. Figure 2 is a typical chromatogram. The time of the cuts was chosen to obtain a fraction containing molecules greater than 1500, one containing polypeptides, and one containing low molecular weight molecules. Each fraction was lyophilized immediately after collection. The freeze-dried fractions were weighed and stored in closed vials at 5 °C until use.

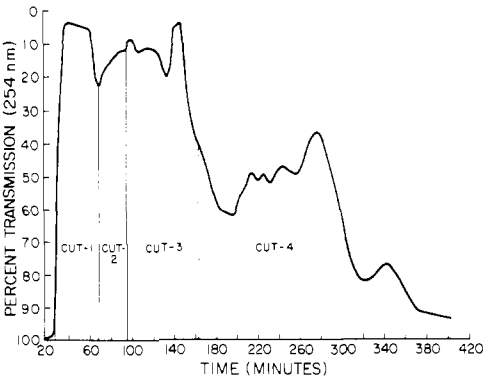


**Figure 2.** Chromatograph of supernatant (cuts 1–4) on G-15 Sephadex (see text for conditions).

**Table I. Packing Parameters of Four-Column Chromatograph**

| Column | Packing | Height, cm | Vol, mL |
|---|---|---|---|
| 1 | Sephadex G 10, 25[a] | 13.1 | 3.70 |
| 2 | Sephadex G 50, 75[a] | 12.6 | 3.56 |
| 3 | Bio-Gel A-0.5 m | 11.7 | 3.31 |
| 4 | Bio-Gel A-5 m | 11.5 | 3.24 |
| | Total | 58.9 | 13.82 |

[a] Mixed to give equal bed volumes.

Crude protein, micro-Kjeldahl, was determined on each fraction. Percent nitrogen values were corrected for moisture by measuring loss on drying over 24 h at 100 °C.

**Gel Chromatography.** Analytical gel chromatography was performed on cuts 1–3 as described (Fishman, 1976) with the following modifications. Five or fewer samples were injected serially with an automatic sample loading system (M.E.R. Chromatographic, Mountain View, Ca.). Sample loops were calibrated, with a microliter syringe (Hamilton Co., Reno, Nev.), to hold 0.200 mL.

Four 330 × 6 mm columns with adjustable end fittings (6 μm disc) (LDC, Riviera Beach, Fla.) were packed and connected in series. The packing procedure was to fill the column with a concentrated slurry of gel and degassed solvent, pump the slurry under a flow of 0.12 mL/min until all but the fine particles settled and only a light blue haze remained above the gel bed. Then, the outlet was closed, the pump disconnected, and excess fluid was withdrawn until only a few centimeters remained above the gel bed. The procedure was repeated until the desired bed height was obtained. Packing and column parameters are given in Table I. The operating range, as determined by the calibration curve in Figure 3, is 669 × $10^3$ to 0.426 × $10^3$

