Effect of the Herbicide Metribuzin on the Nitrogenous Constituents of Potatoes

Nell I. Mondy*,† and Cyrus B. Munshi‡

Division of Nutritional Sciences, Institute of Food Science and Institute of Toxicology, and Department of Food Science, Cornell University, Ithaca, New York 14853

The effect of the herbicide metribuzin on the nitrogenous constituents of five potato cultivars was investigated in a 2-year study. Metribuzin was sprayed on the plots at the rate of 1.5 kg/ha AI (1.3 lb/acre) in two different ways: treatment 1 (Tr 1), application to the soil at preemergence; treatment 2 (Tr 2), application to the soil at both pre- and postemergence of the plant. Both Tr 1 and Tr 2 resulted in significant increases in total nitrogen and protein content over the controls, and the increases were greater with Tr 1 than with Tr 2. Significant decreases in nitrate nitrogen content occurred following Tr 1 whereas Tr 2 resulted in significant increases in nitrate nitrogen content as compared to controls. Essential amino acids such as valine, methionine, and leucine were greatly increased up to 19.7% by Tr 1 and decreased by 20.6% by Tr 2.

Chemical weed control using herbicides is widely practiced to minimize damage to the potato plant. However, little research has been done on the effect of herbicides on potato quality. Herbicides are categorized into several classes on the basis of their chemical properties. The herbicide metribuzin used in this study belongs to the class of triazines (subclass triazinones) categorized as powerful inhibitors of photosynthesis. Metribuzin interferes with the photochemically induced electron transport in plants (Geissbuhler, 1979; Tischer and Strotmann, 1977). Metribuzin is very effective for control of grass and broadleaf weeds infesting agricultural crops such as potatoes, sugarcane, soybeans, wheat, lentils, etc. Since metribuzin is a very active herbicide, preemergence and postemergence applications have shown good selectivity and provided excellent control of many important leaf weeds.

Triazine herbicides are known to have a profound effect on the nitrogen metabolism of treated plants. Simazine significantly increased the total nitrogen content of leaves of sweet lime and sour orange (Goren and Monselise, 1966), corn (Fink and Fletchall, 1967; Ries and Gast, 1965; de Vries, 1963), pine (de Vries, 1963), and leaf nitrogen of peach and apple (Ries, et al., 1963). Total nitrogen was also increased when atrazine was applied to corn (Fink and Fletchall, 1967) and corn and johnsongrass (Gramlich and Davis, 1967). Triazines are also known to exhibit the so-called protein effect. Simazine increased the protein content of the leaves of sweet lime and sour orange (Goren and Monselise, 1966), rye and peas (Ries et al., 1967), and forage grasses (Allinson and Peters, 1970) but had no significant effect on the total protein content of coastal Bermunda grass (Monson et al., 1971) and wheat (Ries et al., 1970). However, increases in seed protein of wheat due to simazine resulted in higher yields of the second generation (Ries et al., 1970). Triazines also increase nitrate uptake in plants (Fink and Fletchall, 1967; Gramlich and Davis, 1967).

The protein content of the potato is nutritionally well balanced, containing substantial levels of essential amino acids such as lysine and leucine, making potatoes a good complement to cereals. Scrimshaw and Young (1976) found the essential amino acid content of potatoes to be adequate in tryptophan but poor in methionine and cystine. According to Rexen (1976) the limiting amino acid was methionine for some varieties and isoleucine for others.

Nitrates are potential toxicants in the tubers since they serve as precursors of nitrites. The occurrence of nitrites in foods is a cause for concern because they oxidize ferrous hemoglobin to ferric hemoglobin, subsequently inhibiting oxygen transport through the body and resulting in methemoglobinemia (Hartman, 1982). Nitrites also react with secondary or tertiary amines to form carcinogenic and mutagenic *N*-nitroso compounds (Walters et al., 1979). According to White (1975), potatoes contribute approximately 14% of the per capita ingestion of nitrates in the United States.

This investigation was carried out in order to study the efffect of metribuzin on the total nitrogen, protein, nitrate nitrogen, and amino acid contents on potato tubers.

