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Extractability and Solubility of Leaf Protein

Antoinette Betschart*[1] and John E. Kinsella

A laboratory-scale extraction method was developed which maximized the extraction of leaf protein while minimizing the possibility of denaturation. The method consisted of homogenizing leaves in a micromill at 6° with 0.1 *M* Tris buffer, pH 7.4 containing 0.5 *M* sucrose, 7.5 m*M* ascorbic acid, 6.6 m*M* cysteine-HCl, and 14.2 m*M* mercaptoethanol. Protein (TCA insoluble) nitrogen equivalent to 60.8% of total leaf nitrogen was extracted from alfalfa leaves by this method. The protein content of cowpea, peanut, and soybean leaves was also investigated. The solubility of total and protein nitrogen of soybean leaf extracts was studied as a function of pH. Both the total and protein nitrogen were most soluble at pH 2.0 and 6.0 and above. Minimum solubility occurred between pH 3.2 and 3.7.

The inadequate supply of good quality protein for human consumption poses one of the major challenges of this era (Autret, 1970; Pirie, 1970). Leaf protein affords good potential as a protein supplement (Kinsella, 1970; Pirie, 1970). The quantity of leaf materials which can be ingested by humans is limited due to the presence of fiber and toxic substances. To facilitate the consumption of leaf protein it must be extracted, thoroughly washed, and concentrated. The nutritive value, high yields, and simplicity of extraction and preparation suggest that leaf protein can be an effective and feasible source of proteins for humans (Lexander *et al.*, 1970; Oelschlegel *et al.*, 1969; Stahmann, 1968). Several large scale processes have been developed for the extraction of leaf protein (Chayen *et al.*, 1961; Hollo and Koch, 1971; Knuckles *et al.*, 1971; Kohler and Bickoff, 1971; Pirie, 1971). These processes extract from 35 to 80% of the total leaf protein.

The extractability of leaf protein is influenced by a multitude of factors. Since cell walls and chloroplasts must be disrupted to extract the proteins effectively, the influence of the various factors is undoubtedly dependent upon their ability to disintegrate these cellular and subcellular membranes. A variety of the methods used to rupture cell walls have been reviewed by Stahmann (1963). Some of the factors which have been reported to influence the extraction of leaf proteins are: leaf species and stage of maturity (Boyd, 1968; Chayen *et al.*, 1961); the presence of mucilagenous material (Nazir and Shah, 1966); postharvest treatment (Huang *et al.*, 1971); pH and composition of the extractant, flotation ratios, and extraction time and temperature (Lu and Kinsella, 1972; Nazir and Shah, 1966; Poppe *et al.*, 1970). In general, higher protein yields are obtained by extracting tender leaves shortly after harvest in an alkaline medium.

The presence of endogenous proteolytic and oxidative enzymes in leaf extracts may partially impair the recovery of protein. Phenoloxidase and peroxidase catalyze the reduction of *o*-diphenols to quinones. The latter, in the presence of oxygen, polymerize and complex with the protein, thus impairing the solubility and digestibility of the protein (Horigome and Kandatsu, 1966; Loomis and Battaile, 1966; Stahmann, 1963). The formation of these complexes is hindered by extracting the leaves in the presence of reducing agents and by removing the phenolic sub-

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stances with polyvinylpyrrolidone (PVP) (Anderson and Rowan, 1967; Loomis and Battaile, 1966).

The yield of total extractable protein has been a major objective in leaf protein investigations (Boyd, 1968; Lexander *et al.*, 1970; Oelschlegel *et al.*, 1969). Less importance has been placed upon the possible deleterious effects of an extraction process on the native protein structure and subsequent functional properties which are important in food applications. The present study was undertaken to develop a procedure to maximize the extraction of leaf protein while attempting to minimize denaturation, determine the protein extractability of a limited number of species using the method developed, and examine the solubility of the leaf protein extracts at various pH values.