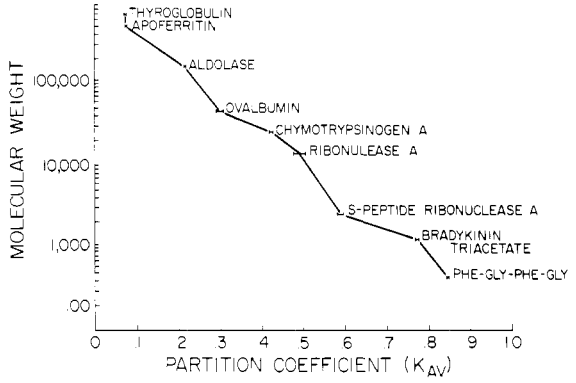


**Figure 3.** Calibration curve for four-column gel chromatograph.

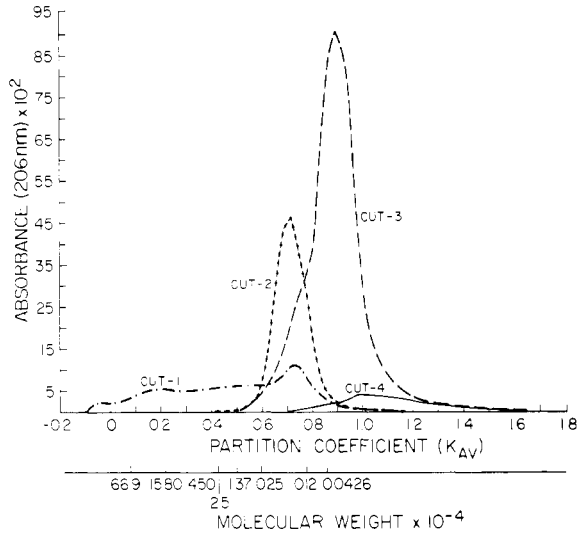


**Figure 4.** Gel chromatogram of soluble fractions. Each cut was run separately on four column chromatograph. Extractant contains 1% SDS.

for globular proteins and polypeptides. Molecular weights were calculated on the assumption that intervals between calibration points were linear. The absorbance of chromatographed materials was measured and recorded at 206 and 254 nm with a flow-through monitor (LKB, Rockville, Md.) connected to a dual-channel potentiometric recorder. The wavelength at 206 nm was chosen to measure amide linkages in the proteins, while 254 nm was chosen to measure aromatic groups.

Flow rate and elution volumes were measured by collecting the eluent with a fraction collector (LKB, Rockville, Md.) in drop counting mode. An event marker on the recorder was signaled every five drops. The drop counter was calibrated by collecting the eluent in a centrifuge tube and measuring the volume of 20 drops with a microliter syringe. The long-term flow rate ranged from 0.108 to 0.0917 mL/min. Void ($V_o$) and total bed volume ($V_T$) were determined periodically by measuring the elution volume of a mixture of Blue Dextran and Sodium Azide at peak maximums. The results of 17 measurements gave 6.76 ± 0.27 mL for $V_o$ and 16.84 ± 0.53 mL for $V_T$. The maximum length of a run (injection to baseline return) was 192 min. Based on triplicate measurements of ribonuclease A, which penetrates about 50% of the gel pores, the height equivalent to a theoretical plate (HETP) was 1.45 ± 0.28 nm and the maximum number of resolvable peaks was 5.01 ± 0.35 (for calculations see Martin and Synge, 1941; Giddings, 1967).

The long-term pressure was 60 to 90 psi as measured by a Bourdon gauge (LDC, Riviera Beach, Fla.) depending

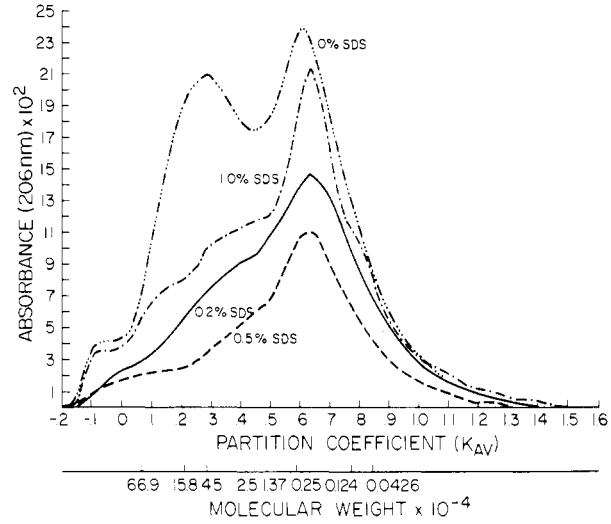


**Figure 5.** Gel chromatograms of cut 1 extracted with various concentrations of SDS in extractant. Wavelength of detection 206 nm. Chromatograms have not been normalized for concentration (3–6 mg/mL).