MATERIALS AND METHODS

Five potato cultivars were used in this study. Katahdin, Lemhi Russet, and Hampton cultivars grown at the Cornell Vegetable Research Farm in Freeville, NY, were used in the first year and Katahdin, Norking Russet, and Shepody in the second year of the study. Metribuzin was sprayed on the soil at the rate of 1.5 kg ha^{-1} active ingredient (AI) at two different times: (1) at the preemergence stage to one group of potatoes (Tr 1); (2) to the soil at preemergence and again after emergence (Tr 2) to the other group of potatoes. The postemergence application was carried out 3 weeks following emergence of the plants. Three replicates were made on each treatment. Tubers were harvested 18 weeks after planting and stored in the dark at 5 °C and 95% RH until analyzed. Analyses were made within 1 week following the harvest. Lyophilized potato tissue was used in the determination of total nitrogen, nonprotein nitrogen, protein, and amino acid contents.

The tubers of size C (approximately 7.2-cm diameter) were cut longitudinally from bud to stem end and the slices (2 mm thick) separated into cortex (including the peel) and pith sections along the vascular ring. Two samples, each consisting of eight tubers, were analyzed for each treatment. Duplicate extrac-

† Institute of Food Science and Institute of Toxicology.
‡ Department of Food Science.

0021-8561/90/1438-0636$02.50/0

tions were made on each sample. Both cortex and pith tissues were analyzed during each of the two years of the study.

Determination of Total Nitrogen. Total nitrogen was determined on lyopholized potato powder according to the micro-Kjeldahl method described by the AOAC (1975).

Determination of Nonprotein Nitrogen. The modified method of Desborough and Weiser (1974) using trichloroacetic acid precipitation was used for nonprotein nitrogen determination.

Determination of Nitrate Nitrogen. Nitrate nitrogen analyses were done on aqueous extracts of fresh tubers with use of phenoldisulfonic acid as described by Ulrich et al. (1959).

Determination of Protein Nitrogen and Protein Content. Protein nitrogen was determined by subtracting nonprotein from total nitrogen. Protein content was calculated by multiplying protein nitrogen content by the micro-Kjeldahl conversion factor of 7.5 for potato protein as indicated by Desborough and Weiser (1974).

Amino Acid Analysis. The precolumn derivatization method of amino acid analysis using high-performance liquid chromatography (HPLC) was used (Bidlingmeyer et al., 1984). The derivatization reagent consisted of ethanol–triethylamine (TEA)–water–phenyl isothiocyanate (PITC) in the ratio 7:1:1:1. The free amino acid pool was extracted with 2 mL of distilled water, and 20 μL of the supernatant was derivatized. The derivatized volume was reconstituted to 200 μL, and 20 μL of this volume was injected into the HPLC. The amino acid content was also assayed on the hydrolyzed fraction of the aqueous extract. Hydrolysis was carried out by adding 0.1 M HCl to the extract and heating the mixture to 150 °C for 95 min. The hydrolyzed sample was derivatized and reconstituted to 200 μL, and 20 μL of this mixture was injected into the HPLC. Cortex tissue of a white (Katahdin) and russet (Lemhi Russet) cultivar was analyzed for amino acids.

Statistical Analysis. Complete random design was employed, and statistical significance of the data was determined using analysis of variance (ANOVA) with a protected LSD test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Both treatments with metribuzin resulted in significant increases in total nitrogen and protein content of tubers over the controls (Figure 1). The increases were greater with Tr 1 than with Tr 2. Significant decreases in nitrate nitrogen content of tubers as compared with controls occurred following Tr 1 (Table I). However, significant increases in nitrate nitrogen content resulted from Tr 2. Metribuzin had no effect on yield or size of tubers. Plant growth was normal, and no chlorosis was observed.

It is likely that the effect of metribuzin on nitrogen metabolism may have been due to its effect on the activity of the enzyme nitrate reductase. Mohandas et al. (1978) reported an increase in nitrate reductase, glutamine synthetase, and glutamate synthetase activities due to atrazine, a triazine herbicide similar to metribuzin. Nitrate reductase is required for reduction of nitrate to ammonium ions incorporated into the carbon skeletons of amino acids. Glutamine synthetase and glutamate synthetase are required for the incorporation of ammonium ions into the carbon skeletons of amino acids. Therefore, increases in the activities of these enzymes would enhance protein synthesis. When metribuzin was applied to the soil at preemergence (Tr 1), a decrease in tuber nitrate occurred. It is likely that increased NRA of the plant enhanced nitrate reduction. The increased ion concentration resulting from nitrate reduction would result in increased protein synthesis. When metribuzin was applied after the plants had emerged (Tr 2), it could contact some of the leaves. Since metribuzin is a potential inhibitor of photosynthesis, lower amounts of photosynthates would result in decreased protein synthesis. Lower photosynthates result in the accumulation of ammonium ions and, in turn, inhibit NRA resulting in the accumulation of nitrate ions.