EXPERIMENTAL SECTION

Leaf Material. Alfalfa (*Medicago sativa* L., Saranac) and soybean (*Glycine max.* L., Hark) leaves were obtained from field plots in nearby Caldwell Field (Cornell University). The harvested leaves were immediately brought to the laboratory, individually removed from the stem, cleaned by removing extraneous material with paper towels, and extracted within 2 hr after harvest. Some samples were freeze-dried at shelf temperatures of $\leq 7°$ and stored until use at $-30°$.

The alfalfa plants were prebloom or early bloom, from the first and second cuttings, and 30 to 50 cm in height. The alfalfa samples (*Medicago sativa* L., Saranac, Mark II, and Multileaf) which were used to compare varietal influence were first cutting, prebloom, and 40 to 60 cm in height. The soybean leaves were lush and tender, bean pods were small and immature, and the plants were 50 to 60 cm tall. The peanut (*Arachis hypogaea* L.) plants were in bloom when the leaves were harvested. The leaves of the cowpea (*Vigna sinensis*, Savi) were tender and the plants were from 30 to 40 cm in height.

Factors Influencing Leaf Protein Extractability. Several factors which affected the extractability and subsequent yield of protein were evaluated. The conditions under which the individual parameters were tested are described below. Unless otherwise specified, the extraction procedure was that of the method which was adopted (Figure 1).

pH. Duplicate 5-g samples of fresh soybean leaves were extracted in a Waring Blendor with pH adjusted distilled deionized water at pH values ranging from 1.0 to 12.0. Appropriate quantities of 1 *N* HCl and 1 *N* NaOH were used to adjust the pH. The final volume of the extract was 50 ml. Total soluble nitrogen and trichloracetic acid (TCA)-insoluble nitrogen were determined by the microKjeldahl method (McKenzie and Wallace, 1954).

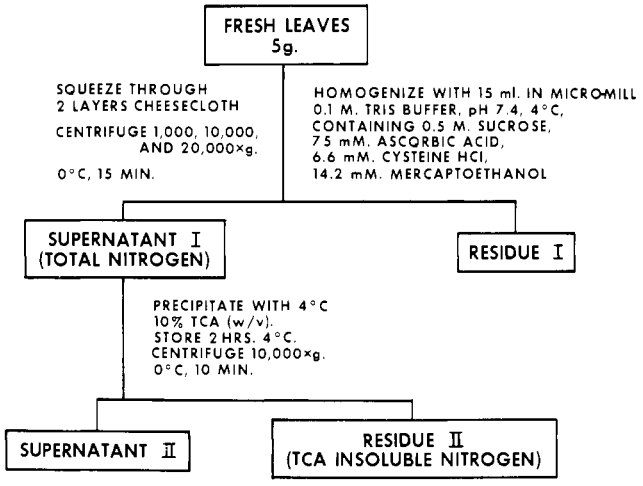

Figure 1. Extraction procedure which gave optimum yields of leaf protein.

Nature of Maceration and Extractant. The quantity of protein (TCA insoluble nitrogen × 6.25) extracted by various methods of maceration was evaluated. Those methods studied were the mortar and pestle, Waring Blendor, Waring Blendor with a stainless steel attachment, and a micromill (Chemical Rubber Co., Cleveland, Ohio). In addition, several different aqueous extracting media were explored. Those extractants which were most effective were compared, using the above methods of maceration (Table I).

Sample Size, Freeze Drying, and pH. The extraction procedure and extractant, *i.e.*, 0.1 *M* Tris buffer system, were those of the adopted method. Fresh 5 and 10-g samples of alfalfa leaves were extracted with 15 and 30 ml of buffer, respectively. Freeze-dried alfalfa leaf samples equivalent to 10 g fresh weight were also extracted with 30 ml. The influence of pH was observed by adjusting the pH of the 0.1 *M* Tris buffer system to 6.7, 7.0, 7.5, 8.0, and 8.5 with 1 *N* NaOH or 1 *N* HCl.