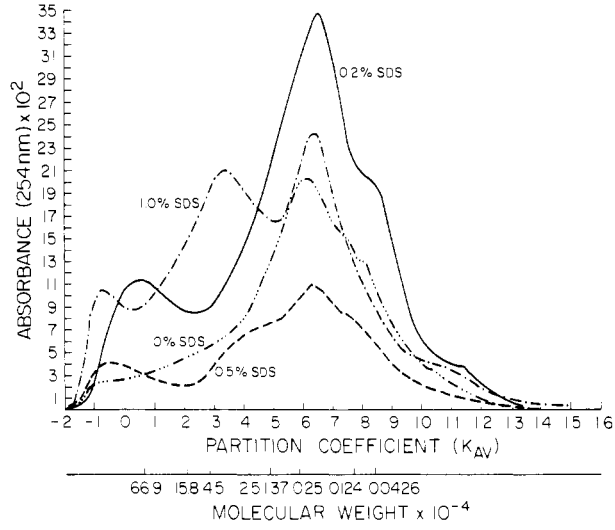


**Figure 6.** Gel chromatograms of cut 1 extracted with various concentrations of SDS in extractant. Wavelength of detection 254 nm. Chromatograms have not been normalized for concentration (3–6 mg/mL).

on minor adjustments of the peristaltic pump (Buchler Inst., Fort Lee, N.J.), each time the tubing was changed. It was necessary to elute with 0.8 N NaCl to prevent retardation of the large proteins (e.g., apoferritin or aldolase).

For comparison, chromatograms were replotted by a computer in terms of the column partition coefficient, $K_{AV}$ (Figures 4, 5, and 6). Molecular size distributions of nitrogenous constituents are characterized by the first and second moments of the gel curve, $\bar{K}_{AV_1}$ and $\bar{K}_{AV_2}$, respectively. The apparent first moment molecular weights, $M_{A_1}$, were from $\bar{K}_{AV_1}$ and calibration data in Figure 3. Information retrieval from chromatograms, mathematical definitions of $\bar{K}_{AV_1}$, $\bar{K}_{AV_2}$, and $K_{AV}$ were reported elsewhere (Fishman, 1976).

## RESULTS AND DISCUSSION

**Extractability and Initial Solubility of Nitrogeneous Constituents.** Figures 7 and 8 show the effects of SDS concentration on the extractability and solubility of nitrogeneous constituents in Coastal Bermuda grass. $R_I$, residue I (Figure 1), is the insoluble residue of Coastal

Table II. Moments and Molecular Weights from Gel Chromatography

| % SDS | Cut | 206 nm $\bar{K}_{AV_1}$ | 206 nm $\bar{K}_{AV_1}/\bar{K}_{AV_2}$ | 206 nm $M_{A_1} \times 10^{-3}$ | 254 nm $\bar{K}_{AV_1}$ | 254 nm $\bar{K}_{AV_1}/\bar{K}_{AV_2}$ | 254 nm $M_{A_1} \times 10^{-3}$ |
|---|---|---|---|---|---|---|---|
| 0 | 1 | 0.446 ± 0.041[a] | 0.880 ± 0.021 | 21.2 ± 1.2 | 0.566 ± 0.059 | 0.907 ± 0.027 | 5.2 ± 6.7 |
| 0.2 | 1 | 0.520 ± 0.021 | 0.900 ± 0.020 | 10.5 ± 2.4 | 0.572 ± 0.024 | 0.883 ± 0.018 | 4.6 ± 2.7 |
| 0.5 | 1 | 0.565 ± 0.009 | 0.915 ± 0.003 | 5.4 ± 1.0 | 0.557 ± 0.015 | 0.870 ± 0.014 | 6.3 ± 1.7 |
| 1.0 | 1 | 0.525 ± 0.030 | 0.896 ± 0.009 | 9.9 ± 3.4 | 0.492 ± 0.037 | 0.870 ± 0.013 | 13.7 ± 5.0 |
| 0 | 2 | 0.752 ± 0.045 | 0.984 ± 0.007 | 1.78 ± 0.20 | 0.750 ± 0.049 | 0.984 ± 0.006 | 1.79 ± 0.22 |
| 0.2 | 2 | 0.724 ± 0.014 | 0.983 ± 0.005 | 1.90 ± 0.06 | 0.734 ± 0.014 | 0.984 ± 0.006 | 1.85 ± 0.06 |
| 0.5 | 2 | 0.751 ± 0.017 | 0.992 ± 0.006 | 1.78 ± 0.08 | 0.756 ± 0.019 | 0.992 ± 0.007 | 1.19 ± 0.08 |
| 1.0 | 2 | 0.762 ± 0.043 | 0.983 ± 0.008 | 1.73 ± 0.19 | 0.777 ± 0.059 | 0.985 ± 0.006 | 1.19 ± 0.67 |
| 0 | 3 | 0.965 ± 0.089 | 0.986 ± 0.008 | <0.426 | 0.961 ± 0.087 | 0.985 ± 0.011 | <0.426 |
| 0.2 | 3 | 0.892 ± 0.045 | 0.977 ± 0.004 | <0.426 | 0.910 ± 0.043 | 0.975 ± 0.009 | <0.426 |
| 0.5 | 3 | 0.858 | 0.981 | <0.426 | 0.866 ± 0.044 | 0.971 | <0.426 |
| 1.0 | 3 | 0.866 ± 0.037 | 0.980 ± 0.009 | <0.426 | 0.847 ± 0.039 | 0.980 ± 0.008 | <0.426 |