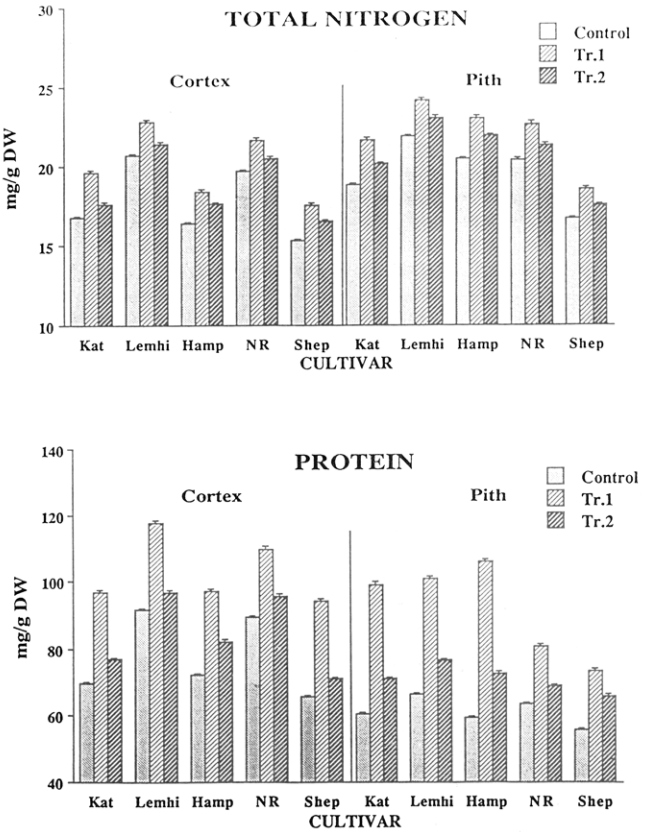

Figure 1. Effect of preemergence (Tr 1) and pre- plus postemergence (Tr 2) applications of metribuzin on total nitrogen and protein content of Katahdin (Kat), Lemhi Russet (Lemhi), Hampton (Hamp), Norking Russet (NR), and Shepody (Shep) potato cultivars.

Table I. Nitrate Nitrogen (ppm FW) of Potatoes As Affected by Metribuzin[a]

	cortex			pith		
	C	Tr 1	Tr 2	C	Tr 1	Tr 2
Katahdin	38.21	20.27[a]	41.76[b]	23.61	15.02[a]	27.26[b]
Lemhi Russet	60.27	30.72[a]	71.26[b]	34.65	29.66[a]	39.97[b]
Hampton	48.20	33.75[a]	53.76[b]	30.76	21.26[a]	35.70[b]
Norking Russet	59.72	21.21[a]	63.76[b]	33.01	23.70[a]	38.88[b]
Shepody	49.20	31.26[a]	52.96[b]	21.20	15.20[a]	23.88[b]

[a] Key: C, control; Tr 1, treatment 1 (preemergence treatment); Tr 2, treatment 2 (pre- plus postemergence treatment). (a) Significantly different from the controls at $p < 0.01$. (b) Significantly different from the controls at $p < 0.05$.

Metribuzin applications resulted in substantial changes in the dietary essential amino acids threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine. All nonessential amino acids were also affected.

Free Amino Acids. Treatment 1 increased most essential amino acids while treatment 2 decreased them (Table II). However, cultivar differences were also observed. Treatment 1 decreased the leucine and phenylalanine content of the Katahdin cultivar and methionine, valine, isoleucine, and phenylalanine content of the Lemhi Russet cultivar. Treatment 2 increased the threonine and tryptophan content of the Katahdin cultivar. Overall, Tr 1 increased the essential amino acid content by 6.05% in the Katahdin cultivar and 8.85% in the Lemhi Russet cultivar while Tr 2 decreased the amino acid content by 20.6% in the Katahdin cultivar and 18.2% in the Lemhi Russet cultivar.

