Effect of Low-Dose Irradiation on Soybean Protein Solubility, Trypsin Inhibitor Activity, and Protein Patterns Separated by Polyacrylamide Gel Electrophoresis

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Five soybean cultivars were used in this study to investigate changes in soybean protein solubility, protein patterns separated by SDS-PAGE and Poro-PAGE, and activity of trypsin inhibitor after exposure to γ irradiation. SDS-PAGE proved that changes in protein subunits patterns could be identified in the Clark cultivar. Three high molecular weight protein bands were detected in irradiated soybean cultivars by using Poro-PAGE.

The use of radiation for food preservation is considered a peaceful application of nuclear energy (Urakami et al., 1959, 1961; Richardson et al., 1960; Diehl, 1973; Diehl et al., 1978; Merritt et al., 1979; Simic, 1978; Ward, 1978). Production of certain irradiated foods for human consumption has been approved in several countries (Nelson et al., 1959; Ley and Hickman, 1960; Azamar et al., 1957; Cabrera and Carrasco, 1978; Whitburn et al., 1982; Loaharanu, 1985).

A number of investigations have shown that changes in chemical and physical properties of proteins occur after irradiation both in dilute aqueous solution and in the solid state (Errera, 1951; Alexander et al., 1956; Garrison, 1968; Simic, 1978; Hafez et al., 1985).

This investigation studies the effect of γ irradiation on soybean protein solubility, trypsin inhibitor activity, and protein patterns separated by SDS-PAGE and Poro-PAGE.

MATERIALS AND METHODS

Materials. Mature soybean seeds from the cultivars Kent, Clark, Lower, York, and Duglas, harvested in 1986, were supplied by the Faculty of Agriculture, Cairo University. The samples were exposed to γ radiation from a ^{60}Co unit γ cell-22 (Department of Nuclear Physics, Nuclear Research Center, Egyptian Atomic Energy Authority). The doses were calculated as kilorads, and the samples were exposed to the following radiation doses: 10, 20, and 30 krad at 12 rad/s.

Methods. The irradiated and the control materials were milled in an electric mill (Janke and Kunkel Micro-Fein Mühle Culalli) to pass through a 1.5-mm sieve. The seed meal was defatted with a mixture of chloroform–benzene (Preston et al., 1975). The results were calculated as the average of three samples in each case.

Protein Extraction (Water Extract). Water extractions of each defatted meal were carried out with distilled water according to the method of Hu and Esein (1981).

Protein Determination. Total protein was determined in the water extract of defatted meal according to the method of de Wreede and Stegemann (1981). Total nitrogen was determined in the defatted meal according to the method of Kjeldahl (AOAC, 1975).

Trypsin Inhibitor Activity. Trypsin inhibitor activity was determined in the water extract of the defatted meal according to the method of Hamerstrand et al. (1981).

Electrophoresis. SDS-PAGE was carried out according to the method of Laemmli (1970). Samples were subjected to electrophoresis in 15% PAA for 16 h at 100 V and then stained with Coomassie Brilliant Blue R 250 (0.1% in 10% acetic acid and 20% methanol). Gels were destained in 10% acetic acid and 20% methanol.

Poro-PAGE (5–25% PAA in Tris–borate buffer, pH 8.9) was carried out according to the method of Margolis and Kenrick (1968). Staining and destaining were carried out as described for SDS-PAGE.

Electrophoresis was carried out on POOMA-PHOR apparatus (Labor Müller-3510 Hann-Münden).

Statistical Analysis. The data were subjected to statistical analysis using two-way analysis of variance and further analysis using Duncan's multiple-range t test.

RESULTS AND DISCUSSION

Five soybean cultivars (Kent, Lower, York, Clark, Duglas) were used in this study. The changes in soybean protein solubility, protein patterns as shown by SDS-PAGE and Poro-PAGE, and activity of trypsin inhibitor were shown before and after exposure to γ irradiation.