[a] Standard error of six measurements (three extractions × two chromatographs) for cuts 1 and of three measurements (three extractions × one chromatograph) for cuts 2 and 3.

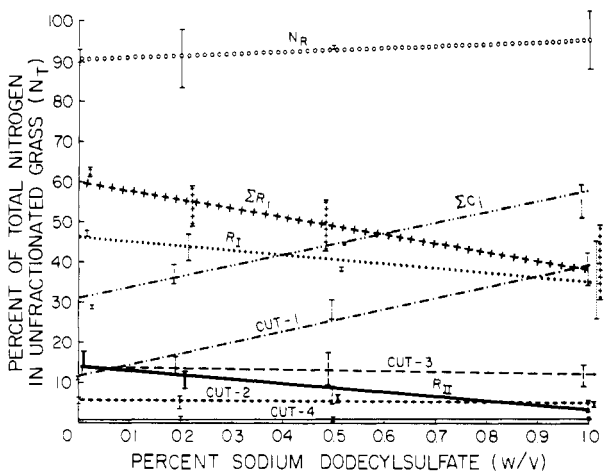


**Figure 7.** Dependence of percent total nitrogen ($N_T$) in each fraction on percent sodium dodecyl sulfate in extractant; $\Sigma R_i$ is sum of $R_I$ and $R_{II}$; $\Sigma C_i$ is sum of cuts 1–4; $N_R$ is sum of all fractions.

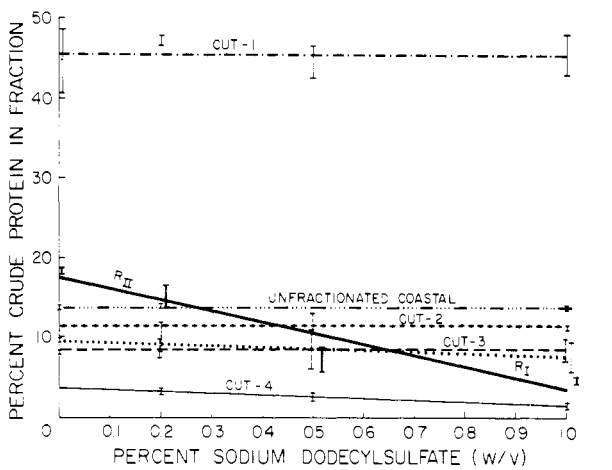


**Figure 8.** Dependence of crude protein composition in each fraction on percent SDS in extractant (see Figure 7 for meaning of symbols).

