gas chromatograph coupled to a Nuclide 90G double-focusing mass spectrometer. The $^1H$ NMR spectra were obtained with a Varian HA-100 instrument from solutions in chloroform-*d* with tetramethylsilane as an internal standard.

**1-Deoxy-1-sarcosino-D-fructose.** Sarcosine (20 g) and D-glucose (100 g) were reacted in 300 mL of dimethylformamide as previously described (Klemer and Micheel, 1956). The concentrated reaction mixture was dissolved in 1.4 L of water and placed on 454 g of wet packed Dow 50W-4X ($H^+$) column. The column was eluted with 2 L of water and then with 0.1 M pyridine. The first 3 L of eluate ws discarded and then 15-mL fractions were collected. Those fractions that gave positive ninhydrin and alkaline ferricyanide reactions were combined after analytical chromatography on No. 1 Whatman paper, using 1-butanol/acetic acid/water (4:1:1) as the developing solvent; this showed them to contain only the Amadori compound. After concentration, the syrup was dried by azeotropic distillation of ethanolic solutions. The final residue was taken up in methanol and the solvent was removed; this step was repeated three times. Finally, upon cooling, the product separated from the fourth methanolic solution; mp 82–85 °C (d), yield 32 g.

Anal. Calcd. for $C_9H_{17}O_7N$:$CH_3OH$: C, 42.40; H, 7.42; N, 4.95. Found: C, 41.87% H, 7.21; N, 4.97.

**Pyrolysis.** The Amadori compound was decomposed in 10-g amounts at 140 °C and 0.1 torr for 5 h, and the resulting distillate was isolated in a trap cooled in ethanol–dry ice. After warming, the receiver was rinsed with chloroform, and the sample was isolated after drying this solution (sodium sulfate) and removal of the solvent in vacuo at 0 °C; yield, 0.30 g. The oil was then examined by GLC–MS. When required, individual components were isolated by GLC.

**3,6-Dimethyl-2,5-dioxopiperazine (Sarcosine Anhydride).** Sarcosine (5 g) was heated at its melting point (210 °C) for 3 h. The fused mass was dissolved in water, and the water was then extracted with ethyl acetate. After drying of the organic phase (sodium sulfate) and solvent removal by evaporation, a crystalline precipitate formed; yield 0.360 g, mp 142.5–143 °C. Mass spectrum: *m/e* (rel %) 142 (100), 114 (5), 113 (9), 85 (14), 58 (3), 57 (81), 56 (7), 44 (49), 43 (72).

***N*-*N*-Dimethyl-2-furfurylamine (4) and *N*,*N*-Dimethyl-5-methyl-2-furfurylamine (8).** An excess of thionyl chloride was added to the appropriate 2-furoic acid that was dissolved in benzene. After 6 h of reflux, the solvent and excess thionyl chloride were removed by distillation. The residue was dissolved in chloroform and an excess of *N*,*N*-dimethylamine was added. After stirring for 2 h, the solution was filtered and the solvent removed in vacuo. Proton magnetic resonance, GLS–MS showed the isolate in each preparation to contain only the respective amide (90% yield).

The amide (0.5 g) was reduced using sodium borohydride and pyridine (Kikugawa et al., 1969). Mass spectrum: **4**, *m/e* (rel %) 125 (100), 111 (9), 96 (52), 84 (44), 81 (11), 73 (12), 69 (25), 68 (30). Mass spectrum: **8**, *m/e* (rel %) 139 (100), 124 (8), 110 (26), 95 (6), 82 (30), 81 (10), 57 (16), 55 (27), 44 (55), 47 (88).

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LITERATURE CITED

Bruggemann, J., Erbersdobler, *Z. Lebensm.-Unters. Forsch.* **137**, 137 (1968).
Hodge, J., "Chemistry and Physiology of Flavors", Schultz, H. W., Day, E. A., Libbey, L. M., Eds., Avi, Westport, CT, 1967, Chapter 22.
Hodge J. E., Mills, F. D., Fisher, B. E., *Cereal Sci. Today* **17**, 34 (1972).
Kikugawa, Y., Ikegami, S., Yamada, S., *Chem. Pharm. Bull. (Tokyo)* **17**, 98 (1969); CA 70, 87491C.
Klemer, A., Micheel, F., *Chem. Ber.* **89**, 1242 (1956).
Ledl, F., Schnell, W., Severin, T., *Z. Lebensm.-Unters. Forsch.* **160**, 367 (1976).
Mills, F. D., Baker, B. G., Hodge, J. E., *J. Agric. Food Chem.* **17**, 723 (1969).
Mills, F. D., Baker, B. G., Hodge, J. E., *Carbohydr. Res.* **15**, 205 (1970).
Mills, F. D., Hodge, J. E., *Carbohydr. Res.* **51**, 9 (1976).
Reynolds, T. M., *Food Technol. Aust.* **22**, 610 (1970).
Shigematsu, H., *Nippon Sembai Kosha, Chuo Kenkyiyo Kenkyo Nokoy* **11B**, 119 (1976).
Shigematsu, H., Shibata, S., Kurata, T., Kato, H., Fujimaki, M., *Agric. Biol. Chem. (Jpn)* **41**, 2377 (1977).

