Table IV. Isoflavone Analysis of Five Soybean Protein Concentrates

isoflavone	mg/100 g, as is, of concentrate[a]				
	K	L	M	N	O
daidzin	3	59	9	4	76
glycitein 7-β-glucoside	1	22	2	1	13
genistin	4	124	19	6	191
daidzein	11	20	12	2	11
glycitein	1	tr	tr	1	4
genistein	1	22	1	2	22
total	21	247	43	16	317

[a] Average of two replicates. Relative standard error per mean is 13.7%. Least significant ratio (0.05 level) of two means is 1.5.

Table V. Isoflavone Analysis of Five Soybean Protein Isolates

isoflavone	mg/100 g, as is, of isolate[a]				
	P	Q	R	S	T
daidzin	16	14	23	20	30
glycitein 7-β-glucoside	3	4	4	3	6
genistin	67	59	80	66	55
daidzein	8	12	18	10	21
glycitein	2	1	2	1	3
genistein	22	13	18	5	17
total	118	103	145	105	132

[a] Average of two replicates. Relative standard error per mean is 18.1%. Least significant ratio (0.05 level) of two means is 1.6.

indicates that lesser amounts of genistein may be needed to cause an effect in rat uteri. Our studies show low levels of daidzein and genistein in soybean protein products but large amounts of the isoflavone glucosides. The total isoflavone glucosides and aglycons measured in this study in hexane-defatted soybean meal appears to be approximately 2500 μg/g. Further research is needed to study these soybean constituents as a source of estrogenic response in animals.

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LITERATURE CITED

Bickoff, E. M.; Livingston, A. L.; Hendrickson, A. P.; Booth, A. N. *J. Agric. Food Chem.* **1962**, *10*, 410.

Circle, S. J.; Smith, A. K. "Soybeans: Chemistry and Technology. Proteins"; Smith, A. K.; Circle, S. J., Eds.; Avi Publishing Co.: Westport, CT, 1972; Vol. 1, Chapter 9.

Drane, H. M.; Patterson, D. S. P.; Roberts, B. A.; Saba, N. *Food Cosmet. Toxicol.* **1980**, *18*, 425.

Eldridge, A. C. *J. Chromatogr.* **1982**, *234*, 494.

Eldridge, A. C.; Kalbrener, J. E.; Moser, H. A.; Honig, D. H.; Rackis, J. J.; Wolf, W. J. *Cereal Chem.* **1971**, *48*, 640.

Kitts, D. D.; Krishnamurti, C. R.; Kitts, W. D. *Can. J. Anim. Sci.* **1980**, *60*, 531.

Naim, M.; Gestetner, B.; Zilkah, S.; Birk, Y.; Bondi, A. *J. Agric. Food Chem.* **1974**, *22*, 806.

Pratt, D. E.; Birac, P. M. *J. Food Sci.* **1979**, *44*, 1720.

Wyman, J. G.; VanEtten, H. D. *Phytopathology* **1978**, *68*, 583.

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Effect of pH on the Extraction and Fractionation of Dry Matter and Crude Protein from Coastal Bermuda Grass and White Clover

John J. Evans

Freeze-dried Coastal Bermuda grass (CBG) and white clover (WC) were extracted at pHs ranging from 4 to 10 and fractionated into four distinct fractions: chloroplastic (CHL), cytoplasmic (CYT), nonprotein nitrogen (NPN), and residue (RES). Dry matter (DM) and crude protein (CP) distributions in the fractions were influenced by pH. At pH 4, the greatest amount of CHL protein was extracted from CBG while the least amount was extracted from WC. At pHs ranging from 6 to 10, the CHL CP extracted remained constant for each forage with WC having twice the CHL CP as CBG. CYT CP extractability exhibited a quadratic effect ($P < 0.001$) due to pH; the pH optima for extraction of CYT proteins occurred at pHs 7 and 8 for CBG and WC, respectively. The amounts of CYT CP extracted from CBG and WC at their optimal pH were equivalent. The NPN fractions increased in CP with increasing pH while the CP in the RES fractions decreased with increasing pH for both forages. In general, the DM distribution paralleled the CP distribution.

