forms the active glutamic conjugate, making it difficult to understand why the glutamic conjugate would be more active than 2,4-D in this tissue. Studies (Feung *et al.*, 1973b) with 2,4-D-[1-^{14}C]-glutamic acid indicate a more rapid conversion of it to the inactive 4-OH-2,3-D or 4-OH-2,5-D than conversion to 2,4-D itself. This is again inconsistent with the higher activity of the 2,4-D-glutamic conjugate. It thus seems possible that the conjugated form of 2,4-D could be exclusively the physiologically active form. In any event some of these active conjugates may be more suitable auxins for plant tissue cultures than either NAA or 2,4-D.

Different responses were observed for the various conjugates; however, it is possible that at the subcellular level no real differences exist. The observed differences in physiological responses may reflect variations in permeability and/or metabolism which thus affect micro-pool environments.

Amino acid conjugates of auxins can effect responses at very low concentrations and it has been postulated one site of action is at the nucleic acid level. Cellular localization of 2,4-D in the nucleus (Liao and Hamilton, 1966) as well as in nuclioli (Zwar and Brown, 1968) has been shown. Key and Shannon (1964) reported that IAA and 2,4-D, at the concentration which promoted cell elongation, enhanced ^{14}C-nculeolide incorporation into ribonucleic acid (RNA) of excised soybean hypocotyl tissue, whereas inhibitory levels decreased incorporation. It has in general been concluded (Key, 1969) that RNA and protein synthesis are essential for the process of cell elongation to proceed at the normal rate. The observed differences in physiological response may also reflect a difference in binding to a protein and/or a selectivity in an enzyme reaction leading to biological function. These amino acid conjugates could compete with amino acid or specifically react with transfer ribonucleic acid to affect selective protein synthesis. Labeled 2,4-D has been reported bound to macro-molecules (Galston and Davies, 1969). Additional studies are needed to elicit the function of these amino acid conjugates of auxins in plants.

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LITERATURE CITED

Feung, C. S., Hamilton, R. H., Mumma, R. O., *J. Agr. Food Chem.* 21, 632 (1973a).
Feung, C. S., Hamilton, R. H., Mumma, R. O., *J. Agr. Food Chem.* 21, 637 (1973b).
Feung, C. S., Hamilton, R. H., Witham, F. H., *J. Agr. Food Chem.* 19, 475 (1971).
Feung, C. S., Hamilton, R. H., Witham, F. H., Mumma, R. O., *Plant Physiol.* 50, 80 (1972).
Filner, P., *Exp. Cell Res.* 39, 33 (1965).
Galston, A. W., Davies, P. J., *Science* 163, 1288 (1969).
Gamborg, O. J., Ojima, K., Proceedings of the Sixth International Conference on Plant Growth Substances, Carleton University, Ottawa, Canada, 1967.
Harvey, A. E., *Can. J. Bot.* 43, 1783 (1967).
Key, J. L., *Annu. Rev. Plant Physiol.* 20, 449 (1969).
Key, J. L., Shannon, J. C., *Plant Physiol.* 39, 360 (1964).
Krewson, C. F., Drake, T. F., Neufeld, C. H. H., Fontaine, T. D., Mitchell, J. W., Preston, W. H., Jr., *J. Agr. Food Chem.* 4, 140 (1956).
Liao, S., Hamilton, R. H., *Science* 151, 822 (1966).
Lieb, H. B., Ray, T. B., Still, C. C., *Plant Physiol.* 51, 1140 (1973).
Lieb, H. B., Ray, T. B., Still, C. C., Journal Paper, New Jersey Agricultural Experiment Station, unpublished communication, 1972, 15 pp.
Miller, C. O., *Modern Methods Plant Anal.* 6, 194 (1963).
Wang, H. C., Hamilton, R. H., Deering, R. A., in "Biochemistry and Physiology of Plant Growth Substances," Wightman, R., Setterfield, G., Ed., Runge Press, Ottawa, Canada, 1968, p 685.
Witham, F. H., *Plant Physiol.* 43, 1455 (1968).
Wood, J. W., Fontaine, T. D., *J. Org. Chem.* 17, 891 (1952).
Wu, L., Li, H. W., *Cytologia* 36, 411 (1971).
Yatazawa, M., Furuhashi, K., Shimizu, M., *Plant Cell Physiol.* 8, 363 (1967).
Zwar, J. A., Brown, R., *Nature (London)* 220, 500 (1968).