Method of Extraction. As a result of several preliminary tests, the extraction procedure outlined in Figure 1 was adopted. The samples were macerated in a micromill. Briefly, the micromill is a laboratory-scale apparatus which can homogenize samples of from 20 to 50 ml in size. The stainless steel grinding chamber contains a hard-faced stainless steel blade which rotates at speeds up to

Table I. Effect of Extractant and Method of Maceration on Extractability of Alfalfa Leaf Protein (TCA insoluble N × 6.25)

Composition of extractant	pH of extractant	Method of maceration: Mortar and pestle	Waring Blendor	Waring Blendor + attachment	CRC micromill
		mg of protein extracted/g of fresh leaves[b]			
0.1 *M* Tris buffer[a] 0.5 *M* sucrose 75 m*M* ascorbic acid 6.6 m*M* cysteine–HCl 14.2 m*M* mercaptoethanol	7.4	4.95 ± 0.55	9.30 ± 0.30	8.35 ± 0.35	23.63 ± 3.86
0.5% Sodium deoxycholate[c]	7.2	6.60 ± 0.10	8.20 ± 0.98	8.25 ± 0.55	14.93 ± 1.07
0.1 *M* borate HCl[d]	8.0		9.70 ± 0.20	7.80 ± 0.60	12.50 ± 0.60
0.1 *M* Hepes buffer[e] 0.1 m*M* dithiothreitol	7.4	4.45 ± 0.25	7.30 ± 0.20	8.74 ± 0.35	

[a] Stavely and Hanson (1967). [b] Values represent mean and standard error of three extractions; each extraction was analyzed in triplicate. [c] Hilty and Schmitthenner (1966). [d] Mukhopadhyay and Millikan (1967). [e] McCown *et al.* (1968).

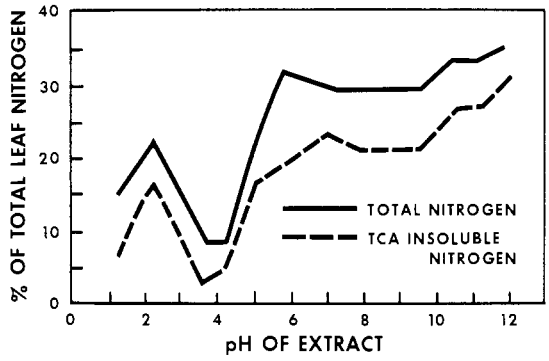

Figure 2. Extractability of total and TCA-insoluble nitrogen from fresh soybean leaves using a Waring Blendor and pH adjusted water.

20,000 rpm. The 5-g leaf samples were macerated for two 15-sec intervals interrupted by a 15-sec pause to preclude the excessive formation of heat. In addition, cool water (4.5°) was continuously circulated through the heat exchanger which encircles the grinding chamber and cover in order to maintain low temperature during extraction. The extracts were squeezed through two layers of cheesecloth into centrifuge tubes which were immersed in ice. An automatic refrigerated centrifuge (Sorvall RC2-B, Ivan Sorvall, Inc., Norwalk, Conn.) was used. Samples were centrifuged at the initial low *g* force to remove cell debris; the respective supernatants were then sequentially centrifuged at 10,000 and finally at 20,000 × *g*.

Total and TCA nitrogen were determined by the microKjeldahl method of McKenzie and Wallace (1954), except that digestion was carried on for 30 min after fuming. Duplicate glycine controls (ammonia-free) (Nutritional Biochemicals Corporation, Cleveland, Ohio) were carried through each series of analyses. Recovery based on glycine nitrogen was 98 ± 2.1%. Unless otherwise noted, triplicate analyses were conducted on each sample.