The economical production of leaf protein concentrates from forages is desirable since forages can yield more dry matter and crude protein than any other crop (Pirie, 1979). The fractionation of forage proteins into green chloroplastic fractions for use in animal feeds and nearly white cytoplasmic fractions for human use has increased the importance of forages as sources of protein (Subba Rau et al., 1969; Evans et al., 1974; Horigome, 1977; Hanna and Ogden, 1980). Consequently, many different extraction and fractionation procedures have been described (Spencer et al., 1970, 1971; Pirie, 1971; Fishman and Burdick, 1977; Ostrowski, 1979) in an attempt to efficiently extract proteins from different plants. Chloroplastic and cytoplasmic proteins have been separated mainly on the basis of differential heat treatment of the expressed plant juices (Byers, 1967; Lexander et al., 1970; de Fremery et al., 1973; Edwards et al., 1975; Miller et al., 1975). These extraction

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and fractionation procedures were usually performed at endogenous or slightly elevated pHs.

The objective of the present work was to determine the effect of pH on the dry matter and crude protein extractability and distribution into four different, easily obtained fractions from Coastal Bermuda grass and white clover. These two forages were chosen because of their widely divergent characteristics. Coastal Bermuda grass is a warm-season grass which has a relatively high stem to leaf ratio, is less digestible, and usually contains less protein than white clover. White clover is a cool-season, succulent legume, with the edible parts being primarily leaves and petioles.

MATERIALS AND METHODS

Plant Material. Coastal Bermuda grass (*Cynodon dactylon* [L.] Pers.) was harvested in mid-June 1979 (first cutting) from a well-established experimental plot at Athens, GA, and was approximately 10 cm in height. The grass was fertilized with 13–13–13 (N–P–K) at a rate of 1090 kg/ha 1 mo prior to cutting. White clover (*Trifolium repens* L.) was grown in a greenhouse, at Athens, GA, from seed in 40 × 30 × 7 cm plastic containers. The clover was fertilized weekly with Hoagland's solution (Machlis and Torrey, 1956) and routinely cut every 3 weeks until it was established. White clover material used in these experiments was from a 3-week regrowth of the established clover cut in mid-June 1979. The forages were lyophilized immediately after cutting and were ground in a Wiley mill to pass a 40-mesh screen. The samples were stored in the dark at −20 °C until used.

Extraction and Fractionation Procedure. The plant material (15 g) was extracted in duplicate with 150 mL of H_2O at 21 °C for 1 h after adjustment of the pH by dropwise additions of 50% NaOH or concentrated HCl, with continuous stirring. The pH of the green slurry was monitored continuously with a Corning Model 110 pH meter, and additional acid or base was added to maintain the desired pH. The endogenous pHs of the WC and CBG were 5 and 6, respectively, and therefore adjustment of the pH was not necessary. The green slurry was filtered through a 45-μm nylon cloth, resulting in a green suspension and the insoluble "residue" fraction left in the cloth. The green suspension was centrifuged for 30 min at 30000*g*, resulting in a green "chloroplastic" fraction (ppt) and a straw-colored supernatant. The "cytoplasmic" fraction was obtained by heating the above supernatant to 100 °C in a water bath for 10 min and cooling to 10 °C to precipitate the cytoplasmic proteins. After removal of the cytoplasmic proteins by centrifugation, a straw-colored supernatant remained that was designated the "nonprotein nitrogen" fraction. For determination that all of the cytoplasmic proteins were precipitated, trichloroacetic acid was added (10% w/v) to portions of the nonprotein nitrogen fractions and heated to 100 °C in a water bath for 10 min and then cooled to 10 °C. No additional protein was precipitated. All fractions were freeze-dried until used.

By use of these extraction and fractionation procedures, both dry matter (DM) and crude protein (CP) recoveries were 95–99% for CBG and WC.