Amino Acid Profile on Hydrolysis. In both cultivars Tr 1 increased all the essential amino acids except

Table II. Effect of Preemergence (Tr 1) and Pre- plus Postemergence (Tr 2) Treatments on the Free Amino Acid Pool of Katahdin and Lemhi Russet Potatoes

amino acid[a]	Katahdin			Lemhi Russet		
	C	Tr 1	Tr 2	C	Tr 1	Tr 2
Asp	2.67	1.98	1.75	3.11	7.07	2.97
Glu	2.03	1.10	1.29	2.44	2.39	2.56
Ser	0.73	0.80	0.66	0.77	0.81	0.72
Asn	11.73	16.31	12.98	11.29	11.92	11.23
Gly	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Gln	2.66	4.34	3.37	4.86	4.40	4.41
His	0.49	0.56	0.42	0.53	0.46	0.39
Thr*	0.35	0.44	0.39	0.39	0.42	0.38
Ala	0.20	0.34	0.19	0.32	0.29	0.25
Arg	4.34	4.76	4.45	5.09	4.39	3.82
Pro	0.13	0.18	0.15	0.69	0.57	0.44
Tyr	0.77	0.69	0.55	0.85	0.80	0.78
Val*	1.25	1.39	1.04	1.39	1.19	1.15
Met*	0.32	0.39	0.28	0.15	0.12	0.15
Cys	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Ile*	0.45	0.48	0.34	0.49	0.46	0.37
Leu*	0.21	0.19	0.13	0.28	0.29	0.25
Phe*	0.44	0.41	0.26	0.61	0.49	0.55
Trp*	<0.02	<0.02	<0.04	<0.04	<0.03	<0.03
Lys*	0.62	0.56	0.45	0.89	0.86	0.71

[a] Amino acids data reported as milligrams per gram dry weight. Asterisks indicate essential amino acids.

Table III. Effect of Preemergence (Tr 1) and Pre- plus Postemergence (Tr 2) Applications of Metribuzin on Amino Acid Content of Katahdin and Lemhi Russet Potatoes Derived upon Hydrolysis

amino acid[a]	Katahdin			Lemhi Russet		
	C	Tr 1	Tr 2	C	Tr 1	Tr 2
Asx	12.79	14.64	13.08	10.88	13.07	11.35
Glx	6.27	7.43	6.65	7.07	7.76	7.89
Ser	2.33	2.45	2.31	1.66	2.63	1.75
Gly	1.73	1.88	1.81	1.49	1.60	1.62
His	0.19	0.96	0.85	0.78	0.81	0.64
Arg	5.94	5.66	5.64	5.41	5.56	5.12
Thr*	1.90	2.11	1.97	1.47	1.72	1.34
Ala	1.46	1.58	1.49	1.15	1.33	1.12
Pro	1.98	2.36	2.19	2.29	2.22	2.31
Tyr	1.32	1.79	1.49	1.21	1.62	0.99
Val*	2.97	3.15	2.89	2.61	2.88	2.47
Met*	1.16	1.35	1.23	0.91	1.06	1.00
Cys	0.58	0.85	0.77	0.65	0.62	0.65
Ile*	1.77	1.78	1.66	1.39	1.70	1.19
Leu*	2.79	3.05	2.88	2.26	2.66	2.13
Phe*	1.90	1.97	1.86	1.57	2.01	1.39
Lys*	1.73	1.60	1.55	1.33	1.79	0.91

[a] Amino acids data reported as milligrams per gram dry weight. Asterisks indicate essential amino acids.

lysine, which was lowered in the Katahdin cultivar (Table III). Treatment 2 decreased most essential amino acids of both cultivars. Some essential amino acids were, however, increased by Tr 2. They are threonine, methionine, and leucine of the Katahdin cultivar and lysine of the Lemhi Russet cultivar. Overall Tr 1 increased the essential amino acid content by 5.56% in the Katahdin cultivar and 19.75% in the Lemhi Russet cultivar while Tr 2 decreased the amino acid content by 1.27% in the Katahdin cultivar and 9.62% in the Lemhi Russet cultivar.

SUMMARY

When metribuzin was applied prior to plant emergence to the soil in which potatoes were grown (Tr 1 or preemergence treatment), the quality of the tubers was superior to that in which pre- plus postemergence applications (Tr 2) of metribuzin were used. The tubers resulting from preemergence treatment were higher in total nitrogen and protein content and lower in nitrates than tubers resulting from the pre- plus postemergence treatments. The preemergence treatment also resulted in greater increases of amino acids than the pre- plus postemergence treatments. Therefore, application of metribuzin to the soil prior to the emergence of the plants is more beneficial and results in tubers that are safer and more nutritious.