Bermuda grass; $R_{II}$, residue II, is the extractable but insoluble fraction (predominantly chloroplasts or fragments thereof). Cut 1 (Figures 1 and 2) is comprised predominantly of molecules over molecular weight 1500; cut 2 about 1500; cut 3 less than 1500; and cut 4 those molecules retarded by adsorption to the G-15 packing. A previous study (Fishman and Evans, 1974) indicated that rapid separation of cut 1 from lower molecular weight fractions stabilized cut 1 from continuing degradation and precipitation. Cuts 2–4 were chosen arbitrarily. Initially cuts 1–4 are soluble. Figure 7 shows that little or no correlation between detergent concentration and nitrogen content ($N_T$) in cuts 2 and 4. Increasing the detergent concentration decreases $N_T$ slightly in cut 3 but increases it markedly in cut 1, which in turn causes $N_T$ in the total solubles ($\Sigma$Ci) to rise. $R_I$ and $R_{II}$ contribute equally to the accumulation of $N_T$ in cut 1 with increasing detergent. The decrease in $N_T$ from the sum of the insolubles ($\Sigma$Ri) is also shown in Figure 7, where $N_R$ is the percent nitrogen recovered from all fractions. Figure 8 shows that crude protein (CP) is independent of detergent concentration in cuts 1–3 and $R_I$, while CP decreases slightly in cut 4 and markedly in $R_{II}$. CP values for cut 1 at all levels of detergent and for $R_{II}$, extracted without detergent, are significantly higher than values from unfractionated Coastal, but all other values are lower. The initial solubilities of cuts 1–4 indicate they are low in fiber, a necessary but not sufficient condition for feeding these cuts to monogastric animals.

The foregoing results suggest that SDS enhances the extraction and disruption of the plant cells and of the chloroplasts within them. Further, the ratio of nitrogeneous to nonnitrogeneous soluble macromolecules (cut 1) is nearly constant (slope cut 1 $\simeq$ 0, Figure 8) regardless of origin in the plant cell. SDS-solubilized macromolecules from $R_I$ may arise from chloroplasts which were not extracted at 0% SDS. This is indicated by the decrease in the green color of $R_I$ with increasing SDS until at 1:1 (wt grass:wt SDS), $R_I$ is off-white.

**Molecular Weight Distribution from Gel Chromatography.** In Figure 4, gel chromatographs of the four soluble fractions of Coastal Bermuda grass, extracted with buffer containing 1% SDS and chromatographed separately on the multicolumn system (Table I), were superimposed. Since cut 4 (<1.27% $N_T$) is retarded by adsorption, gel chromatography gives no information on its size distribution. Table II contains the first moment, $\bar{K}_{AV_1}$, the ratio of first to second moments $K_{AV_1}/K_{AV_2}$, and the first moment apparent molecular weight, $M_{A_1}$, for soluble fractionates 1–3. Cuts 2 and 3 are narrow size distributions (i.e., $\bar{K}_{AV_1}/\bar{K}_{AV_2}$ is 0.98–0.99; see Fishman, 1976) with $M_{A_1}$ in the range 2–1.5 × $10^3$ and <456.5, respectively. Molecular size and weight parameters for cuts 2 and 3 are independent of detergent concentration and wavelength of detection. The molecular size ($K_{AV_1}$) and weight ($M_{A_1}$) parameters of cut 1 in Table II are dependent on SDS concentration and wavelength. At 206 nm, the $M_{A_1}$–SDS concentration relationship passes through a minimum. At 254 nm, the $M_{A_1}$–SDS function is constant over 0–0.5 g/100 $cm^3$ and more than doubles at 1.0% SDS. Moreover, at 0 and 0.2% SDS, $M_{A_1}$ is larger at 206 nm than at 254 nm. A similar result was found for the unbuffered