Analyses. Moisture was determined by Method 44-15A of the American Association of Cereal Chemists (1969). CP ($N \times 6.25$) was determined on the whole forage and each fraction in duplicate by micro-Kjeldahl analysis (McKenzie and Wallace, 1954). The nonprotein nitrogen fractions were also expressed in terms of CP ($N \times 6.25$) to enable a valid CP materials balance. The amino acid composition of all fractions extracted at each pH for both forages was also determined and will be reported in a subsequent publication. The differences in CP distribution ($N \times 6.25$) observed in this study were verified by the amino acid analyses.

Table I. Dry Matter Distribution in Coastal Bermuda Grass Fractions Obtained at Different pHs

	% for fraction[a]			
pH	chloroplastic	cytoplasmic	NPN[b]	residue
4.0	3.06^{f}	0.09^{e}	14.48^{e}	82.38^{e}
5.0	1.74^{e}	0.49^{f}	15.05^{e}	82.73^{e}
6.0[c]	2.99^{f}	1.37^{g}	14.13^{e}	81.52^{e,g}
7.0	2.66^{f}	1.71^{h}	15.58^{e,f}	80.06^{f,g}
8.0	3.04^{f}	0.76^{i}	17.27^{g,f}	78.92^{f}
9.0	3.23^{f}	0.35^{j,f}	17.50^{g}	78.95^{f}
10.0	3.25^{f}	0.26^{j,e}	18.28^{g}	78.23^{f}
SEM[d]	0.07	0.02	0.18	0.21

[a] Duplicate analyses. [b] Nonprotein nitrogen. [c] Endogenous pH. [d] Standard error of the mean ($P < 0.05$). [e–j] Means within columns having a common superscript are not different ($P < 0.01$).

The CP contents of the whole, unfractionated CBG and WC were 18.61 ± 0.33 and 29.66 ± 0.19%, respectively.

Statistical Methods. Analysis of variance, multiple-comparisons procedures (least significant difference), and orthogonal polynomial comparisons for equally spaced treatments were performed on the data according to Steel and Torrie (1960).

RESULTS AND DISCUSSION

The extraction and fractionation of CBG and WC resulted in four distinct fractions: chloroplastic (CHL), cytoplasmic (CYT), nonprotein nitrogen (NPN), and residue (RES). The CHL fractions were green in color while the CYT fractions were cream colored; both were light, fluffly powders after lyophilization. The NPN fractions were yellow-orange in color and appeared crystalline after lyophilization while the RES fractions could not be distinguished from the starting material.

Extraction of CBG at different pHs and subsequent fractionation resulted in differences in DM distribution (Table I). The CHL fractions from CBG showed similar DM extractabilities ($\bar{x}$ = 3.04% of the total DM) at all pHs except pH 5 where there was a decrease ($P < 0.01$). DM extractabilities in the CYT fractions from CBG increased with increasing pH, reaching a maximum at pH 7 and decreasing thereafter. DM content in the CYT fractions increased 19-fold between pH 4 and pH 7 and decreased 6.6-fold between pHs 7 and pH 10. The DM in the NPN fractions from CBG increased with increasing pH while the percentage of initial DM in the RES decreased with increasing pH, resulting in a maximum DM extractability of 21.77% at pH 10 (Table I).

The DM distribution in WC fractions obtained from whole clover extracted at different pHs is given in Table II. The DM extracted in the CHL fractions of WC increased with increasing pH, resulting in a 3.4-fold increase in CHL DM extracted between pH 4 and pH 10. This increase with increasing pH is in contrast to the CHL fractions from CBG (Table I) where there was no increase in DM extracted with increasing pH. At pH 10, 2.7 times more CHL DM was extracted from clover than from the CBG. DM in the WC CYT fractions increased with increasing pH, reaching a maximum at pH 8 and decreasing thereafter. The maximum amount of DM obtained in the WC CYT fraction of pH 8 was almost twice that extracted in the CBG CYT fraction of pH 7, the optimal pH for CBG CYT DM extractability. The DM extracted into the WC NPN fractions remained relatively constant from pH 4 to pH 9 with an increase ($P < 0.01$) at pH 10. This was in