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Extraction of Nitrogenous Constituents from the Jack Bean (*Canavalia ensiformis*)

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Studies were carried out on the nitrogen (protein) solubility of the Jack bean (*Canavalia ensiformis*). Using a solvent-to-meal ratio of 100:6, the optimum conditions for a one-step protein extraction, using an aqueous system, were pH 13, 70° for 1 hr. The point of minimum solubility was found at pH 4.9, and nitrogen solubility increased toward the acidic and basic sides. Jack bean meal proteins displayed a different pH-nitrogen solubility profile in 0.5 *N* NaCl solution. At pH 4.9, 70% of the extracted nitrogen was recovered. The recovered proteins showed a higher essential amino acid content than the original meal. Methionine was the most limiting amino acid in both cases. When using methionine, the chemical score for the protein concentrate was lower than for the original meal. The reverse was true when the second most limiting essential amino acid was used, indicating a better quality protein for the concentrate once the methionine deficiency is overcome.

Jack bean (*Canavalia ensiformis*) can be grown relatively easily, producing high yields in regions of low altitude, high temperature, and relative humidity, inadequate for the growth of other edible legume foods such as *Phaseolus vulgaris* (Rachie, 1973). Thus, the Jack bean has a high potential in regions of varying climates and altitudes like the Central American area where it would not compete with the black bean (*Phaseolus vulgaris*) and could yield an additional protein source.

Although the Jack bean has a relatively high protein

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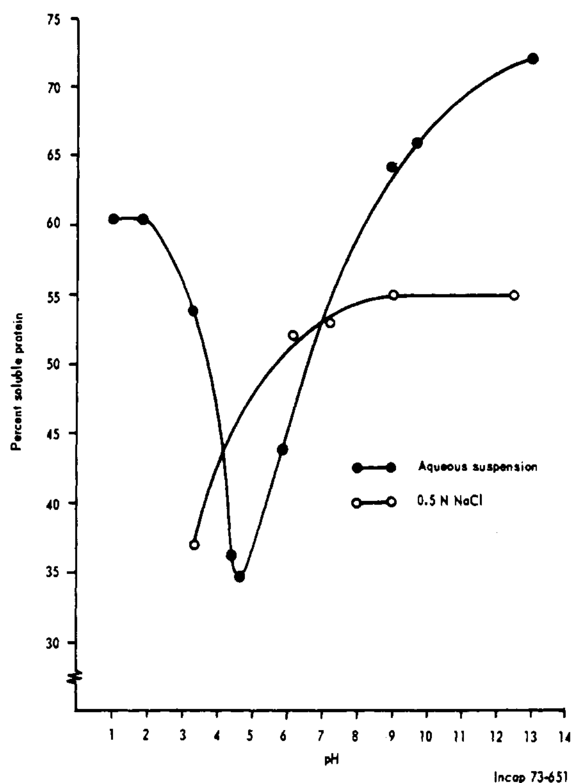

Figure 1. Protein solubility profiles of Jack bean meal in aqueous and 0.5 *N* NaCl solutions at varied pH values adjusted by addition of NaOH or HCl.

content (around 30%), its use as human food in Central America is limited both by the presence of growth-inhibiting factors (Borchers and Ackerson, 1950; Orru and Demel, 1941) and, mainly, by the regional dietetic habits which do not include the Jack bean as part of the diet.

In order to increase the utilization of this legume food as a protein source in the area, two main possibilities have been considered: (1) its use as partial substitute of the black bean in the manufacture of precooked black bean flours; and (2) the production of a protein concentrate from Jack bean which could be used in several food formulations.

The present work was undertaken to establish the optimum conditions for the extraction and recovery of the protein from the Jack bean through a simple low-cost process.

EXPERIMENTAL SECTION

The Jack bean used in this study was grown at INCAP's experimental farm "San Antonio Pachalí," Guatemala, at an altitude of 4500 ft above sea level and corresponded to the 1971 crop. The seed was milled in a hammer mill, equipped with a 30-mesh screen, to obtain the meal which was used throughout the study.

Nitrogen, ash, ether extract, moisture, crude fiber, and total solids were determined in duplicate according to the AOAC (1970). Protein estimations were carried out by multiplying the nitrogen content by the customary conversion factor, 6.25.

All the extractions were carried out using a reciprocal water bath shaker (New Brunswick Scientific, Model R-76) with a constant agitation equivalent to 120 strokes per minute. The NaOH, HCl, and NaCl used to adjust pH and/or sodium chloride concentration in the suspension were all reagent grade. The recuperation of the extracts was carried out by centrifugation, using a basket centrifuge (International, Model CH-33918 H) at 4000 rpm. The protein concentrate was obtained through isoelectric point precipitation. The protein concentrate thus obtained was then washed three times with water adjusted to a pH equivalent to the isoelectric point and dried under vacuum at 60 ± 1° for 36 hr prior to further analysis.