Table III. Solubility and Purity Parameters from Gel Chromatography

| % SDS | Cut | $A_{206} \times 10^2$[a] | $A_{254} \times 10^2$[a] | $A_{206}/A_{254}$ | $F_A$[b] | $F_S$[c] |
|---|---|---|---|---|---|---|
| 0 | 1 | 395 ± 46[d] | 46.7 ± 18.7 | 8.5 ± 1.6 | 1.0 | 1.00 |
| 0.2 | 1 | 206 ± 25 | 38.6 ± 4.5 | 5.3 ± 0.1 | 1.6 ± 0.3 | 0.52 ± 0.12 |
| 0.5 | 1 | 82 ± 30 | 21.3 ± 8.3 | 3.8 ± 0.3 | 2.2 ± 0.5 | 0.21 ± 0.10 |
| 1.0 | 1 | 111 ± 17 | 31.2 ± 4.0 | 3.7 ± 0.2 | 2.3 ± 0.6 | 0.28 ± 0.08 |
| 0 | 2 | 381 ± 15 | 70.8 ± 1.7 | 5.1 ± 0.1 | 1.0 | 1.00 |
| 0.2 | 2 | 408 ± 49 | 83.3 ± 2.7 | 4.7 ± 0.3 | 1.1 ± 0.1 | 1.07 ± 0.17 |
| 0.5 | 2 | 305 ± 13 | 60.3 ± 2.5 | 5.1 ± 0.1 | 1.0 ± 0.0 | 0.80 ± 0.07 |
| 1.0 | 2 | 337 ± 29 | 67.8 ± 3.6 | 4.9 ± 0.3 | 1.0 ± 0.1 | 0.88 ± 0.11 |
| 0 | 3 | 532 ± 22 | 64.4 ± 7.4 | 8.5 ± 0.6 | 1.0 | 1.00 |
| 0.2 | 3 | 486 | 75.1 ± 6.9 | 7.0 ± 0.3 | 1.2 ± 0.1 | 0.91 |
| 0.5 | 3 | 547 | 61.6 | 8.9 | 1.0 | 1.02 |
| 1.0 | 3 | 531 ± 17 | 52.1 ± 4.7 | 9.9 ± 2.9 | 0.9 ± 0.3 | 1.00 ± 0.07 |

[a] Units are mL/mg (N × 6.25); A refers to area; subscript refers to wavelength in nanometers. [b] Fractional increase in absorbance due to aromatics (see eq 1 in text). [c] Fraction soluble (see eq 2 in text). [d] Standard error of six measurements (three extractions × two chromatographs) for cuts 1 and of three measurements (three extractions × one chromatograph) for cuts 2 and 3.

aqueous extract of Coastal (Fishman, 1976), but in this work the reverse occurs at 1.0% SDS. These trends in $M_{A_1}$ are strongly influenced by changes in the amount of material which chromatographs in the range of $K_{AV}$'s between 0.2 and 0.6; that material corresponds to a molecular weight range of (186–2.43) × $10^3$. At 206 nm and 0% SDS there is a peak at a $K_{AV}$ of 0.294 ± 0.042 and $M_{A_1}$ of 50 × $10^3$ that becomes less prominent when SDS is used (Figure 5). At 254 nm and 1% SDS there is a peak at a $K_{AV}$ of 0.391 ± 0.042 and 28.7 × $10^3$ (Figure 6). This peak is not apparent at 0% SDS but gradually appears with increasing SDS concentration. The significance of these shifts will be discussed in the next two sections.

**Aromatic Content.** Of the four extractants, the 0% SDS solution favors the extraction of protein that is low in aromatic groups. The inclusion of SDS in the extract may dissociate the protein that is extractable at 0% SDS into polypeptides, or may extract other polypeptides similar in composition but lower in molecular weight. Eventually, SDS increases the aromatic groups in cut 1, as indicated by the decrease in $\bar{K}_{AV_1}$ at 1% SDS and 254 nm (see Table II). Simultaneously there is an increase in the solubilization of chloroplastic protein. It is believed that quinones, phenols, and other tanning agents in the chloroplasts could combine with proteins (Loomis and Battaile, 1966). Such reactions could explain the increase in absorbance at 254 nm, as confirmed by the data in Tables II and III, and possibly increase protein precipitation through crosslinking (Fishman and Evans, 1974).