This extraction method was used to determine the influence of species (*Medicago sativa* L., Saranac; *Glycine max.* L., Hark; *Vigna sinensis*, Savi; *Arachis hypogaea* L.) and variety (*Medicago sativa* L., Saranac, Mark II, Multileaf) on the protein content of a limited number of samples. The influence of freeze-drying on protein extractability was evaluated in two leaf species, *i.e.*, alfalfa (*Medicago sativa* L., Saranac) and soybean (*Glycine max.* L., Hark). The crude protein (N × 6.25) of the leaves was determined on 100-mg samples by the microKjeldahl method.

Solubility of Soybean Leaf Extracts. The relative solubility of the total nitrogen and protein (TCA insoluble) nitrogen present in leaf extracts was studied at various pH values. A large quantity of leaves was extracted in order to provide sufficient extract to conduct the solubility studies. Therefore, a Waring Blendor was used to extract the soluble proteins of 29 g of freeze-dried soybean leaves with 400 ml of deionized distilled water. The final pH of the extract was 6.1. Duplicate 10-ml aliquots of the soluble leaf protein extract were adjusted to pH values from 1.0 to 12.0 with 0.1 *N* HCl or 0.1 *N* NaOH. The pH adjusted suspensions were shaken for 1 hr at room temperature (25°) on an Evapo-Mix (Buchler Instruments, Fort Lee, N. J.) and subsequently centrifuged at 10,000 × *g* for 15 min. The quantities of total and protein nitrogen which were solubilized in the supernatants at the various pH values were determined in duplicate by the microKjeldahl method. The results were expressed as percent of total nitrogen or protein nitrogen, respectively, which was initially soluble in the extract at pH 6.1.

RESULTS AND DISCUSSION

Development of the Extraction Method. Several factors were investigated in an effort to maximize the aqueous extractability of leaf protein while minimizing conditions which favor denaturation. The extractability of both total and TCA-insoluble nitrogen was markedly influenced by pH; maximum quantities of both were extracted at pH 12.0 (Figure 2). The enhanced extractability of leaf protein with increasing alkalinity has been observed by others (Festenstein, 1961; Lu and Kinsella, 1972; Poppe *et al.*, 1970). Leaf protein is more soluble and chloroplasts are disrupted more effectively at higher pH values (Chayen *et al.*, 1961; Stahmann, 1963).

The extractability profile also suggests that extraction at pH values of 7.0 to 8.0 would be quite effective. The additional extractability that might be gained by using a strongly alkaline medium was deemed insufficient in the present study to compensate for the possible denaturation of the extracted proteins.

Minimum extractability occurred near the isoelectric point; for total nitrogen it ranged from pH 3.7 to 4.2, whereas that of TCA-insoluble nitrogen was 3.7. Under the conditions of this experiment, *i.e.*, use of a Waring Blendor and distilled deionized water (25°) as the extractant, the maximum TCA-insoluble nitrogen extracted was less than one-third of the total leaf nitrogen. It was apparent that the leaves were not thoroughly macerated, for distinct particulate matter was observed in the extract. Since the characteristics of the composite leaf proteins were to be studied rather than a selective portion which was most readily extractable, alternative means of maceration were investigated.

Of the methods of maceration investigated, the micromill was the most efficient in extracting protein from fresh alfalfa leaves (Table I). The inadequacy of the mortar and pestle may be due to the smooth slippery nature of the leaves in the presence of an extractant and the resultant difficulty of attaining thorough maceration. The efficiency of the micromill may be attributed to the small grinding chamber and effective maceration, which gave a very homogeneous final extract.

Based upon the extractability profile (Figure 2), extractants ranging from pH 7.2 to 8.0 were explored (Table I). Buffers were investigated since they tend to counteract the pH drift which occurs during maceration as a result of the release of organic acids from the ruptured vacuoles. In the absence of this buffering action, the resultant lower pH would probably render the proteins less soluble.