Protein Solubility. The results in Table I show that the total protein content was not affected by γ irradiation. Significant differences were noticed only due to the main effect of different cultivars (Table II). Significant total water-soluble protein differences were noticed as a function of both cultivar and dose of irradiation (Table II). Duncan's multiple-range t test showed significant differences between two mean values of total soluble protein of each of two cultivars in each interval. These results are in agreement with those found by Diehl et al. (1978) and Alexander et al. (1956), who cited that irradiation of globular protein causes formation of protein aggregates. Hafez et al. (1985) reported that microwave heating decreases soybean protein solubility from 80 to 17%. The decrease in protein solubility after freezing and heating was reported by Dowdie and Biede (1983) and Davis and Anderson (1984).

Trypsin Inhibitor Activity. From the results in Table I it is observed that the trypsin inhibitor activity of five soybean cultivars ranged from 40.68 (cV. York) to 48.79 units/mg (cV. Kent). These results are in agreement with those of Roy and Bhat (1974) who cited that trypsin inhibitor activity in five soybean ranged from 37.2 to 38.0. Hafez et al. (1985) found TIA of the Essex cultivar was 52.74.

The results indicated that the different doses of γ radiation caused a significant difference in the activity of TIA due to soybean cultivars and dose of irradiation Table II. The decrease in TIA in soybean extract after γ irradiation was cited also by Lynn and Raoult (1975) and Roy and Bhat (1974).

Electrophoretic Separation. The water-soluble protein extracted from soybean after irradiation and the

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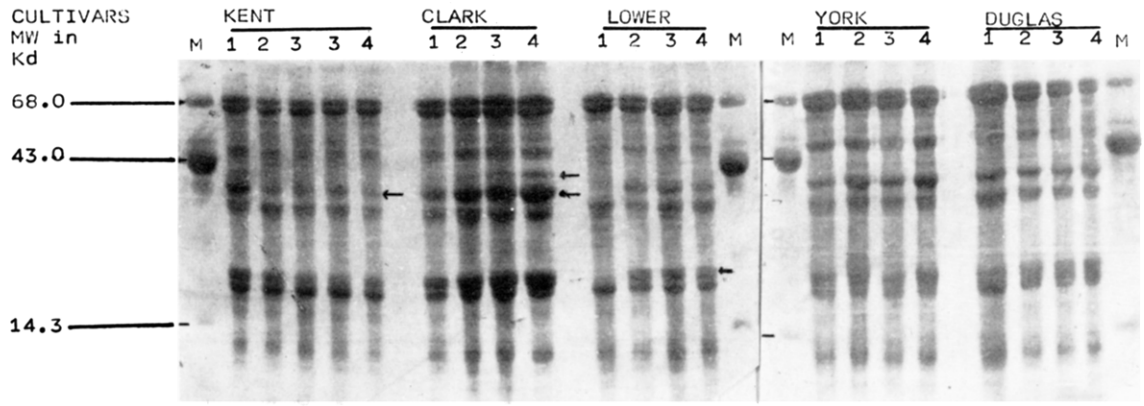

Figure 1. SDS-PAGE (Laemmli, 1970) of 5 and 15% PAA in Tris–glycine buffer (pH 8.3): 1, control; 2, soybean exposed to 10 krad; 3, soybean exposed to 20 krad; 4, soybean exposed to 30 krad.