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# Peanut Leaf Extract. Chemical Composition and Protein Characterization

A peanut leaf extract obtained from fresh leaves contained 42.2% carbohydrate, 25.4% ash, 18.0% protein, 8.8% fat, 4.6% moisture, and 0.8% fiber on a dry basis. Over 65% of the fatty acids comprised linolenic, palmitic, and linoleic acids; the concentration of oleic acid was higher than in most leaf tissues. Amino acid composition of protein in peanut leaf extract was comparable to that of similar extracts derived from other leaf tissues lacking in sulfur-containing amino acids. Calculations from amino acid data showed that the estimated biological value of the extracted leaf protein was less than that of casein or egg. Serological and electrophoretic analyses of protein in the peanut leaf extract showed several associative species, some of which exhibited antigenic identity but varied in electrophoretic mobility and molecular size.

In recent years, several investigators have emphasized the unconventional use in human nutrition of protein from various leaves (Betschart and Kinsella, 1973; Edwards et al., 1975, Kohler et al., 1977; Pirie, 1971). Because of the tremendous worldwide supply of leafy crops produced each year, it seems logical that much of the protein therefrom

Table I. Proximate Principles in PLE

| analysis | quantity[a] |
|---|---|
| ash | 14.9[b] |
| fat | 8.8 |
| moisture | 4.6 |
| fiber | 0.8 |
| carbohydrate | 42.2[c] |
| protein | 18.0[d] |

[a] Percent of freeze-dried material. [b] Corrected for buffer salts calculated at 10.4%. [c] Calculated by difference. [d] Percent N × 6.25.

could be processed for human consumption. Protein isolates from leaves of nine plant species have been reported to have acceptable biological values; however, very little differences were observed in their amino acid composition (Gerloff et al., 1965; Lexander et al., 1970). For most plant species, the nutritional quality of leaf proteins is better than of seed proteins, but not so good as that of egg and milk (Pirie, 1971). Some reports have indicated that protein foods prepared from leaves are economically attractive only if combined with byproducts applicable to animal feeding programs (Bray, 1977; Vosloh et al., 1976).

The objective of this study was to obtain a crude peanut leaf extract (PLE) by use of an aqueous buffered system, determine its chemical composition in relation to nutritional quality, and characterize the protein components within the fraction by serological and electrophoretic methods. PLE is not to be confused with leaf protein concentrates (LPC) reported in this area of research. These concentrates usually contain over 50% protein.

## EXPERIMENTAL SECTION

**Leaves.** Mature leaves, excluding stems, were excised from variety Virginia 56-R peanut plants that were cultivated under normal agronomic conditions in southeast Louisiana. Foundation seed stock was obtained from the Crops Research Division of the U.S. Department of Agriculture, Beltsville, MD.

**Extraction Procedure.** A single batch of freshly harvested leaves (385 g) was homogenized in a Waring blender in 1400 mL of phosphate buffer, pH 7.9, ionic strength 0.2, at 25 °C. The homogenate (final volume of 1800 mL) was forced through six layers of cotton cheesecloth (Type II-Class 2, Kendall Co., Boston). The green filtrate (PLE) was freeze-dried for later chemical analyses.

**Analytical Methods.** Proximate principles, fatty acid profile, and amino acid content were determined by AOAC (1975) procedures. The amino acid procedure was developed by Kaiser et al. (1974). Immune serum against protein from PLE was prepared from rabbits by Antibodies, Inc., Davis, CA. Disc electrophoresis on 7.5% acrylamide gels was performed according to Godshall and Roberts (1978) and immunoelectrophoresis in agar was carried out by the method of Grabar and Williams (1953).

