Table III. Results of Nitrogen Studies with Growing Rats

Processing	Apparent Digestibility, %	True Digestibility, %	Biological Value, %
	CORN		
Control	83.4	91.1	48.0
Cooked, 4 minutes, 15 pounds	83.1	92.4	45.8
Irradiated, 2.8×10^6 rad	84.3	92.9	47.1
Irradiated, 9.3×10^6 rad	76.4	86.3	46.3
	WHEAT GLUTEN		
Control	91.8	98.7	41.6
Cooked, 4 minutes, 15 pounds	91.6	98.5	41.4
Irradiated, 2.8×10^6 rad	91.4	99.1	41.7

ysis of variance showed no difference in digestibility (99%) or biological value (42%) in wheat gluten owing to processing—heat or irradiation. The limiting amino acid in wheat gluten is lysine. Heat processing of proteins has been reported to bring about reduced availability of lysine probably owing to the formation of a new peptide linkage between ε-amino group of lysine and a free carboxyl group of other amino acids. There was apparently no such chemical change due to irradiation as no reduction in the biological value was observed when wheat gluten in water suspension was autoclaved for 4 minutes at 15 pounds pressure or was irradiated at 2.8 million rad. The nitrogen metabolism data on wheat gluten are in excellent agreement with the reported values (*8*). The lysine content of wheat gluten (2.2%) did not change owing to heat cooking or irradiation.

Thus, lysine in corn or wheat gluten is not damaged due to irradiation sterilization. There was no change in the digestibility or the biological values of the proteins of the corn and wheat gluten when processed at 2.8 million rad gamma irradiation.

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SEED PROTEIN SOLUBILITY

Comparison of Solubility Characteristics of Selected Seed Proteins

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IN A RESEARCH program on the chemical composition of seeds from plants not now cultivated as economic crops in the United States (*18*), information was desired permitting selection of seed species containing protein constituents extractable in high yield under mild conditions. It was also desirable to classify and characterize protein systems under study, at least on an empirical basis. To this end, meals from a number of seed species were extracted with a series of solvents and the percentage of extractable nitrogen was determined.

The suitability of proteins for given uses is difficult to define in terms of fundamental structure, composition, and properties in the same fashion that amino acid content is related to nutritive value. Present commercial uses for protein have generally been developed empirically (*12*). Practically all current industrial applications for proteins, such as production of fibers, sizes, adhesives, ingredients of coatings, emulsifiers, and food additives depend upon bringing proteinaceous material into solution. The material is usually derived in high yield from some high protein source. Hence a knowledge of solubility would appear to be an important factor in selection of vegetable proteins for possible industrial applications. Some of these applications would also depend on use of the mildest possible extraction conditions.

Selection of Materials. Factors considered in selecting seed species for these extractions were: high protein and/or oil content, general prominence or promise of the botanical family to which the species belongs, and desirability of varying widely the spectrum of plant families included. Emphasis was placed on little-investigated seed species; certain extensively studied ones, such as wheat, corn, soybeans, and flax, were included for comparison.

Method. To compare solubility characteristics of seed protein constituents, the following solvents were selected: 0.01*M* sodium hydroxide (pH 11.7 to 11.9); 0.5*M* disodium phosphate (pH 8.9); 0.5*M* sodium chloride; 70% ethyl alcohol, and water. These selections were more or less arbitrary and changes in concentration of solutions used as extractants would doubtless have produced different results. As a measure of nonprotein nitrogen the trichloroacetic acid extraction procedure of Becker,

Extractability of the protein and other nitrogenous constituents of 41 species of seeds, representing 21 plant families, has been studied. Considerable diversity in solubility characteristics was found, judging from the differing patterns of solubilities encountered in extraction with 0.01*M* sodium hydroxide, 0.5*M* disodium phosphate, 0.5*M* sodium chloride, water, 70% ethyl alcohol, and 0.8*M* trichloroacetic acid. These data provide information that will permit selection of species producing seed protein constituents which are extractable in high yield under mild conditions and that will allow an empirical grouping of similar plant materials as an aid in future studies.

Milner, and Nagel (*2*), which employs 0.8*M* trichloroacetic acid, was used in the modified form as described. This extraction procedure was developed for use with the soybean, but was thought to serve for purposes of comparison. In any case, nonprotein nitrogen in vegetable materials includes a very heterogeneous group of substances which will respond differently to various extractants or precipitants (*16*).