Table I. Mean ± SE of Total Protein (TP), Total Soluble Protein (TSP), and Trypsin Inhibitor Activity (TIA) in Soybean Cultivars after Exposure to Ionizing Radiation[a]

cultivar		0.0 krad	10 krad	20 krad	30 krad
Duglas	TP	43.29 ± 0.069 A	43.27 ± 0.121 A	43.27 ± 0.127 A	43.29 ± 0.063 A
	TSP	33.97 ± 0.127 A,a	32.78 ± 0.242 A,b	30.51 ± 0.173 A,c	29.98 ± 0.127 A,c,d
	TIA	47.07 ± 0.063 A,a	46.57 ± 0.190 A	46.57 ± 0.386 A	45.67 ± 0.485 A,d
Clark	TP	47.94 ± 0.185 B	47.93 ± 0.173 B	47.93 ± 0.167 B	47.93 ± 0.144 B
	TSP	37.58 ± 0.237 B,a	37.92 ± 0.117 B,a	33.21 ± 0.138 B,c	33.00 ± 0.288 B,c,d
	TIA	40.88 ± 0.365 B,a	40.18 ± 0.625 B	39.78 ± 0.525 B	39.38 ± 0.265 B,d
Kent	TP	48.85 ± 0.109 C	48.83 ± 0.075 C	48.87 ± 0.138 C	48.85 ± 0.231 C
	TSP	38.95 ± 0.238 C,a	38.45 ± 0.254 B,a	39.37 ± 0.173 C,c,a	34.20 ± 0.130 C,d
	TIA	48.97 ± 0.519 C,a	47.69 ± 0.190 A,b	46.89 ± 0.288 A,b,c	46.59 ± 0.545 A,b,c,d
York	TP	45.32 ± 0.063 D	45.30 ± 0.127 D	45.33 ± 0.138 D	45.34 ± 0.081 D
	TSP	35.87 ± 0.467 D,a	35.21 ± 0.127 C,b	33.99 ± 0.242 D,c	33.70 ± 0.291 C,c,d
	TIA	40.68 ± 0.485 B,a	40.07 ± 0.081 B,a,b	39.27 ± 0.265 B,b,c	38.87 ± 0.485 B,c,d
Lower	TP	50.77 ± 0.404 E	50.76 ± 0.346 E	50.78 ± 0.444 E	50.79 ± 0.519 E
	TSP	39.52 ± 0.128 C,a	39.61 ± 0.244 D,a	36.91 ± 0.288 E,c	36.95 ± 0.251 D,c,d
	TIA	43.83 ± 0.190	42.72 ± 0.285 C,b	41.93 ± 0.486 C,b,c	41.53 ± 0.525 C,c,d

[a] A–E: Different letters in the same column are significant ($P < 0.01$). a–e: Different letters in the same row are significant ($P < 0.01$).

Table II. Summary of Two-Way Analysis of Variance for Testing the Significance of Difference between the Mean Values of the Various Cultivars for Total Protein, Total Soluble Protein, and Trypsin Inhibitor Activity

source of variation[a]	SS	dF	MS	F value
		Total Protein		
A	419.111	4	104.777	671.84[b]
B	0.00364	3	0.0012	0.0076
A × B	0.00464	12	0.000386	0.00247
error	6.23812	40	0.155953	
		Total Soluble Protein		
A	375.1313	4	93.78	629.40[b]
B	120.3278	3	40.11	269.19[b]
A × B	54.46388	12	4.54	30.47[b]
error	5.9802	40	0.149	
		Trypsin Inhibitor Activity		
A	618.6275	4	154.657	349.5[b]
B	28.188	3	9.396	21.24[b]
A × B	2.4243	12	0.173	0.391
error	17.697	40	0.442	

[a] Key: A, cultivar; B, radiation dose. [b] $P < 0.01$.

control were analyzed by SDS-PAGE and Poro-PAGE to follow changes in protein patterns.