The amino acid analyses were carried out at "Alimentos Balanceados de México" (ALBAMEX), México, D. F., using an amino acid autoanalyzer and following the method of Spackman *et al.* (1958).

Urease activity was tested according to the method described by Sumner (1955).

RESULTS AND DISCUSSION

The proximate composition (on "as is" basis) of the 30-mesh Jack bean meal revealed a high protein (29.3%) and fiber (10.0%) content. The high fiber content can be explained by the fact that the meal was prepared from the whole seed, including the shell. The percent moisture, ether extract, ash, and nitrogen-free extract found for the meal sample was 12.3, 2.7, 2.6, and 43.1, respectively.

In order to determine the minimum time required for maximum protein extraction, an aqueous suspension of the meal was prepared at an adjusted pH of 9.0 using a 6:100 meal-to-solvent ratio and an extraction temperature of 25°. The results indicate that the maximum protein extraction efficiency (63.0 ± 0.46%) is obtained after 1 hr of treatment under the specified conditions. Analysis of the data showed that there was not a statistically significant difference between the extraction efficiency obtained at 1 hr from that obtained at longer periods up to 2.5 hr, while there was a statistically significant difference ($p < 0.05$) from the efficiency obtained at a period of 0.5 hr (61.03 ± 0.39%). For this reason, 1 hr of treatment was used in all subsequent experiments.

In order to study the effect of pH on the protein extraction yields, the pH of the aqueous suspension was varied by the addition of NaOH and/or HCl prior to the extraction, which was carried out at 25° for 1 hr using a meal-to-solvent ratio of 6:100. The results are shown in Figure 1. As may be observed, the protein extraction is favored either by an acid pH (between 1 and 2) or by an alkaline pH (higher than 12), the maximum extraction yield being obtained at a pH value of 13. Such results appear to be in accordance with those reported for several legume seeds (Evans and Kerr, 1963; Hang *et al.*, 1970a; Pant and Tulsiani, 1969; Powrie, 1961; Smith *et al.*, 1959). Practically no pH drift was observed during the protein extraction treatment.

The low protein solubility at the pH values of 4.0 to 6.0 may be attributed to the intermolecular attraction of proteins in the isoelectric zone. However, part of this low solubility could also be due to the formation of protein–phytic acid complexes as reported for navy beans (*Phaseolus vulgaris*) at similar pH values (Powrie, 1961). The different pattern of protein solubility found when using acidic 0.5 *N* NaCl solutions (Figure 1) seems to support such a possibility, since in navy beans (*Phaseolus vulgaris*) the NaCl ions in acid solvent and at similar concentrations seem to restrict the binding of phytic acid by proteins (Powrie, 1961).

It should be noted that the maximum protein extraction achieved with 0.5 *N* sodium chloride solutions at different pH values (approximately 55%) never reached the levels obtained when using an aqueous system and a pH higher than 9.0 (65 to 74% of the original protein). Nevertheless, the change in protein solubility pattern at different pH values using solvents containing ions like sodium chloride could be of some nutritional significance. This assumption is based on the fact that in other materials it has been shown that when the protein extraction is effected at pH values higher than 12, the nutritive value of the extracted protein is compromised (De Groot and Slump, 1969; Pant and Tulsiani, 1969).

Due to the high protein extraction efficiency found when using an aqueous solvent at an alkaline pH, it was