Seeds were ground in a hammer mill, extracted overnight with petroleum ether (30 to 60° C.), and reground and/or re-extracted if required to produce an essentially oil-free meal of approximately 100 mesh. Oil and protein analyses of seed meals, as initially obtained, were carried out by standard procedures (*1*, *5*); results for proteins are listed in Table I.

Oil-free meals were placed under vacuum to remove traces of solvent, then equilibrated with atmospheric moisture for several hours before use. Separate samples of the meals were extracted with each solvent. Duplicate samples of about 1 gram were weighed accurately and introduced into 250-ml. centrifuge bottles; 50 ml. of extractant was pipetted into each and the mixture was shaken mechanically for 1 hour, then centrifuged. All extractions were carried out at room temperature, 25° C., without constant temperature regulation. Supernatants were filtered when necessary to remove floating particles. The nitrogen content of the supernatants was determined by a micro-Kjeldahl procedure (*17*). Results of duplicate extractions which did not check within 5% were rejected. The total nitrogen content of the oil-free meals was determined by a macro-Kjeldahl method (*1*). Results of extractions were expressed as percentage of total nitrogen in the meal extracted by a given solution. The pH of the sodium chloride, disodium phosphate, and water extracts was determined routinely.

Heat-coagulable, water-soluble protein was determined by heating the aqueous extract obtained as described in the preceding paragraph at reflux for 15 minutes, centrifuging to sediment the coagulum, if any, and determining the nitrogen remaining in the supernatant. Values in Table I for this water-soluble, heat-coagulable protein were then determined by difference.

Results and Discussion

Results obtained by application of the above method are summarized in Table I. Data are grouped to point out correlations between botanical relationships and solubility characteristics, and other differences or similarities between the species studied. Solubility of protein constituents as determined in this procedure may be affected considerably by other constituents present. The results are therefore considered as indicative of the solubility or dispersibility of protein in various seeds, not as precise values characteristic of purified proteins.

Prolamines should be included in nitrogen measured by the 70% ethyl alcohol extraction. Because the alcohol soluble nitrogen figure for the seeds studied was appreciably higher than the trichloroacetic acid–soluble nitrogen only for those which were already known to contain prolamines—i.e., those of the Gramineae or grass family—there is no evidence for any appreciable amount of prolamine in any of the seeds studied other than those of the grass family. It is inferred that the nitrogen extracted by 70% ethyl alcohol was, in most cases, only nonprotein nitrogen. This inference is in accord with the results discussed by Vögeli (*16*) in a recent paper on yeast protein.

A number of seeds, especially in the legume family, contain nearly as much water-soluble as salt-soluble protein, or even more.

Routine determination of the pH of the disodium phosphate extracts revealed little variation in values from one species to another, because of the high buffering capacity of the extractant; most were within the range 8.4 to 8.6. There was considerably more variation in pH of extracts obtained with sodium chloride solution or with water. The largest numbers were within the range of 6.1 to 6.8. Some were as low as 5.0; none were higher than 7.1.

Differences between Plant Families. Considerable diversity in the solubility characteristics of seed proteins is indicated. Although broad generalizations cannot be made on the basis of the data in Table I, certain correlations may be noted among the solubilities within the individual families. The legume, cucurbit, and mallow families are notable for the generally high solubilities of their nitrogenous seed constituents in sodium hydroxide, disodium phosphate, and sodium chloride solutions at the concentrations used. Solubilities among cucurbits were especially high.

In contrast with these families having quite soluble seed proteins, species belonging to certain others—e.g., the Scrophulariaceae, Euphorbiaceae, Umbelliferae, Papaveraceae, Chenopodiaceae, and especially the Iridaceae—have seed proteins of low solubilities under the conditions employed. Only a single species in some of these families was studied, however.

Relative Efficacy of Different Extractants. As might be expected, the extractant of highest pH, 0.01*M* sodium hydroxide, extracted the largest proportion of protein from the various seeds in most cases. The next largest amounts of protein were, in general, extracted by sodium chloride or disodium phosphate. In contrast, certain species were encountered in which the largest amount of seed protein was extracted by 0.5*M* sodium chloride rather than by 0.01*M* sodium hydroxide. In such cases the disodium phosphate value was intermediate. Species exhibiting this behavior were members of the Cruciferae, the closely related family Capparidaceae, and onion seed (*Allium porrum*) of the Liliaceae family.