The parameter $A_{206}/A_{254}$ in Table III is independent of sample weight or protein concentration. Changes in the ratio with SDS concentration result from changes in the composition of the functional groups which absorb. The fractional change ($F_A$) in absorbing functional groups that occur at 0 to finite SDS concentration is given by the following equation:

$$F_A = (A_{206}/A_{254})_{SDS=0}/(A_{206}/A_{254})_{SDS=X} \quad (1)$$

where $(A_{206}/A_{254})_{SDS=0}$ is the ratio of areas at 0% SDS; $(A_{206}/A_{254})_{SDS=X}$ is the ratio of areas at finite SDS concentration.

If it is assumed that all changes in $A_{206}$ are changes in solubility (see next section), $F_A$ is the fractional change in absorption of aromatic groups from Table III, and the $F_A$ of cut 1 increases with increasing SDS to 0.5 and levels off. The aromatic character of cuts 2 and 3 appears to be independent of detergent concentration. Nevertheless, $A_{206}/A_{254}$ values are lower for cut 2 than for cut 3, therefore aromatic composition is higher. Moreover, the $A_{206}/A_{254}$ value for cut 3 is similar to that of cut 1 at 0% SDS. All fractions are higher in aromatics than the standard proteins in Table IV which suggests that some of the aromatics isolated in these extracts are contaminants and not aromatic amino acids.

Table IV. Areas of Standards from Gel Chromatography

| Protein | $A_{206} \times 10^2$[a] | $A_{254} \times 10^2$[a] | $A_{206}/A_{254}$ |
|---|---|---|---|
| Thyroglobulin | 358 ± 13[b] | 10.6 ± 0.4 | 33.8 ± 0.6 |
| Aldolase | 339 ± 10 | 4.9 ± 0.1 | 68.2 ± 1.3 |
| Ovalbumin | 365 ± 36 | 5.1 ± 0.5 | 71.3 ± 5.2 |
| Chymotrypsinogen A | 434 ± 16 | 14.0 ± 0.7 | 31.0 ± 0.7 |
| Ribonuclease A | 299 ± 29 | 3.8 ± 0.8 | 79.3 ± 3.1 |
| Ribonuclease A-S-PEPTIDE | 581 ± 37 | 3.4 ± 0.7 | 170.8 ± 3.7 |
| Bradykinin triacetate | 459 ± 19 | 6.2 ± 0.5 | 73.8 ± 2.5 |
| Phe-Gly-Phe-Gly | 598 ± 23 | 8.2 ± 0.5 | 13.0 ± 2.1 |
| Blue dextran | 82 ± 6 | 53.9 ± 3.0 | 1.5 ± 0.06 |
| Sodium azide | 5400 ± 500 | 151.2 ± 12 | 35.8 ± 0.6 |

[a] Units are mL/mg; A is area; subscript is wavelength in nanometers. [b] Standard error of five measurements.

**Solubility.** At 0% SDS, cut 1 is a light, straw-colored extract that freeze-dries to a cream-colored powder, redissolves in 0.8 N NaCl to form a straw colored solution that leaves negligible residue on passing through at 0.45 μm membrane filter. With SDS, cut 1 is a green solution whose ultraviolet absorption spectra is typical of a chlorophyll–protein complex. Lyophilization yields a green powder that partially redissolves in 0.8 N NaCl, leaves an oily green residue on the filter, and gives a filtrate similar to that from the extract at 0% SDS concentration. Cuts 2 and 3 at all detergent concentrations are similar to cut 1 at 0% SDS in color and solubility.