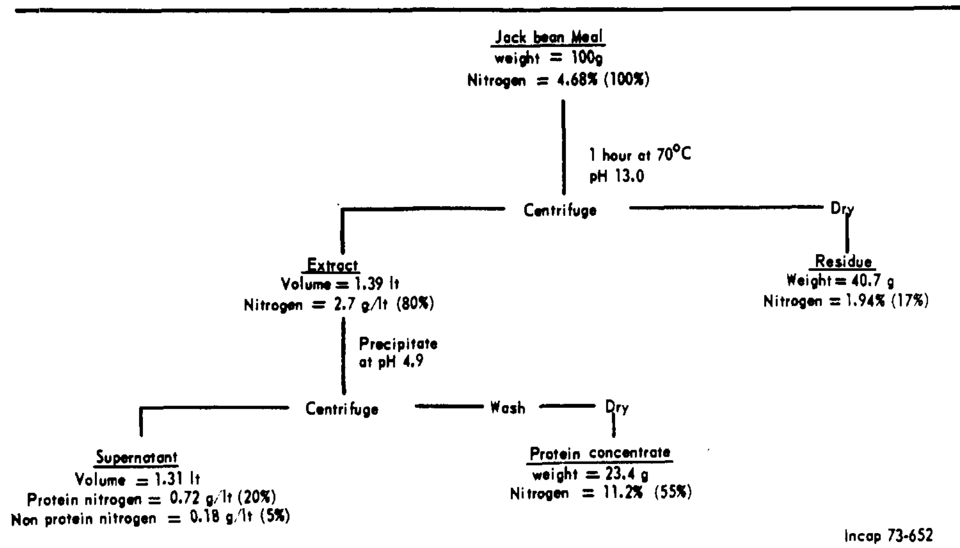

Figure 2. Nitrogen balance of the protein extraction process for Jack bean meal (*Canavalia ensiformis*).

Table I. Effect of Temperature on the Protein Extraction Yield[a]

Temp, °C	Total protein extracted from 100 g of material, g	Protein extraction yield, %
25	21.0	71.7
40	21.7	74.1
50	22.2	75.8
60	23.0	78.5
70	23.4	80.0
80	23.5	80.0

[a] One-hour treatment using a 6:100 meal-to-solvent ratio and a pH of 13.

Table II. Effect of the Meal-to-Solvent Ratio on the Protein Extraction Yield and Total Solids Content of the Extract[a]

Meal-to-solvent ratio, g/ml	Total solids of extract, %	Protein content of extract, %	Protein extraction yield, %
1:100	1.75	0.30	100.0
2:100	2.55	0.60	96.9
4:100	3.86	1.13	89.6
6:100	4.94	1.58	80.0
8:100	6.18	2.00	72.4
10:100	7.31	2.61	65.9
12:100	8.62	3.01	49.3
14:100	10.08	3.63	49.3

[a] One-hour treatment using an extraction temperature of 70° and a pH of 13.

decided to study the temperature effect utilizing a 6:100 meal-to-water ratio and a pH of 13 (Table I). The results indicate that the temperature has an effect on the extraction and that the maximum efficiency (80.0 ± 0.33%) can be obtained when the extraction is carried out at a temperature of 70°. Analysis of the data revealed that the difference between such extraction efficiency and that obtained at 60° (78.5 ± 0.22%) was statistically significant at the 1% confidence level.

Since for other materials it has been found that the meal-to-solvent ratio used in the extraction is a determining factor on the efficiency of the process (Hang *et al.*, 1970b,c; Lu and Kinsella, 1972) it was decided to establish the optimum meal-to-solvent ratio for the present case. The results (Table II) indicate that the extraction efficiency is inversely related to the meal-to-solvent ratio used. The 6:100 meal-to-solvent ratio was adopted for subsequent experiments since, when such a ratio was increased, the fluidity of the suspension was lost, thus compromising the operations during both the extraction and recuperation process of the extract.

As the data show, maximum recuperation, equivalent to 70.3 ± 0.22% of the extracted protein, occurs at a pH of 4.9 ± 0.01. At such pH, 67.5 ± 0.14% of the total solids extracted were recovered. The protein concentrate recovered by this technique was found to have a protein (N × 6.25) content fluctuating between 70 and 80% (dry basis) after three subsequent washings with water adjusted to a pH of 4.9 ± 0.01.

No increment in the percentage of protein recovered was found when the precipitation at pH 4.9 ± 0.01 was effected at temperatures up to 80°

The nitrogen balance for the extraction under the optimum conditions determined is shown in Figure 2.

As can be appreciated, an average of 70% of the extracted nitrogen was recovered by precipitation at pH 4.9 ± 0.01. From the remaining 30% of the extracted nitrogen, 5% was shown to be nonprotein nitrogen according to the trichloroacetic acid test as described by Pant and Tulsiani (1969).

Table III shows the amino acid composition of both the original material and the protein concentrate recovered. By determining the chemical score using egg protein as standard (Pike and Brown, 1967) it was found that although the total essential amino acid content was higher for the protein concentrate (2.71 g/g N) than for the original material (2.36 g/g N), the chemical score of the latter (61%) was higher than that of the former (36%). In both cases methionine was found to be the most limiting essential amino acid. However, in the case of the original material, the second most limiting essential amino acid (isoleucine) gave it a chemical score of 63%, while in the case of the protein concentrate a chemical score of 74% was obtained, with threonine as its second most limiting essential amino acid. Such findings indicate that after correcting the methionine deficiency by fortification or complementation, the protein from the concentrate could be considered chemically of a better quality. Also, it should be pointed out that the chemical score was obtained using only the methionine value and not the one of total sulfur amino acids due to the lack of data for cystine.