Of the extractants used with the micromill, the 0.1 *M* Tris buffer system of Stavely and Hanson (1967) was most efficient in extracting protein (Table I). This is probably due to the presence of sucrose and reducing agents in the buffer system. Sucrose enhances chloroplast disruption by its influence on osmotic pressure. Also, sucrose and other polyhydroxy compounds minimize the aggregation and precipitation of leaf proteins after they are extracted (Heitefuss *et al.*, 1959). The presence of the reducing agents and/or disulfide-splitting agents ascorbic acid, mercaptoethanol, and cysteine hydrochloride would allow the sulfhydryl groups to remain intact and preclude the inter- and intramolecular disulfide bridge formation which is associated with protein–protein aggregation and subsequent precipitation (Heitefuss *et al.*, 1959; Stahmann, 1963). The reducing agents also prevent the oxidation of *o*-diphenols to quinones and the subsequent polymerization and complexing with proteins (Anderson and Rowan, 1967). Conceivably some if not all of these favorable mechanisms functioned to enhance the effectiveness of the 0.1 *M* Tris buffer system as an extractant for leaf protein.

A detergent, 0.5% Na deoxycholate, was also studied as a potential extractant. Approximately equal quantities of leaf protein were extracted by 0.5% Na deoxycholate or the 0.1 *M* Tris buffer system, using all methods of maceration except the micromill (Table I). Others have reported highly efficient extraction of leaf proteins in the

Table II. Effect of Sample Size, Freeze Drying, and pH on Extractability of Alfalfa Leaf Protein (TCA insoluble N × 6.25)

Sample size and state of leaves	pH of extractant[a]	pH of extract	mg of protein/g of fresh leaves[b]	mg of protein/g of freeze-dried leaves[b]
5 g of fresh leaves	6.7	6.7	45.04 ± 0.07	
	7.1	7.1	48.48 ± 0.09	
10 g of fresh leaves	7.0	7.0	37.13 ± 0.38	
	7.5	7.4	37.14 ± 0.51	
	8.0	7.8	36.63 ± 1.02	
	8.5	8.5	36.34 ± 0.88	
10 g of freeze-dried leaves	7.0	7.0	40.63 ± 2.73	163.48 ± 2.05
	7.8	7.7	37.46 ± 0.57	156.71 ± 2.40
	8.1	8.0	38.01 ± 0.14	159.81 ± 1.38
	8.6	8.5	37.94 ± 1.10	158.73 ± 4.63

[a] Extractant was 0.1 *M* Tris buffer (See Table I). [b] Mean and standard error of three extractions.

presence of detergents; this is attributed to the disruptive effects of the detergent upon chloroplasts and other protein-containing bodies (Buraczewski and Bruraczewska, 1964; Firenzuoli and Mastronuzzi, 1966; Hilty and Schmitthenner, 1966). These beneficial disruptive effects may have been overshadowed in the present study by the efficiency of the micromill.

Under the most favorable conditions, *i.e.*, the micromill and 0.1 *M* Tris buffer system, the maximum protein extracted from alfalfa was 2.4% of the fresh leaf weight or approximately 40% of the crude protein (total N × 6.25). Therefore, other aspects of the extraction process, such as additional rinsing of the fibrous residue, sample size, and pH of the 0.1 *M* Tris buffer, were studied. The fibrous residue remaining in the cheese cloth was rinsed a second and third time with 10 ml of 0.1 *M* Tris buffer. This rinsing procedure recovered an additional 4–8% of the crude protein.

Although a flotation ratio (ml of extractant/g of fresh material) of 3:1 was used throughout, 5-g samples were more thoroughly extracted than samples weighing 10 g (Table II). Conceivably, the larger samples may have exceeded the physical capacity of the grinding chambers. Altering the pH of 0.1 *M* Tris buffer from 6.7 to 8.5 had little influence on protein extractability (Table II). Thus, the initial pH of 7.4 continued to be used. As a result of these observations, the extraction procedure was modified to include three rinsings of the fibrous residue with 0.1 *M* Tris buffer and the reduction of sample size to 5 g. With these modifications, from 60 to 62% of the crude protein was extracted.