Table II. Dry Matter Distribution in White Clover Fractions Obtained at Different pHs

pH	% for fraction[a]			
	chloroplastic	cytoplasmic	NPN[b]	residue
4.0	2.61[e]	0.28[e]	30.89[e,f]	66.23[e]
5.0[c]	4.93[f]	0.99[f]	29.71[e]	64.37[e]
6.0	5.88[f,g]	1.49[g]	31.44[f]	61.19[f]
7.0	6.38[g]	2.48[h]	30.26[e,f]	60.90[f]
8.0	8.07[h,i]	3.21[i]	29.69[e]	59.04[f]
9.0	7.06[h,g]	0.58[e,f]	31.52[f]	60.84[f]
10.0	8.91[i]	0.69[e,f]	38.86[g]	51.56[g]
SEM[d]	0.13	0.03	0.16	0.24

[a] Duplicate analyses. [b] Nonprotein nitrogen. [c] Endogenous pH. [d] Standard error of the mean ($P < 0.05$). [e-i] Means within columns having a common superscript are not different ($P < 0.01$).

Table III. Distribution of Crude Protein in Coastal Bermuda Grass Fractions Obtained at Different pHs

pH	% for fraction[a]			
	chloroplastic	cytoplasmic	NPN[b]	residue
4.0	7.56[e]	0.24[e]	13.23[e]	78.97[e]
5.0	3.75[f]	1.85[f]	15.82[e,f]	78.59[e]
6.0[c]	6.04[g,e]	5.94[g]	15.80[e,f]	72.22[f]
7.0	4.97[g,f]	7.11[h]	18.05[f]	69.88[f,h]
8.0	5.66[g]	2.79[i]	23.92[g]	67.63[g,h]
9.0	5.70[g]	0.71[e]	25.14[g,h]	68.46[g,h]
10.0	5.70[g]	0.38[e]	27.73[h]	66.19[g]
SEM[d]	0.18	0.08	0.29	0.39

[a] Duplicate analyses. [b] Nonprotein nitrogen. [c] Endogenous pH. [d] Standard error of the mean ($P < 0.05$). [e-i] Means within columns having a common superscript are not different ($P < 0.01$).

contrast to the CBG NPN fraction (Table I) which increased with increasing pH. The DM in the clover NPN fractions was about twice that in the CBG NPN fractions. This was probably due to the higher sugar and inorganic salt contents of the WC NPN fractions (Gibson and Hollowell, 1966). The distribution of DM in the RES fractions of WC decreased with increasing pH, a maximum of 48.44% of the DM being extracted at pH 10. This contrasts with the CBG RES where only 21.77% of the total DM was extracted at pH 10 (Table I).

The distribution of CP in the four fractions from CBG obtained at the different pHs is in Table III. The greatest amount of CP extracted into the CHL fractions occurred at pH 4. There was a decrease ($P < 0.01$) in CHL CP (50%) when the grass was extracted at pH 5, similar to the decrease in DM (Table I). The CHL CP remained relatively constant from pH 6 to pH 10, accounting for an average of 5.61% of the total CP. The CP in the CBG CYT fractions increased with increasing pH, reaching a maximum at pH 7 and decreasing thereafter. This quadratic effect ($P < 0.001$) on CP content caused by pH paralleled the DM distribution of the CBG CYT fractions (Table I), both having pH optima at pH 7. There was a 30-fold increase in the CBG CYT CP from pH 4 to pH 7 and a 19-fold decrease in CP from pH 7 to pH 10. The CP in the CBG NPN fractions increased with increasing pH, reaching a maximum of 27.73% at pH 10 (Table III). This was a 2-fold increase in CP content compared to that extracted at pH 4 while DM only increased 1.3-fold (Table I). CP in the CBG RES decreased with increasing pH. Approximately 21% of the CBG CP was extracted at pH 4 while 34% of the total CP was extracted at pH 10. In general, the CP distribution in the CBG fractions paralled the DM distribution.