The leucine–isoleucine ratio is quite high both in the original material (2.53) and in the protein concentrate (2.48). Such high ratio could probably compromise the nutritive value of the protein due to an amino acid imbal-

Table III. Amino Acid Composition of the Original Jack Bean Meal *(Canavalia ensiformis)* **and the Protein Concentrate (g/16 g N)**

Amino acid	Jack bean meal	Protein concentrate
Aspartic acid		13.41
Glutamic acid	11.82	12.70
Threonine	4.32	3.30
Serine	5.57	6.25
Alanine	4.36	5.46
Glycine	3.86	4.60
Valine	5.05	5.45
Methionine	1.47	1.00
Isoleucine	3.22	4.82
Leucine	8.15	11.80
Tyrosine	3.96	3.48
Phenylalanine	4.48	5.81
Lysine	5.88	6.22
Histidine	2.77	2.63
Arginine	9.06	5.15
Tryptophan	1.21	1.45

ance. No peak of a lysine–alanine complex was detected in the amino acid chromatogram of the isolate.

Table IV shows the proximate composition (on "as is" basis) of the protein concentrate and the residue from the extraction. As was to be expected, the fiber content of the residue is relatively high (21.6%) while its protein content (12.1%) is quite lower than that of the original material (29.3%). These findings suggest the use of this final residue as animal feed.

The biological evaluation of the protein concentrate is under study, including the nutritional significance of the three amino acids methionine, isoleucine, and threonine. However, since the protein concentrate was found to have no residual urease activity, it is possible that such material could be free of any of the growth-inhibiting factors found in the Jack bean (Borchers and Ackerson, 1950; Orru and Demel, 1941). The effect of the pH and temperature of extraction on the nutritive value of the extracted proteins is under active investigation as well. In this respect, if the extracting pH of 13 compromises the nutritive value of the protein concentrate, preliminary results indicate that a two-stage extraction at lower pH values can give similar yields.

We believe that the findings herein reported show that an increment in the production of a legume seed such as the Jack bean, presently showing a negligible consumption as a food item but being easily grown with high yields, could find some use in the production of protein

Table IV. Percent Composition of the Protein Concentrate and the Residue from the Protein Extraction[a]

	Protein concentrate	Residue
Moisture	4.1	13.8
Ether extract	0.4	1.2
Crude fiber	0.1	21.6
Protein (N × 6.25)	70.3	12.1
Ash	2.4	13.3
Nitrogen free extract	22.7	38.0

[a] Extraction conditions: 1 hr, pH 13, 70°, using a meal-to-solvent ratio of 6:100 with continuous agitation.

concentrates. This, in turn, could be utilized either in the preparation of texturized food items or in several food formulations.

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LITERATURE CITED

Association of Official Agricultural Chemists, "Official Methods of Analysis," 11th ed., 1970.
Borchers, R., Ackerson, C. W., *J. Nutr.* 41, 339 (1950).
De Groot, A. P., Slump, P., *J. Nutr.* 98, 45 (1969).
Evans, R. J., Kerr, M. H., *J. Agr. Food Chem.* 11, 26 (1963).
Hang, Y. D., Steinkraus, K. H., Hackler, L. R., *J. Food Sci.* 35, 318 (1970a).
Hang, Y. D., Wilkens, W. F., Hill, A. S., Steinkraus, K. H., Hackler, L. R., *J. Agr. Food Chem.* 18, 1083 (1970b).
Hang, Y. D., Wilkens, W. F., Hill, A. S., Steinkraus, K. H., Hackler, L. R., *Cereal Chem.* 47, 259 (1970c).
Lu, P. S., Kinsella, J. E., *J. Food Sci.* 37, 94 (1972).
Orru, A., Demel, V. C., *Quad. Nutr.* 7, 272 (1941).
Pant, R., Tulsiani, D. R. P., *J. Agr. Food Chem.* 17, 361 (1969).
Pike, R. L., Brown, M. L., "Nutrition: an Integrated Approach," Wiley, New York, N. Y., 1967, pp 360–363.
Powrie, W. D., *J. Agr. Food Chem.* 9, 67 (1961).
Rachie, K. O., "Seminario sobre Potencial del Frijol y otras Leguminosas Comestibles en América Latina," Cali, Colombia, February–March, 1973.
Smith, C. R., Jr., Earle, F. R., Wolff, I. A., Jones, Q., *J. Agr. Food Chem.* 7, 133 (1959).
Spackman, D. H., Stein, W. H., Moore, S. *Anal. Chem.* 30, 1190 (1958).
Sumner, J. B., *Methods Enzymol.* 2, 378 (1955).

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