ACKNOWLEDGMENT

We acknowledge the assistance of Larry Hymes and Dr. Don Halseth, Department of Vegetable Crops, Cornell University, for arrangements made in growing, harvesting, and transporting the potatoes. This work was supported by the New York State Experiment Station, Cornell University, Ithaca, NY.

LITERATURE CITED

Allinson, D. W.; Peters, R. A. Influence of Simazine on Crude Protein and Cellulose Content and Yield of Forage grass. *Agron. J.* **1970**, *62*, 246–250.

AOAC. *Official Methods of Analysis*, 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1975.

Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. Rapid Analysis of Amino Acids Using Pre-Column Derivatization. *J. Chromatogr.* **1984**, *336*, 93–104.

Desborough, S. L.; Weiser, C. J. Improving Potato Protein: 1. Evaluation of Selection Techniques. *Am. Potato J.* **1974**, *51*, 185–195.

de Vries, M. L. The Effect of Simazine on Monterey Pine and Corn as Influenced by Lime, Bases, and Aluminum Sulfate. *Weeds* **1963**, *11*, 220–222.

Fink, R. J.; Fletchall, O. H. The Influence of Atrazine or Simazine on Forage Yield and Nitrogen Components of Corn. *Weeds* **1967**, *15*, 272–274.

Geissbuhler, H. *Advances in Pesticide Science*, Parts 1–3; Pergamon: Oxford, 1979; 835 pp.

Goren, A.; Monselise, S. P. Some Physiological Effects of Triazines on Citrus Trees. *Weeds* **1966**, *14*, 141–144.

Gramlich, J. V.; Davis, D. E. Effect of Atrazine on Nitrogen Metabolism of Resistant Species. *Weeds* **1967**, *15*, 157–160.

Hartman, P. E. Nitrates and Nitrites: Ingestion, Pharmacodynamics, and Toxicology. In *Chemical Mutagens*; Serres, F. J., Hollander, A., Eds.; 1982; p 211.

Maga, J. A. Potato glycoalkaloids. *CRC Crit. Rev. Food Sci. Nutr.* **1980**, *12*, 371–405.

Mohandas, S.; Wallace, W.; Nicholas, D. J. D. Effects of Atrazine on the Assimilation of Inorganic Nitrogen in Cereals. *Phytochemistry* **1978**, *17*, 1021–1028.

Monson, W. G.; Burton, G. W.; Wilkinson, W. S.; Dumford, S. W. Effect of N Fertilization and Simazine on Yield, Protein, Amino-acid Content, and Carotenoid Pigments of Coastal Bermudagrass. *Agron. J.* **1971**, *63*, 928–930.

Rexen, R. Studies of Protein of Potatoes. *Potato Res.* **1976**, *19*, 189–202.

Ries, S. K.; Gast, A. The Effect of Simazine on Nitrogenous Components of Corn. *Weeds* **1965**, *13*, 272–274.

Ries, S. K.; Larsen, R. P.; Kenworthy, A. L. The Apparent Influence of Simazine on Nitrogen Nutrition of Peach and Apple Trees. *Weeds* **1963**, *11*, 270–273.

Ries, S. K.; Chmiel, H.; Dilley, D. R.; Filner, P. The Increase in Nitrate Reductase Activity and Protein Content of Plants Treated With Simazine. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 526–532.

Ries, S. K.; Moreno, O.; Meggitt, W. F.; Schweizer, C. J.; Ashkar, S. A. Wheat Seed Protein: Chemical Influence on and Relationship to Subsequent Growth and Yield in Michigan and Mexico. *Agron. J.* **1970**, *62*, 746–748.

Scrimshaw, N. S.; Young, V. R. The Requirements of Human Nutrition. *Sci. Am.* **1976**, *235*, 51–64.

Steel, R. G. D.; Torrie, J. M. *Principles and Procedures of Statistics*; McGraw-Hill: New York, 1980.

Tischer, W.; Strotmann, H. Relationship Between Inhibitor Binding by Chloroplasts and Inhibition of Photosynthetic Electron Transport. *Biochem. Biophys. Acta* **1977**, *460*, 113–125.

Ulrich, A.; Rieri, D.; Hidds, F. J.; George, A. G.; Moore, M. A.; Johnson, C. M. Analytical Methods for Use in Plant Analysis. *Bull.—Calif. Agric. Expt. Stn.* **1959**, *No. 766*, 44.