SDS-PAGE showed differences in the amount of water-soluble protein patterns according to irradiation especially in Clark, Kent, and Lower cultivars. The changes in band intensity and the new bands appearing after irradiation were measured by a scanning technique in the visible region of the spectrum (570 nm) (Fishbein, 1972). The intensity of the major subunit band of 11S-2 with 36 kDa insignificantly increased at dose of 30 krad and represented 9.5% compared to the unirradiated cultivar Clark (8.7%; Figure 1, lanes 3 and 4). The subunit band of 40 kDa detected after irradiation with the different doses 10, 20, and 30 krad amounted to 5.7, 5.7, and 6.3% of total bands (Figure 1, lanes 2–4 (Clark); Table III). The major subunit of 36 kDa corresponding to 11S-2 disintegrated after treatment with γ irradiation and amounted to 7.0, 7.2, and 5.0% compared to the unirradiated Kent cultivar (8.2%; Figure 1, lanes 1–4; Table III). On the other hand, bands of 26 and 36 kDa were found in the Lower cultivar as a result of different irradiation doses and represented 7.9, 8.2, 8.77% and 8.1, 8.5, 8.4%, respectively (Figure 1, lane 2–4; Table III). The irradiation of the two cultivars York and Duglas showed slight differences in protein band intensities and gave almost identical patterns. The results are in agreement with the finding of Barron et al. (1955) who showed that irradiation of an aqueous solution of serum albumin produced destruction in its amino acid content. From these results it could be concluded that changes in protein patterns depended almost entirely on the cultivar. Therefore, the results of SDS-PAGE could be used to follow the changes in protein patterns after γ irradiation in some cultivar like Clark, while it could not be used to follow the same changes in the other cultivar (Mäkinen et al., 1979).

Water-soluble protein patterns of soybean cultivars separated by Poro-PAGE showed different profiles after γ irradiation treatments. Three proteins with molecular weights 100, 140, and 330 kDa were detected in irradiated soybean cultivars with 10 and 30 krad. These proteins appeared as strong bands especially in York and Duglas cultivars [Figure 2, lane 4 (York) and lanes 2 and 4

Table III. Mean ± SE of the Percentage of the Major Protein Patterns of Irradiated Soybean Cultivars after SDS-PAGE Separation

MW, kDa	Kent				Clark				Lower			
	0.0[a]	10	20	30	0.0	10	20	30	0.0	10	20	30
79 (α)	13.0 ± 0.2	11.4 ± 1.5	10.1 ± 2.7	9.9 ± 3.2	12.2 ± 0.41	11.8 ± 0.79	11.9 ± 0.85	12.3 ± 1.5	13.5 ± 0.12	13.6 ± 0.03	13.7 ± 0.12	12.9 ± 1.0
67 (α)	11.9 ± 0.42	11.9 ± 0.52	11.6 ± 0.71	10.9 ± 1.01	12.7 ± 1.13	12.8 ± 1.1	12.2 ± 0.9	13.0 ± 1.75	13.5 ± 1.53	13.3 ± 1.39	12.8 ± 0.57	12.7 ± 0.1
50 (β)	9.6 ± 1.11	9.6 ± 1.01	7.6 ± 2.4	7.6 ± 2.4	9.0 ± 0.91	9.5 ± 0.5	9.6 ± 0.52	9.5 ± 0.45	9.5 ± 1.34	9.6 ± 1.25	9.7 ± 1.11	8.9 ± 1.32
40						5.7 ± 0.3	5.7 ± 0.21	6.3 ± 0.22				
36 (11S-2)	8.2 ± 0.41	7.0 ± 1.2	7.2 ± 1.2	5.0 ± 2.8	8.7 ± 1.1	8.9 ± 1.11	8.9 ± 1.2	9.1 ± 1.21		8.1 ± 1.3	8.5 ± 1.11	8.4 ± 1.0
26 (11S-1)	7.5 ± 0.61	7.6 ± 0.72	7.3 ± 1.03	7.3 ± 0.93	7.6 ± 0.52	7.55 ± 0.55	7.5 ± 0.62	7.5 ± 0.71		7.9 ± 1.1	8.2 ± 1.01	8.77 ± 0.9