The distribution of CP in WC fractions obtained from clover extracted at the different pHs is shown in Table IV.

Table IV. Distribution of Crude Protein in White Clover Fractions Obtained at Different pHs

pH	% for fraction[a]			
	chloroplastic	cytoplasmic	NPN[b]	residue
4.0	4.89[e]	0.38[e]	20.01[e]	74.73[e]
5.0[c]	8.42[e,g]	2.25[f]	19.39[e]	69.94[f]
6.0	11.54[f,g]	3.96[g]	24.05[f]	60.47[g]
7.0	11.49[f,g]	6.15[h]	23.96[f]	58.41[g,h]
8.0	13.15[f]	7.97[i]	24.18[f]	54.72[h]
9.0	10.88[f,g]	0.92[e]	30.12[g]	58.09[g,h]
10.0	11.54[f,g]	0.97[e]	44.98[h]	42.52[i]
SEM[d]	0.42	0.09	0.30	0.43

[a] Duplicate analyses. [b] Nonprotein nitrogen. [c] Endogenous pH. [d] Standard error of the mean ($P < 0.05$). [e-i] Means within columns having a common superscript are not different ($P < 0.01$).

Table V. Percent Crude Protein in Coastal Bermuda Grass Fractions Obtained at Different pHs

pH	% for fraction[a]			
	chloroplastic	cytoplasmic	NPN[b]	residue
4.0	45.02[e]	51.15[e]	16.68[e]	17.48[e]
5.0	41.66[e]	72.85[f]	20.27[f]	18.32[f]
6.0[c]	37.23[f]	80.04[g]	20.62[f]	16.34[g]
7.0	35.32[f,g]	78.77[g]	21.92[f]	16.50[g]
8.0	34.42[f,g]	66.47[h]	25.62[g]	15.85[g]
9.0	33.13[g]	39.44[i]	27.04[g]	16.30[g]
10.0	33.29[f,g]	28.68[j]	28.80[g]	16.07[g]
SEM[d]	0.44	0.36	0.36	0.08

[a] Duplicate analyses. [b] Nonprotein nitrogen. [c] Endogenous pH. [d] Standard error of the mean ($P < 0.05$). [e-j] Means within columns having a common superscript are not different ($P < 0.01$).

The CHL CP increased 2.4-fold from pH 4 to pH 6 and remained relatively constant from pH 6 to pH 10 ($\bar{x}$ = 11.72%). The least amount of CHL CP was extracted at pH 4 in contrast to CBG where the most CHL CP was extracted (Table III). Overall, the amounts of CHL CP extracted from WC between pH 6 and pH 10 were about twice that extracted from CBG. CYT CP from WC increased with increasing pH, reaching a maximum at pH 8 and decreasing thereafter. This quadratic effect ($P < 0.001$) due to pH was also observed in the DM distribution (Table II). There was a 21-fold increase in CYT CP between pH 4 and pH 8 and an 8.6-fold decrease between pH 8 and pH 9. Similar quantities of CP were extracted in the CYT fractions for both WC and CBG at their optimum pHs. The CP contents of the NPN fractions from WC increased with increasing pH, reaching a maximum of 44.98% at pH 10 (Table IV). This was more than a 2-fold increase in CP over that extracted at pH 4. In addition, the amount of CP extracted into the NPN fraction of WC at pH 10 was 62% greater than that obtained in the NPN fraction of CBG at pH 10 (Table III). The CP contents of the WC RES fractions decreased with increasing pH (Table IV). Approximately 57.5% of the total CP was extracted from WC at pH 10 compared to 25.3% at pH 4. Total CP extractability at pH 4 was similar for both CBG and WC; however, at pH 10, 1.7 times more CP was extracted from WC. In general, the CP distribution in the WC fractions reflected the DM distribution (Table II).