Walters, C. L.; Carr, F. P. A.; Dyke, C. S.; Saxby, M. S.; Smith, P. L. R. Nitrite Sources and Nitrosamine Formation in vivo. *Food Cosmet. Toxicol.* **1979**, *17*, 473–479.

White, J. W., Jr. Relative Significance of Dietary Sources of Nitrate and Nitrite. *J. Agric. Food Chem.* **1975**, *23*, 886–891.

Received for review April 6, 1989. Accepted November 20, 1989.

Registry No. L-Leu, 61-90-5; L-Phe, 63-91-2; L-Met, 63-68-3; L-Val, 72-18-4; L-Ile, 73-32-5; L-Thr, 72-19-5; L-Trp, 73-22-3; L-Lys, 56-87-1; metribuzin, 21087-64-9; nitrate, 14797-55-8.

Oxygen-17 and Proton Nuclear Magnetic Relaxation Measurements of Soy Protein Hydration and Protein–Protein Interactions in Solution

Lazaros T. Kakalis,† Ion C. Baianu,‡ and Thomas F. Kumosinski*,†

Eastern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, Philadelphia, Pennsylvania 19118, and Department of Food Science, Agricultural and Food Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

The amount and mobility of "bound" water in neutral aqueous suspensions of a commercial soy protein isolate were quantitated by means of transverse and longitudinal ^{17}O NMR relaxation measurements at 54.2 MHz and 21 °C. Data analysis yielded 33.2 g of "bound" water/100 g of protein and correlation times of 14.2 ns and 32–34 ps for the motions of "bound" water. The hydration of the same protein isolate was investigated by ^{1}H NMR transverse relaxation measurements at 10 MHz and 22 °C. Up to 10% (w/w) protein aqueous dispersions were measured at pH 4.5, 7, 9.1, and 11 in the absence and presence of NaCl. In all cases, a nonlinear protein concentration dependence was observed for relaxation rates. Protein–protein interactions were quantitated by fitting the ^{1}H NMR data by a virial expansion. Transverse ^{1}H NMR relaxation rates showed a linear dependence on protein activity. Data interpretation was based on the effects of the NMR measurements of the ionization of protein groups, the state of protein aggregation, and the binding of salt by the protein.

1. INTRODUCTION

Protein–water interactions have been extensively studied because of their fundamental role in biological systems (Kuntz and Kauzmann, 1974). Recently, soybean protein has become increasingly important as a food ingredient, with its performance in food systems being intimately related to its hydration properties (Kinsella et al., 1985; Chou and Morr, 1979). Water vapor sorption isotherms of various soy protein preparations have been reported (Hagenmaier, 1972; Puri and Bala, 1975; Hermansson, 1977; Hansen, 1978; Chou and Morr, 1979). The amount of water that remains unfrozen in the presence of soy protein has been estimated by using differential scanning calorimetry (DSC) (Muffett and Snyder, 1980) or nuclear magnetic resonance (NMR) (Hansen, 1978; Derbyshire, 1982). Other pertinent work involved measurements of the spontaneous uptake of liquid water by protein powders (Hermansson, 1972; López de Ogara et al., 1987) or the amount of water retained by the insoluble protein after centrifugation of a protein dispersion (Fleming et al., 1974; Hutton and Campbell, 1981) as well as other empirical approaches to the determination of soy protein hydration (Elgedaily et al., 1982).

Nuclear magnetic resonance relaxation is a noninvasive technique that can provide information about the amount and the mobility of "bound" water (i.e., the fraction of water that is significantly perturbed by the protein) and the extent of various intermolecular interactions (Derbyshire, 1982; Bryant and Halle, 1982; Pessen and Kumosinski, 1985).

The purpose of the present study is to calculate the hydration parameters of soybean protein from ^{17}O NMR relaxation measurements; we also aim to quantitate protein–protein interactions using ^{1}H NMR at various values of pH and ionic strength.

2. THEORY

Among the three different nuclei that can be used in NMR studies of the molecular properties of water (i.e., ^{1}H, ^{2}H, and ^{17}O), oxygen-17 is the one whose NMR relaxation is free from the complications of cross-relaxation and chemical exchange, thus providing the most direct

* To whom correspondence should be addressed at the Eastern Regional Research Center, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, PA 19118.
† U.S. Department of Agriculture.
‡ University of Illinois at Urbana-Champaign.

0021-8561/90/1438-0639$02.50/0