A considerable variation in nonprotein nitrogen content among the various seeds was noted, most of the higher values being found in the legume family

Comparison with Literature Values. Comparatively little has been systematically recorded on the solubilities of seed proteins *per se*. Most of the available data on the distribution of seed proteins in the various species into the different solubility classes have been summarized recently by Brohult and Sandegren (*3*). In their investigation of the effect of certain variables in the extraction of soybean meal, Smith, Belter, and Johnsen (*13*) found that the percentage of nitrogen extracted was affected by temperature, method of stirring, and pH of the system, as well as age and variety of the beans used. A few results may be cited in which conditions and extractants used by others approximated those used in the present work. Values for the soybean are in general agreement with data of Smith and Circle (*14*) and of Nagel, Becker, and Milner (*2*, *7*).

Table I. Protein Content and Extractability of Seeds Studied

Botanical Name	Common Name	Protein Dry Basis, %	Percentage of Nitrogenous Materials of Seed Meals Extracted by Selected Solvents: NaOH, 0.01M	Na_2HPO_4, 0.5M	NaCl, 0.5M	H_2O	Heat-coag. H_2O[a]	EtOH, 70%	TCA, 0.8M
Leguminosae									
Subfamily Papilionoideae									
Astragalus cicer	Milk vetch	39.9	90.9	60.5	41.4	31.7	0	9.7	21.7
Astragalus falcatus		41.0	94.2	62.9	40.5	33.4	2.5	7.2	11.8
Clitoria ternatea	Butterfly pea	46.9	83.9	72.4	71.9	50.8	...	7.6	7.7
Cyamopsis tetragonolobus	Guar	28.4	85.9	67.8	52.0	34.4	10.7	9.0	9.5
Dalea alopecuroides		36.6	85.6	61.4	63.4	43.8	19.7	6.8	9.7
Glycine max	Soybean	41.2	97.2	75.4	72.3	84.1	...	2.8	3.2
Hedysarum boreale	Northern sweet vetch	43.0	87.0	73.4	54.1	44.1	11.5	13.2	22.3
Lathyrus cicer	Vetchling	34.7	87.4	73.3	76.1	61.9	0.6	8.6	16.1
Lespedeza cuneata	Sericea lespedeza	40.5	83.5	64.4	45.6	36.7	3.8	5.3	10.7
Lespedeza stipulacea		52.0	87.1	70.4	70.4	42.2	0	4.7	9.9
Lupinus albus	White lupine	38.8	93.3	84.1	82.3	35.3	10.9	6.7	9.4
Lupinus angustifolius	Blue lupine	35.3	95.6	80.1	80.3	27.8	8.9	4.2	8.3
Lupinus luteus	Yellow lupine	44.1	90.4	85.1	82.4	30.3	8.6	3.9	7.5
Medicago sativa	Alfalfa	37.0	78.4	61.4	56.6	43.9	16.0	7.1	12.3
Melilotus alba	Sweet clover	42.8	76.4	61.2	53.8	41.2	0	6.7	12.0
Onobrychis viciaefolia	Sainfoin	40.9	83.5	70.0	47.6	31.3	10.0	7.8	11.7
Phaseolus aureus	Mung bean	28.2	95.6	74.3	79.1	74.0	...	3.6	6.5
Phaseolus vulgaris	Kidney bean	24.2	96.4	68.6	76.2	74.9	0	8.2	10.7
Sesbania macrocarpa		38.2	76.6	52.6	21.7	29.2	...	9.9	20.5
Trigonella foenum-graecum	Fenugreek	39.8	74.1	59.9	65.8	61.7	3.6	7.1	10.3
Subfamily Caesalpinioideae									
Cassia occidentalis	Coffee senna	22.5	68.1	62.9	41.0	34.8	1.3	5.2	8.1
Gleditsia tricanthos			89.7	66.9	40.9	27.0	...	7.1	12.8
Subfamily Mimosoideae									
Acacia occidentalis		18.9	96.0	79.8	81.6	79.8	22.0	25.3	29.6
Desmanthus illinoensis	Prickle weed	34.4	65.2	58.0	57.4	20.2	6.6	6.8	10.0
Cucurbitaceae									