It was coincidentally observed that freeze drying did not impair the extractability of protein from alfalfa leaves (Table II). This was of practical interest since fresh samples were not always available.

Protein Content of Some Varieties and Species. The extraction method developed (Figure 1) was used to determine the protein content of a limited number of varieties and species. It was also used to extract samples in preparation for a study on the effects of storage on the nutritive value of leaf protein concentrate (LPC) to be reported elsewhere. LPC is prepared by acid or heat coagulation of supernatant I.

Of the species and varieties investigated, the largest quantities of extractable protein were obtained from fresh and freeze-dried alfalfa (*Medicago sativa* L., Saranac) and fresh soybean (*Glycine max.* L., Hark) leaves (Table III). Either of these would be good sources for leaf protein preparations. Although cowpea leaf protein was the most easily extracted, the total extractable protein was less than the above-mentioned species because of the lower initial content of crude protein.

Freeze drying impaired the extraction of protein from soybean leaves (Table III). This is in contrast to the negligible influence of freeze drying upon the extractability of alfalfa leaf protein (Tables II and III). Both species of leaves were harvested under nearly comparable conditions, *i.e.*, they were not fully mature and both contained from 73 to 77% moisture. The differences in the nature of the leaves may account for some of the observed variability in the extraction of protein from the freeze-dried samples. Alfalfa leaves are small and more readily frozen than

Table III. Crude and Extractable Protein of Fresh and Freeze-Dried Leaves

Scientific name	Common name	No. of samples	Crude protein content of leaves (N × 6.25)[a]		No. of samples	Extractable leaf protein (TCA insoluble N × 6.25)[a]		Extractable protein N – % of total leaf N
			mg/g of fresh leaves	mg/g of dried leaves		mg/g of fresh leaves	mg/g of dried leaves	
Fresh leaves								
Medicago sativa L., Saranac	Alfalfa	5	73.0 ± 1.3		10	44.4 ± 1.6		60.8
Glycine max. L., Hark	Soybean	5	76.4 ± 0.7		5	37.0 ± 0.6		48.4
Vigna sinensis. Savi	Cowpea	5	45.7 ± 0.8		5	29.1 ± 1.0		63.7
Arachis hypogaea L.	Peanut	5	52.1 ± 1.2		5	24.9 ± 0.9		47.8
Freeze-dried leaves								
Medicago sativa L., Saranac	Alfalfa	3	67.5 ± 2.3	394.0 ± 4.2	3	39.0 ± 0.6	163.1 ± 2.0	57.8
Medicago sativa L., Mark II	Alfalfa	3	55.4 ± 1.2	318.3 ± 2.4	3	19.7 ● 1.1	112.6 ± 3.1	35.6
Medicago sativa L., Multileaf	Alfalfa	3	59.2 ± 0.9	383.9 ± 3.6	3	27.7 ± 1.3	121.2 ± 2.7	46.8
Glycine max. L., Hark	Soybean	3	65.3 ± 1.0	340.2 ±1.8	10	23.4 ± 0.9	97.7 ± 1.3	35.9

[a] Mean and standard error.