## RESULTS AND DISCUSSION

On a dry basis, PLE represented 32% of the total weight of leaves extracted. Compositional data in Table I shows that carbohydrates, protein, and ash constituted the largest proportions of the dried material. Less than one-half (10.4%) of the ash content is attributed to residual phosphate salts from the extraction procedure. The relatively high content of moisture illustrates the hygroscopic nature of the freeze-dried material. These data are comparable to alfalfa soluble fractions (residual soluble protein left after heat coagulation), reported by Kohler (1977) except that a higher content of fat is present in PLE.

Table II. Fatty Acid Profile of Lipid in PLE

| methyl ester[a] | acid | quantity[b] |
|---|---|---|
| 12:0 | lauric | 1.4 |
| 14:0 | myristic | 1.4 |
| 16:0 | palmitic | 17.3 |
| 18:0 | stearic | 5.8 |
| 24:0 | lignoceric | 5.8 |
| 16:1 | palmitoleic | 10.1 |
| 18:1 | oleic | 10.1 |
| 18:2 | linoleic | 14.4 |
| 18:3 | linolenic | 36.1 |

[a] Number of carbons/number of double bonds. [b] Percent by weight.

Table III. Amino Acid Content and Chemical Score of Protein in PLE

| | quantity[a] | | |
|---|---|---|---|
| amino acid | FAO[c] | PLE | chemical score[b] |
| Ala | | 60 | |
| Val | 50 | 54 | 108 |
| Gly | | 54 | |
| Ile | 40 | 45 | 113 |
| Leu | 70 | 85 | 122 |
| Pro | | 48 | |
| Thr | 40 | 47 | 188 |
| Ser | | 45 | |
| Met + Cys | 35 | 6 + 5[d] | 31 |
| Phe + Tyr | 60 | 57 + 38 | 158 |
| Asp | | 92 | |
| Glu | | 124 | |
| Lys | 55 | 59 | 107 |
| His | | 20 | |
| Arg | | 50 | |
| Trp | 10 | | |

[a] Milligrams/gram of protein. [b] Percent of required essential amino acids. [c] Provisional scoring pattern (FAO, 1973). [d] Value of whole leaf protein (FAO, 1970).

Relative concentration of the major fatty acids in the lipid portion of PLE is shown in Table II. Linolenic acid, palmitic acid, and linoleic acid are the predominant components and their values resemble data from lipids in leaf protein concentrates of rye grass and wheat (Lima et al., 1965). The distribution of fatty acid is in general agreement with literature reports on leaf tissue fatty acids. Compared to lipids in leaf protein concentrates from clover, corn, turnip, and wheat, however, PLE has a much higher content of oleic acid (Lima et al., 1965).

The data in Table III show that the amino acid composition of protein in PLE, except for the sulfur amino acids, is comparable to that of protein in leaf protein concentrates from other sources (Gerloff et al., 1965; Lexander et al., 1970). The quality of the protein, based on the ratios of the content of each essential amino acid in PLE to that of the provisional FAO pattern is fairly good. Although methionine is severely limiting, the other essential amino acids, especially lysine, are adequate. No determinations were obtained for cystine and tryptophan which are probably low, as they are for other leaf proteins (Gerloff et al., 1965). How a given protein fulfills nutritional requirements depends on the total nitrogen intake derived from essential amino acids. Hence, grams of essential amino acids per gram of nitrogen ($E_T$ ratio) is another way of expressing estimated protein quality (FAO, 1965). For the protein in PLE, this ratio is 2.78, which is less than for eggs and milk (Jacks et al., 1972).

Characterizations of protein in PLE by immunoelectrophoresis and disc electrophoresis are shown in Figure 1. The data are intended to show the homogeneity of the

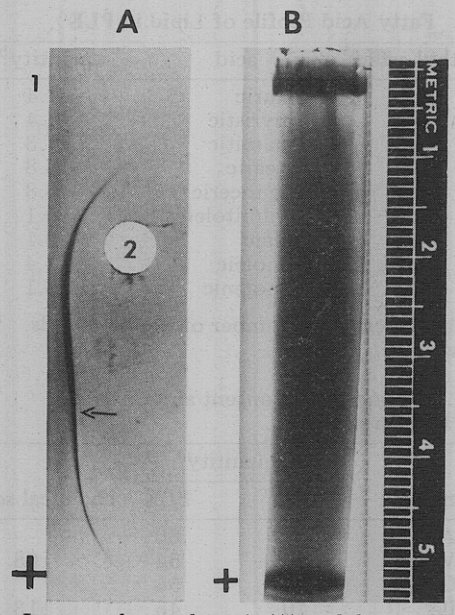


**Figure 1.** Immunoelectrophoresis (A) and disc electrophoresis (B) of protein in PLE. In A, 1.0 mg of protein was applied to the well (2), and immune serum was applied to the trough (1) after electrophoresis. In B, 1.25 mg of protein was applied over the stacking gel (migration from top to bottom). Precipitin lines and protein zones on disc gel were stained with amido black.