Curcurbita pepo	Pumpkin	39.3	92.5	90.7	63.9	13.8	0.1	2.7	5.5
Luffa acutangula	Luffa	27.5	95.3	71.4	70.0	16.4	0	2.8	6.6
Marah gilensis		27.8	96.1	89.4	93.7	31.1	10.2	3.1	7.8
Marah macrocarpa		29.4	99.2	95.8	98.1	37.2	...	3.1	9.5
Momordica balsamina		29.5	76.9	72.8	70.5	48.7	25.4	8.1	6.3
Umbelliferae									
Daucus carota	Carrot; Queen Anne's lace	26.9	31.4	25.7	26.8	24.3	0	4.9	7.6
Foeniculum vulgare	Common fennel	20.1	50.8	36.0	39.1	40.2	0	6.1	10.9
Euphorbiaceae									
Euphorbia variegata	Snow-on-the-mountain	21.4	80.0	57.2	51.9	38.8	16.0	5.8	13.7
Euphorbia heterophylla	Painted leaf	25.2	84.2	22.1	22.5	17.3	1.0	4.1	8.4
Labiatae									
Perilla frutescens		31.8	94.8	86.6	72.4	15.7	0	2.8	7.3
Gramineae									
Triticum vulgare	Wheat	16.8	94.9	20.3	30.9	20.8	5.1	39.4	6.4
Zea mays	Corn	10.2	49.2	11.9	13.4	8.2	0.7	27.6	5.2
Solanaceae									
Datura fastulosa	Jimson weed	12.5	23.5	17.5	16.7	15.3	1.1	4.0	5.4
Rosaceae									
Sanguisorba minor	Burnet	13.5	67.8	11.3	12.2	12.2	3.0	6.1	8.7
Compositae									
Carthamus tinctorius	Safflower	17.4	87.0	38.5	76.7	34.1	11.5	10.7	12.6
Dimorphotheca aurantiaca		37.8	79.9	67.3	72.0	60.4	5.9	12.8	15.3
Guizotia abyssinica	Niger	21.1	83.9	54.3	45.0	12.8	2.9	3.1	5.4
Liatris spicata		35.6	85.4	57.9	65.7	62.2	30.0	8.2	5.1
Vernonia anthelmintica	Ironweed	18.1	75.1	20.4	25.7	13.9	0	5.6	9.2
Iridaceae									
Iris germanica		14.8	22.8	8.3	6.2	5.4	0.8	2.5	4.6
Ginkgoaceae									
Ginkgo biloba	Ginkgo	10.2	93.9	71.6	73.6	68.9	7.4	21.6	26.4
Papaveraceae									
Bocconia cordata		17.6	46.0	27.4	40.5	12.8	1.4	2.3	6.5
Linaceae									
Linum usitatissimum	Linseed; flax	25.6	84.6	60.5	75.2	54.5	2.9	2.4	7.2
Liliaceae									
Allium porrum	Onion	25.9	55.5	63.1	67.1	20.7	1.8	11.5	14.3
Chenopodiaceae									
Beta vulgaris	Beet	15.4	50.9	31.0	31.9	16.8	...	5.2	9.5
Polygonaceae									
Fagopyrum tataricum	Buckwheat	11.7	72.7	46.1	47.3	38.2	6.6	3.9	8.5
Malvaceae									
Hibiscus cannabinus	Kenaf	27.9	89.2	77.6	78.0	39.1	1.3	5.3	7.0
Gossypium sp.	Cotton	37.1	85.3	74.4	78.4	23.1	0	3.6	6.1
Amaranthaceae									
Amaranthus caudatus		17.5	94.2	39.9	49.6	27.1	8.5	7.0	11.6
Scrophulariaceae									
Digitalis purpurea	Foxglove	16.8	79.0	63.6	66.5	55.1	1.3	4.8	12.2
Penstemon murraya x grandiflora		19.7	55.7	46.6	48.9	16.5	...	3.1	5.6

(Continued on page 136)

Table 1. (*Continued*)

Botanical Name	Common Name	Protein Dry Basis, %	Percentage of Nitrogenous Materials of Seed Meals Extracted by Selected Solvents: NaOH, 0.01M	Na_2HPO_4, 0.5M	NaCl, 0.5M	H_2O	Heat-coag. H_2O[a]	EtOH, 70%	TCA, 0.8M
Cruciferae									
Brassica campestris	Rape	22.6	65.8	66.0	68.8	31.1	11.1	12.2	8.5
Brassica carinata		34.4	63.2	80.3	82.5	33.0	9.1	9.5	10.8
Eruca sativa	Garden rocket	37.4	45.6	59.4	68.3	16.1	3.0	14.5	11.3
Raphanus sativus	Radish	33.2	75.4	89.3	90.6	23.0	4.6	12.0	12.6
Capparidaceae									
Cleome serrulata	Stinking clover	23.1	48.9	64.4	66.0	10.1	1.1		9.0
Cleome pungens	Bee plant	19.8	54.0	69.0	75.6	12.9	2.1	8.2	6.6

[a] Values represent water-soluble protein coagulated by heat (obtained by difference).