Table IV. Quantity of Protein Extracted from Various Leaf Species

Scientific name	Common name	State of leaves	Extractable protein N – % of total leaf N	Reference
Medicago sativa L.	Alfalfa	Fresh	37.0	Valli Devi *et al.* (1965)
Medicago sativa L.	Alfalfa	Fresh	42.2	Singh (1964)
Medicago sativa L.	Alfalfa	Fresh	67.6	Huang *et al.* (1971)
Medicago sativa L., Saranac	Alfalfa	Fresh	60.8	Present study
Medicago sativa L., Saranac	Alfalfa	Freeze-dried	57.8	Present study
Medicago sativa L., Multileaf	Alfalfa	Freeze-dried	46.8	Present study
Medicago sativa L., Mark II	Alfalfa	Freeze-dried	35.6	Present study
Vigna sinensis, Savi	Cowpea	Fresh	52.0	Byers (1961)
Vigna sinensis, Savi	Cowpea	Fresh	63.7	Present study
Glycine hispida	Soybean	Fresh	31.0	Valli Devi *et al.* (1965)
Glycine max. L., Hark	Soybean	Fresh	48.4	Present study
Glycine max. L., Hark	Soybean	Freeze-dried	35.9	Present study
Arachis hypogaea L.	Peanut	Fresh	18.5	Gonzalez *et al.* (1968)
Arachis hypogaea L.	Peanut	Fresh	27.9	Byers (1961)
Arachis hypogaea L.	Peanut	Fresh	47.8	Present study

the larger thicker soybean leaves. The alfalfa proteins may, therefore, be less altered during freeze drying than those of soybean. The physical nature of the large freeze-dried soybean leaves may also be more difficult to rehydrate and thus hinder protein extraction.

Several investigators have screened leafy materials for their protein content (Boyd, 1968; Byers, 1961; Gonzalez *et al.*, 1968; Lexander *et al.*, 1970). The quantities of protein extracted from fresh and freeze-dried leaves in this study compared favorably with reported values where protein nitrogen was determined by heat precipitation or TCA insolubility (Table IV). Also, the amount of protein extracted from fresh alfalfa (*Medicago sativa* L., Saranac), cowpea, and soybean leaves was nearly equivalent to large scale extraction methods which usually extract 55 to 75% of the total leaf protein (Pirie, 1971). The quantities of protein extracted in the present study were encouraging since many of the factors which enhance protein extractability but may also promote denaturation (*i.e.*, a strong alkaline medium, high temperatures, and lengthy extraction times) were not employed. The proteins extracted by the method described in this study should, therefore, have potential in terms of functional properties. For this potential to be realized it is imperative that these functional properties be retained during the isolation process. This suggests that methods of isolation other than heat coagulation be explored.

Solubility of Soybean Leaf Protein. The solubility profile of extracted leaf protein reveals its isoelectric point for future precipitation studies as well as its native solubility prior to any major denaturation. The isoelectric point of TCA-insoluble nitrogen was between pH 3.2 and 3.7 (Figure 3). This is slightly lower than the pH values of 4.0 to 4.5 used by others to precipitate leaf protein (Chayen *et al.*, 1961; Cowlishaw *et al.*, 1956; Subba Rau and Singh, 1970). The slight discrepancy may be due to differences in the protein extractants. Most studies have used water as the extractant or simply extract the leaf juices. The isoelectric point is known to be a function of the nature and concentration of solutes present in the protein solution (Mahler and Cordes, 1966). The 0.1 *M* Tris buffer, sucrose, and small amounts of ascorbic acid, cysteine hydrochloride, and mercaptoethanol used in this study might, therefore, be expected to alter the isoelectric point.

The solubility curves (Figure 3) also suggest that acid precipitation would be an efficient method of isolating leaf protein. Less than 5% of the TCA-insoluble nitrogen remained in solution at the isoelectric point.

Prior to isolation or denaturation, leaf proteins are very soluble at pH 2.0 and 6.0 and above. In this state leaf proteins offer some potential for incorporation into foods. It would be of interest to compare the solubility profiles of the protein extract with that of LPC after it has been isolated by such methods as acid or heat. The denaturation which accompanies heat coagulation would be expected to greatly impair solubility. The solubility profiles of LPC are being studied and will be reported in a subsequent paper.