In Table III, $A_{206}$ and $A_{254}$ refer to areas under the gel curves (See Figures 4, 5, and 6) normalized to unit concentration of protein (N × 6.25) mg/mL. Subscripts refer to the wavelength (nm) of measurements. These areas can be used to measure the changes in solubility of the lyophilized cuts with SDS composition in the extractant, provided changes in the composition of aromatic groups in the protein do not interfere. In Table IV are the normalized areas of the gel curves for a series of proteins. The areas at 206 nm are comparable to cut 1, 0% SDS (see Table III), but are smaller by a factor of 5 to 10 at 254 nm. Cut 1, 0% SDS, is chosen as the basis of comparison because it is the only high molecular weight fraction that is completely soluble by visual observation. If it is assumed that the 206 nm areas are not affected by changes in aromatic composition, it follows that the fraction soluble ($F_S$) at each detergent concentration in a cut can be computed from:

$$F_S = (A_{206})_{SDS=X}/(A_{206})_{SDS=0} \quad (2)$$

where $(A_{206})_{SDS=X}$ is the area at finite detergent concentration; $(A_{206})_{SDS=0}$ is the area at 0 detergent concentration.

From Table III, $F_S$ decreases as SDS increases to 0.5 and then levels off for cut 1. Moreover, the arithmetic products of $F_S$ and total nitrogen (i.e., total nitrogen resolubilized) in cut 1 are 11.8, 9.6, 6.4, and 11.7 mg, respectively at 0, 0.2, 0.5, and 1% SDS. Therefore, SDS increases extractability and initial solubility, but decreases resolubility for cut 1 (see $F_S$, Table III). Cuts 2 and 3 are completely soluble at all detergent concentrations within experimental error. The solubility results for all cuts agree with qualitative visual observations.

## CONCLUSIONS

The nitrogenous constituents of freeze-dried Coastal Bermuda grass can be classified as insoluble ($R_I$), extractable-insoluble ($R_{II}$), extractable-temporarily soluble (SDS extractable cut 1), extractable-soluble (cut 1 at 0% SDS, cut 2, and cut 3). These classifications serve as a functional method of determining the end use of forage protein for maximum utilization as food or feedstuff. Cut 1 soluble ($N_T \simeq 11\%$); and possibly cuts 2 and 3 ($N_T \simeq 18\%$) might be made suitable for human use. Cut 1, SDS extractable ($N_T \simeq 28\%$ at 1% SDS), cut 2 and 3, and possibly $R_{II}$ ($N_T \simeq 5\%$ at 1% SDS) might serve as a protein source for monogastric animals and $R_I$ and $R_{II}$ ($N_T \simeq 40\%$ at 1% SDS) for ruminants.

The degradation or precipitation of cut 1 could be minimized by separating it, before the chloroplastic proteins are solubilized and with the utmost rapidity, from cuts 2 and 3. Here, cut 1 was separated from cuts 2 and 3 in less than 4 h from the start of the extraction. Since $N_T$, percent CP, molecular weight distribution parameters (Table II), solubility parameters (Table III), and the content of aromatics (Table III) are independent of SDS concentration for cuts 2 and 3, a two-stage procedure apparently would be appropriate: stage 1, extract with buffers, antioxidant and mild conditions to separate $R_I$ and $R_{II}$ from cuts 1–3; stage 2, extract $R_I$ and $R_{II}$ with harsher conditions (e.g., detergent, pressure, heat, organic solvents, etc.) which would separate maximum chloroplastic protein.

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# Determination of *all-trans* and *13-cis* Vitamin A in Food Products by High-Pressure Liquid Chromatography

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A high-pressure liquid chromatography (HPLC) procedure has been developed for the determination of retinol and its esters in food products. The method quantitates both the all-trans and 13-cis isomers. The presence of the 13-cis isomer in a number of food products was demonstrated. Recovery studies on different food products showed an average recovery of 94.6 ± 6.6%. Reproducibility data were generated showing a pooled relative standard deviation of 3.9%. The HPLC procedure was compared with an AOAC colorimetric procedure for six products; there was no statistical difference between the means.

A major challenge facing the food chemist is the development of more accurate and cost effective methods for nutrient analysis. High-pressure liquid chromatography (HPLC) is a technique which is finding many applications in this area. The technique is potentially fast, specific, and sensitive; it offers many advantages over more commonly used chemical methods (Clifford, 1976).

HPLC has been used extensively for the analysis of fat-soluble vitamins in concentrates, multivitamin preparations, and to a certain degree in food products. These

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