MW, kDa	York				Duglas			
	0.0	10	20	30	0.0	10	20	30
79 (α)	13.2 ± 0.3	13.8 ± 0.11	14.4 ± 0.25	13.8 ± 1.3	13.8 ± 1.1	14.8 ± 1.3	14.9 ± 0.9	14.8 ± 1.4
67 (α)	14.1 ± 1.3	14.1 ± 1.7	13.9 ± 0.95	14.2 ± 1.73	14.4 ± 0.92	14.1 ± 0.72	14.6 ± 1.2	14.3 ± 0.9
50 (β)	11.2 ± 1.22	11.4 ± 1.63	11.3 ± 1.22	11.0 ± 1.23	11.4 ± 1.3	9.8 ± 1.1	9.8 ± 0.75	9.3 ± 1.3
40								
36 (11S-2)	6.5 ± 0.71	6.5 ± 0.65	6.5 ± 0.72	6.6 ± 0.67	6.4 ± 0.4	6.5 ± 0.4	6.5 ± 0.47	6.4 ± 0.4
26 (11S-1)	6.7 ± 0.8	6.7 ± 0.8	6.7 ± 0.82	6.7 ± 0.79	7.0 ± 0.75	7.3 ± 0.46	7.4 ± 0.48	7.5 ± 0.4

[a] Doses in kilorads.

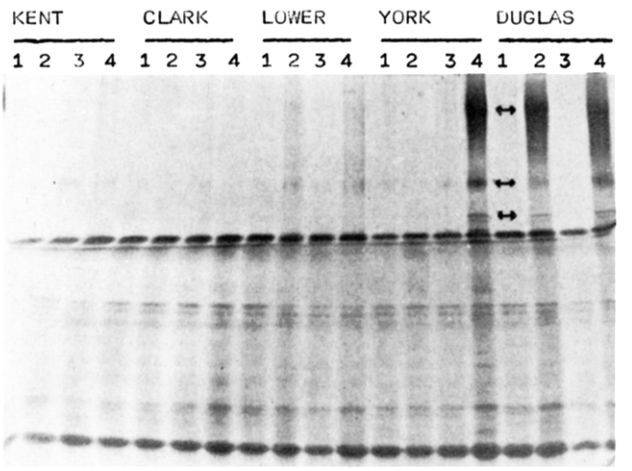

Figure 2. Poro-PAGE of 5–25% PAA in Tris–borate buffer (pH 8.9). Samples numbered as in Figure 1.

(Duglas)]. The other major protein patterns were similar in all cultivars either irradiated or not. The appearance of high molecular weight protein was in agreement with the aggregation of a special protein to produce high molecular weight proteins as a result of γ irradiation (Alexander et al., 1956; Drake et al., 1957; Diehl et al., 1978).

The changes in protein patterns after SDS-PAGE and Poro-PAGE separation in soybean cultivar after irradiation may be due to partial protein deamination, scission of peptide and disulfide bonds, and addition to aromatic and heterocyclic amino acid residues (Whitcher et al., 1953; Taub et al., 1976; Simic, 1978).

In conclusion, irradiation effects on the protein composition of soybean cultivars may cause aggregation to high molecular weight proteins, producing new subunit bands. This effect depended on the cultivar and the exposure dose. These results need further investigation to examine the proteins produced as a result of γ irradiation and its biological effect on experimental animals.

ABBREVIATIONS

krad = kilorad; PAGE = polyacrylamide gel electrophoresis; PAA = polyacrylamide; SDS-PAGE = sodium dodecyl sulfate PAGE; Poro-PAGE = porosity gradient PAGE.

Registry No. Trypsin inhibitor, 9035-81-8.

LITERATURE CITED

Alexander, P.; Fox, M.; Stracey, K. A.; Rosen, D. "Comparison of Some Direct and Indirect Effect of Ionizing Radiation in Protein". *Nature (London)* **1956**, *178*, 846–849.

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

Azamar, B. J. A.; Carrasco, A. H.; Cabrera, M. L. de F. M. V.; Loyola, V. V. M. "Preservation of Fruits and Vegetables by Irradiation". Transaction of american Nuclear Society Conference on Nuclear in Latin American, TANASAO 21 (supplement 2), 1975, pp 1–62.