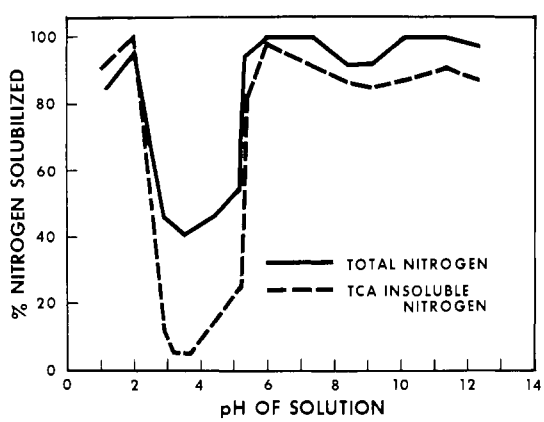

Figure 3. Solubility at room temperature (25°) of total and TCA-insoluble nitrogen of soybean leaves. Values are expressed as percent of the total and TCA-insoluble nitrogen which was originally soluble in the extract at pH 6.1.

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Determination of Mineral Elements in Plant Tissues Using Trichloroacetic Acid Extraction

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An extraction method is described for quantitatively determining Mg, K, Na, Zn, and Mn in plant tissues. The tissue is extracted with 2% trichloroacetic acid and the elements are determined on the filtrate by atomic absorption spectrophotometry. Results compare favorably with those determined after wet ashing with nitric and perchloric acids. In addition, trichloroacetic acid extracts the same fraction of P as does acetic acid and quantitatively extracts total Ca from plant tissues not high in oxalate.

The mineral content of plant samples is usually determined by analyzing the liquid phase after dry ashing or wet ashing ground dried plant materials. The procedures and the problems of fume disposal and special laboratory equipment required for the methods have been discussed (Johnson and Ulrich, 1959). Nicholas (1951) surveyed the literature on extraction methods for tissue analysis in determining the nutrient status of various crops. Many reports indicate good correlations between nutrients extracted and fertilizer applications, but only a few compare the extracted values to the total content. Nicholas (1948a,b) extracted plant tissue with acetate, citrate, malonate, and succinate buffers (pH 4.8). The concentrations determined by extraction were correlated with the total concentrations in the tissue, but the two sets of values were not directly compared. Greweling (1962) and Baker and Greweling (1967) analyzed the EDTA extracts from a variety of plant tissues for several mineral elements and compared the results with those obtained after dry ashing. In most cases the extracted and ashed values agreed closely.

Analysis of acetic acid extracts of plant tissues for Zn and Mn indicated that extraction of these elements was essentially complete from most but not all plant materials. Consequently, a stronger acid, trichloroacetic, was used and the number of elements determined extended to include Ca, Mg, K, Na, Cu, Fe, and P. This report deals with the use of trichloroacetic acid for extracting the total contents of several mineral elements from various plant tissues and a study of some of the factors affecting its use.

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METHODS AND MATERIALS

Plant Samples. Forty-two samples representing 20 different crops (Table I) were analyzed for their total content of several mineral elements by wet ashing and for the amount of the various constituents extracted in various solutions. All samples were washed in demineralized water, dried at about 60°, and ground in an all-steel Wiley mill to pass a 40-mesh stainless steel sieve. The ground material was redried for at least 2 hr at 60° before weighing out samples for analysis.

Extracting Solutions. *Trichloroacetic Acid (TCA) Solution.* A 2% (w/v) solution was prepared by dissolving 20 g of reagent grade TCA in distilled demineralized water and diluting to 1 l.

Acetic Acid (HOAc) Solution. A 2% (v/v) solution was prepared by diluting 20 ml of reagent grade HOAc to 1 l. with distilled demineralized water.

Ammonium EDTA Solution. A 1.0 *M* stock solution was prepared according to the procedure of Baker and Greweling (1967). This solution was diluted with distilled demineralized water to make 0.1 *M* ammonium EDTA as needed.

Extraction of Plant Tissue. Samples (0.5 g) were weighed into wide-mouthed 125-ml linear polyethylene