The percentage CP in the CBG fractions obtained from the grass extracted at different pHs is shown in Table V. Extraction of CBG at pHs 4 and 5 resulted in CHL fractions with the highest percentage CP. The percentage CP in the CHL fractions obtained at pHs 6–10 averaged 34.6%, significantly lower ($P < 0.01$) than at pHs 4 and

Table VI. Percent Crude Protein in White Clover Fractions Obtained at Different pHs

pH	% for fraction[a]			
	chloroplastic	cytoplasmic	NPN[b]	residue
4.0	52.33[e]	38.39[e]	18.13[e]	31.57[e]
5.0[c]	53.05[e]	71.00[f]	20.30[e,f]	33.79[f]
6.0	55.27[e]	74.96[f]	21.59[f,g]	27.88[g]
7.0	52.15[e]	71.96[f]	22.90[f,g]	27.74[g]
8.0	46.60[f]	70.21[f]	23.30[g]	26.52[g]
9.0	44.50[f]	45.89[g]	27.62[h]	27.59[g]
10.0	35.98[g]	39.18[e]	32.16[i]	22.91[h]
SEM[d]	0.49	0.70	0.31	0.18

[a] Duplicate analyses. [b] Nonprotein nitrogen. [c] Endogenous pH. [d] Standard error of the mean ($P < 0.05$). [e-i] Means within columns having a common superscript are not different ($P < 0.01$).

5. The CYT fractions from CBG which gave the highest percentage CP were obtained at pHs 6 and 7 while the fraction obtained at pH 10 had the lowest percentage CP. The percentage CP in the NPN fractions of CBG increased with increasing pH, reaching a maximum of 28.8% CP at pH 10. The percentage CP in the CBG RES (Table V) from the pH 4 extraction was lower ($P < 0.01$) than in the starting material (17.48 vs. 18.6%, respectively). At pH 5, the percentage CP in the RES increased significantly, which coincided with the decreased DM and CP extracted into the CHL fraction at pH 5 (Tables I and III). The percentage CP in the CBG RES decreased ($P < 0.01$) at pH 6 and remained relatively constant to pH 10 ($\bar{x}$ = 16.21% CP).

The percentage CP in the WC fractions obtained at the different pHs is in Table VI. The CHL fractions having the highest percentage CP were those obtained when the clover was extracted at pHs 4–7 ($\bar{x}$ = 53.2% CP). A decrease ($P < 0.01$) was observed at pHs 8 and 9 ($\bar{x}$ = 45.55% CP) with a further decrease occurring at pH 10. CYT fractions obtained by extracting clover at pHs 5–8 gave the highest percentage CP preparations ($\bar{x}$ = 72.03%). The CYT fractions having the lowest percentage CP occurred at pHs 4 and 10. NPN fractions from WC increased in percentage CP as pH increased, which paralleled the increase in total CP (Table IV). The percentage CP in the WC RES fractions increased significantly at pHs 4 and 5 ($\bar{x}$ = 32.68% CP) over that of the starting material (29.66% CP). This increase in percentage CP was due to the small amount of total CP extracted into the CHL and CYT fractions (Table IV) at these pHs and the relatively large amounts of DM (e.g., sugars, salts, etc.) extracted into the NPN fractions at pHs 4 and 5 (Table II). There was a decrease ($P < 0.01$) in percentage CP in the WC RES fractions at pH 6 and this remained relatively constant through pH 9 ($\bar{x}$ = 27.43% CP). A further decrease ($P < 0.01$) occurred at pH 10 which coincided with the large increase in NPN CP at pH 10 (Table IV).