Barron, E. S. G.; Ambrose, J.; Johnson, P. *Radiat. Res.* **1955**, *2*, 145.

Cabrera, L. M.; Carrasco, A. H. "Survey of Food Irradiation Studies in Mexico". *J. Agric. Food Chem.* **1978**, *26*, 3–5.

Davis, C. E.; Anderson, J. G. *J. Food Sci.* **1984**, *49*, 598.

de Wreede, I.; Stegemann, H. "Determination of Proteins in the Presence of Large Quantities of SDS and ME by a Modified Biuret Method". *Z. Anal. Chem.* **1981**, *308*, 431.

Diehl, J. F. "Irradiated Food". *Science (Washington, D.C.)* **1973**, *180*, 214–215.

Diehl, J. F.; Adam, S.; Delincee, H.; Jakubick, V. "Radiolysis of Carbohydrates and Carbohydrate Containing Food Stuffs". *J.*

Agric. Food Chem. **1978**, *26*, 15.
Dowie, O. G.; Biede, S. L. *J. Food Sci.* **1983**, *48*, 804.
Drake, M. P.; Giffee, J. W.; Johnson, D. A.; Koenig, V. L. "Chemical Effect of Ionizing on Proteins. 1. Effect of Radiation on the Amino Acid Content of Insultin". *J. Agric. Food Chem.* **1957**, *79*, 1395.
Errera, M. C. R. "Nature De L'effect Differe. Des Rayons X sur L'acide Desoxyribon Ucleique". *Bull. Soc. Chim Biol.* **1951**, *33*, 555.
Fishbein, W. N. *Anal. Biochem.* **1972**, *46*, 388.
Garrison, W. M. *Top. Radiat. Res.* **1968**, *4*, 43.
Hafez, Y. S.; Mohamed, A. I.; Singh, G.; Hewedy, F. M. "Effect of Gamma Irradiation on Protein and Fatty Acids of Soybean". *J. Food Sci.* **1985**, *50*(2), 1271.
Hamerstrand, G. E.; Black, C. T.; Glover, J. D. *Cereal Chem.* **1981**, *58*(1), 42.
Hu, B.; Esein, A. "Heterogeneity of Soybean Seed Proteins: One-Dimensional Electrophoretic Profiles of Six Different Solubility Fractions". *J. Agric. Food Chem.* **1981**, *29*, 497.
Laemmli, U. K. "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4". *Nature (London)* **1970**, *227*, 680.
Ley, E.; Hickman, J. R. "Processing Irradiation. II. Application to Food Preservation". *Food Res.* **1960**, *13*, 193.
Loaharanu, P. "Food Irradiation Processing". *Proceeding of a Symposium*, March 1985, Washington, DC, p 175.
Lynn, K. R.; Raoult, A. P. D. "Irradiation of Lima Bean Protease Inhibitors in Dilute Aqueous Solution". *Radiat. Res.* **1975**, *65*, 41.
Mäkinen, A.; de Wreede, I.; Stegemann, H.; Heinert, H. H. "Löslichkeit und Gelelectrphoretische Muster Hitzedena Turierter Proteine, auch von Stärkehaltingen Proben nach Einwirkung von Na-dodecylsulphate(SDS)". *Z. Lebensm.-Unters. Forsch.* **1979**, *168*, 282.
Margolis, J.; Kenrick, K. G. "Polyacrylamide Gel Electrophoresis in a Continuous Molecular Sieve Gradient". *Anal. Biochem.* **1968**, *25*, 347.
Merritt, C., Jr.; Angelini, P.; Graham, R. A. "Effect of Radiation Parameters on the Formation of Radiolysis Products in Meat and Meat Substances". *J. Agric. Food Chem.* **1978**, *26*, 29.
Nelson, K. E.; Maxie, F. C.; Enkh, W. "Some Studies in the Use of Ionizing Radiation to Control Botrytis in Table Grapes and Strawberries". *Phytopathology* **1959**, *49*, 475.
Preston, K. P.; Woodbury, W.; Orth, R. A.; Bushuk, W. "Comparison of Gliadin and Glutenin Subunits in the Triticinae by SDS-PAGE". *Can. J. Plan Sci.* **1975**, *55*, 667.
Richardson, L. R.; Ritchey, S. L.; Rigdon, R. H. "A Long Term Feeding Study of Irradiated Foods Using Rats as Experimental Animals". *Fed. Proc.* **1960**, *19*, 1023.
Roy, D. N.; Bhat, R. V. "Trypsin Inhibitor Content in Some Varieties of Soybean (*Glycine max* L.) and Sunflower Seeds (*Hellcanthus annuus* L.)". *J. Sci. Food Agric.* **1974**, *25*, 765.
Simic, M. G. "Radiation Chemistry of Amino Acids and Peptides in Aqueous Solutions". *J. Agric. Food Chem.* **1978**, *26*, 6–14.
Taub, I. A.; Angelini, P.; Merritt, C., Jr. "Irradiation Food: Validity of Extrapolating Wholesomeness Data". *J. Food Sci.* **1976**, *41*, 942.
Urakami, C.; Hallori, H.; Uemura, T.; Takahashi, R. "Effect of Co^{60} Ray on Fried Bean Curd. I. Storage Period and Flavor". *Eiyal Shokuryo* **1959**, *12*(3), 134.
Urakami, C.; Hosomi, M.; Tomonaga, K. "Effect of Co^{60} Rays on Fried Bean Curd. IV. Examination of Heated Soybean Oil and the Oil Absorbed in Outer Crust of the Bean Curd in Relation with the Irradiation Order". *Eiyal Shokuryo* **1961**, *14*(4), 281.
Ward, J. F. "Chemical Irradiating Nucleic Acids". *J. Agric. Food Chem.* **1978**, *26*, 25–28.
Whitburn, K. D.; Shieh, J. J.; Sellers, R. M.; Hoffmann, M. Z.; Taub, I. A. "Redox Transformation in Ferrimyoglobin Induced by Radiation Generated Free Radicals in Aqueous Solution". *J. Biol. Chem.* **1982**, *257*, 1860.
Whitcher, S. L.; Rotheram, M.; Todd, N. C. *Nucleonics* **1953**, *11*(8), 30.