crude protein. The immunoelectrophoretic analysis showed a long continuous precipitin line with an arc near the center (arrow). This indicates a reaction of antigenic identity among major protein species that have different electrophoretic mobilities. Antibody titers for minor constituents were too low for visualization in this experiment. Comparable analysis on disc polyacrylamide gel also showed several zones of protein that had a wide range in electrophoretic migration. Some of the protein remained close to the origin and two distinct zones were observed between 5.0 and 5.5 cm from the origin of electrophoresis. The diffused staining along the entire gel could be attributed to any number of protein species of varying size, shape, and charge. Thus, the proteinaceous portion of PLE exhibits complex behavior. According to theory, the average pore size of a 7.5% polyacrylamide gel is about 50 Å and the gel exhibits extreme frictional resistance to the migration of proteins of molecular weight larger than 156 000 (Ornstein, 1964). Therefore, from our data, some of the proteins in PLE are at least greater than 156 000 and others perhaps as small as 25 000 in molecular weight. Proteins in leaves of Bermuda grass were shown to have a molecular weight range of 7000 to 1 000 000 (Fishman and Burdick, 1977).

In summary, the quality of fatty acids and amino acids in a peanut leaf extract obtained by a relatively simple procedure is sufficiently high for use as a dietary supplement in conventional foods. For use in traditional foods, however, color taste, odor, and functional properties involving finished products would be major considerations. And as pointed out by Vashoh et al. (1976), successful investment in leaf processing requires thorough analysis of a number of factors. These include availability of raw material and energy, competitive prices of feed grains, length of operating season, and plant capacity.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, DC, 1975.
Betschart, A., Kinsella, J. E., *J. Agric. Food Chem.* **21**, 60 (1973).
Bray, W. J., "The Processing of Leaf Protein to Obtain Food-grade Products", Proceedings of the Green Crop Fractionation Symposium, British Grassland Society, 1977, pp 107–116.
Edwards, R. H., Miller, R. E., deFremery, D., Knukles, B. E., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **23**, 620 (1975).
FAO, "Amino Acid Content of Foods and Biological Data on Proteins", Publication No. 24, Rome, 1970, p 94.
FAO/WHO Expert Group, Food Agric. Organization Meetings Report, Series 37, Rome, 1965, pp 1–37.
FAO/WHO, "Energy and Protein Requirements", Technical Report Series No. 522, World Health Organization, Rome, 1973.
Fishman, M. L., Burdick, D., *J. Agric. Food Chem.* **25**, 1122 (1977).
Gerloff, E. D., Lima, I. H., Stahmann, M. A., *J. Agric. Food Chem.* **13**, 139 (1965).
Godshall, M. A., Roberts, E. J., *Proc., 1976 Tech. Sess. Cane Sugar Refining Res.*, 57–72 (1978).
Grabar, P., Williams, C. A., *Biochem. Biophys. Acta* **10**, 193 (1953).
Jacks, T. J., Neucere, N. J., Yatsu, L. Y., *J. Am. Peanut Res. Educ. Assoc.* **4**, 198 (1972).
Kaiser, F. E., Gehrke, C. W., Zumwalt, R. C., Kuo, K. G., *J. Chromatogr.* **94**, 113 (1974).
Kohler, G. O., Knuckles, B. E., *Food Technol.* **31**, 191 (1977).
Lexander, K., Carlsson, R., Schalen, V., Simonsson, A., Lundberg, T., *Ann. Appl. Biol.* **66**, 193 (1970).
Lima, I. H., Richardson, T., Stahmann, M. A., *J. Agric. Food Chem.* **13**, 143 (1965).
Ornstein, L., *Ann. N.Y. Acad. Sci.* **121**, 321 (1964).
Pirie, N. W., Ed., "Leafprotein: Its Agronomy, Preparation, Quality and Use", Blackwell Scientific Publications, Oxford, 1971.
Vosloh, C. J., Edwards, R. H., Enochian, R. J., Kuzmicky, D. D., Kohler, G. O., "Leaf Protein Concentrate (Pro-Xan) from Alfalfa: An Economic Evaluation", National Economic Analysis Division, Economic Research Service, Ag. Econ. Rpt. No. 346, 1976.

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