These results show that the total CP extracted (e.g., CHL plus CYT plus NPN fractions) increased with increasing pH as has been observed by using soybean leaves (Betschart and Kinsella, 1973). However, this increase in total CP was due primarily to an increase in the CP (N × 6.25) in the NPN fractions at the higher pHs (Tables III and IV). Specifically, 27.73 and 44.98% of the total CP extracted at pH 10 from CBG and WC, respectively, were found in the NPN fractions. In CBG, the high-quality CHL and CYT proteins accounted for only 6.08% of the total CP at pH 10 (Table III), and of this total only 0.38% was white CYT protein. Similarly, at pH 10, 12.51% of the total CP from WC (Table IV) was found in the CHL and CYT fractions, less than 1% of the total CP being CYT protein. Extractions of CBG at pHs 6–7 produced the greatest amount of high-quality CHL and CYT protein (ca. 12% of the total CP in the grass), almost twice that obtained at pH 10. Of this 12%, more than half was white CYT protein. Similarly, when WC was extracted at pH 8, 21.12% of the total CP was fractionated as CHL and CYT proteins, 40% of this being white CYT protein.

From these data, it can be seen that pH is an important parameter to consider when extracting and fractionating plant material. Specifically, if a forage is being processed to obtain a green CHL protein and a white CYT protein, selection of the proper pH for maximum yield is essential.

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LITERATURE CITED

American Association of Cereal Chemists "Approved Methods", 7th ed.; AACC: Minneapolis, MN, 1969.
Betschart, A.; Kinsella, J. E. *J. Agric. Food Chem.* **1973**, *21*, 60.
Byers, M. *J. Sci. Food Agric.* **1967**, *18*, 33.
de Fremery, D.; Miller, R. E.; Edwards, R. H.; Knuckles, B. E.; Bickoff, E. M.; Kohler, G. O. *J. Agric. Food Chem.* **1973**, *21*, 886.
Edwards, R. H.; Miller, R. E.; de Fremery, D.; Knuckles, B. E.; Bickoff, E. M.; Kohler, G. O. *J. Agric. Food Chem.* **1975**, *23*, 620.
Evans, J. J.; Landgraff, L. M.; Fishman, M. L. In "Proceedings Fourth Research-Industry Conference Coastal Bermuda Grass Processors' Association, Inc."; Burdick, D., Ed.; Field Crops Laboratory, U.S. Department of Agriculture: Athens, GA, 1974; p 106.
Fishman, M. L.; Burdick, D. *J. Agric. Food Chem.* **1977**, *25*, 1122.
Gibson, P. B.; Hollowell, E. A. *U.S., Dep. Agric., Agric. Handb.* **1966**, *No. 314*.
Hanna, M. A.; Ogden, R. L. *J. Agric. Food Chem.* **1980**, *28*, 1212.
Horigome, T. *Nippon Chikusan Gakkai Ho* **1977**, *48*, 267.
Lexander, K.; Carlsson, R.; Schalen, V.; Simonsson, A.; Lundborg, T. *Ann. Appl. Biol.* **1970**, *66*, 193.
Machlis, L.; Torrey, J. G. "Plants in Action"; W. H. Freeman: San Francisco, CA, 1956.
McKenzie, H. A.; Wallace, H. S. *Aust. J. Chem.* **1954**, *7*, 55.
Miller, R. E.; de Fremery, D.; Bickoff, E. M.; Kohler, G. O. *J. Agric. Food Chem.* **1975**, *23*, 1177.
Ostrowski, H. T. *J. Food Process. Preserv.* **1979**, *3*, 105.
Pirie, N. W., Ed. *Leaf Protein: Its Agron., Prep., Qual. Use* **1971**, *IBP Handbook No. 20*, Chapters 5–9.
Pirie, N. W. *J. Am. Oil Chem. Soc.* **1979**, *56*, 472.
Spencer, R. R.; Bickoff, E. M.; Kohler, G. O.; Witt, S. C.; Knuckles, B. E.; Mottola, A. C. *Trans. ASAE* **1970**, *13*, 198.
Spencer, R. R.; Mottola, A. C.; Bickoff, E. M.; Clark, J. P.; Kohler, G. O. *J. Agric. Food Chem.* **1971**, *19*, 504.
Steel, R. G. D.; Torrie, J. H. "Principles and Procedures of Statistics"; McGraw-Hill: New York, 1960.
Subba Rau, B. H.; Mahadeviah, S.; Singh, N. *J. Sci. Food Agric.* **1969**, *20*, 355.

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