Fair agreement is apparent between the data in this paper and those of Olcott and Fontaine (*8*) for oil-free cottonseed meal. Data comparable to those in Table I are also found for *Phaseolus aconitifolius* (*4*), *Phaseolus vulgaris* (*11*), *Lathyrus sativus* (*6*), *Trigonella foenum-graecum* (*9*, *10*), *Medicago sativa* (*11*), and *Linum usitatissimum* (*11*).

Significance of High Salt Solubility of Crucifer Proteins. More detailed measurement was made of the solubility characteristics of radish (*Raphanus sativus*) seed as an example of crucifer seeds which exhibited higher solubility in salt solutions than in 0.01*M* sodium hydroxide. The solubility of the nitrogenous constituents of radish seed was determined over a wide range of pH values, using dilute hydrochloric acid or sodium hydroxide solutions in appropriate concentrations. Results are summarized in Figure 1, together with comparable data for the soybean from the work of Smith and Circle (*14*). The solubility of the nitrogenous constituents of radish seed in sodium chloride solutions of various concentrations was likewise determined and these values are compared in Figure 2 with similar data for the soybean from the work of Smith, Circle, and Brother (*15*).

Figure 2 shows that changing concentrations of sodium chloride in the range of 0 to 0.1*M* has opposite effects on the solubilities of radish seed and soybean proteins. In this range, the solubility of the latter drops sharply from nearly 90% to less than 50%, whereas the values for the radish rise sharply from about 20% to nearly 50%. The radish seed solubility curve continues to rise to a maximum at 0.68*M*, from whence it drops slightly as the concentration is raised further. In a certain range of concentrations (about 0.1 to 0.4*M* sodium chloride), a bigger percentage of nitrogen is extracted from radish seed than from the soybean.

The curves in Figure 1 have the same general shape. The curve for soybeans rises sharply above pH 5 and has nearly reached a plateau at pH 8. The curve for radish seed, however, rises most sharply between pH 8 and 12. This means that a small change in the

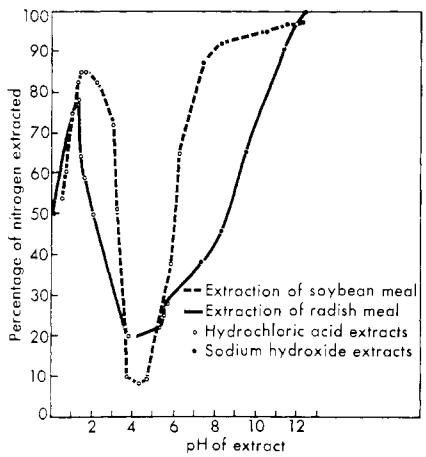

Figure 1. Percentage of total nitrogen extracted from oil-free soybean and radish meals by hydrochloric acid and sodium hydroxide solutions

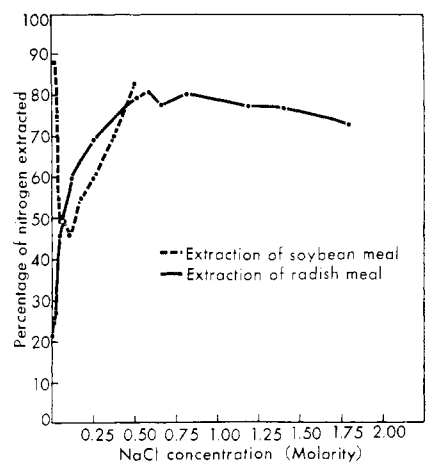

Figure 2. Percentage of total nitrogen extracted from oil-free soybean and radish meals by sodium chloride

pH of the sodium hydroxide extractant in this range will profoundly affect the amount of nitrogen dispersed and, consequently, will determine whether more or less nitrogen is extracted than with sodium chloride solutions covering a considerable range of concentrations (0.5 to 2.0*M*).

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