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Glyphosate Induction of Elevated Levels of Hydroxybenzoic Acids in Higher Plants

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Glyphosate [*N*-(phosphonomethyl)glycine] effects on hydroxybenzoic acid levels in pigweed (*Amaranthus retroflexus* L.), ryegrass (*Lolium perenne* L.), soybean [*Glycine max* (L.) Merr.], velvetleaf (*Abutilon theophrasti* Medic.), and yellow nutsedge (*Cyperus esculentus* L.) were investigated. Leaves were harvested at 3 and/or 6 days after treatment with four levels of glyphosphate. Acid-hydrolyzed extracts were analyzed by HPLC. The concentration of protocatechuic acid in leaf tissue varied among species and was dependent upon the glyphosate dose, duration of exposure, and tissue assayed. Gallic acid levels were higher in four of the five species, and 4-hydroxybenzoic acid was higher in three of the five species treated with glyphosate as compared to controls. Vanillic and syringic acids, which are methylated forms of protocatechuic and gallic acids (respectively), were unaffected by glyphosphate treatment. The data indicate that some hydroxybenzoic acids such as protocatechuic, gallic, and 4-hydroxybenzoic may be directly synthesized from shikimate or shikimate precursors.

The nonselective, broad-spectrum, postemergence herbicide glyphosate [*N*-(phosphonomethyl)glycine] inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EC 2.5.1.19), an enzyme of the shikimic acid pathway (Amrhein, 1986). This results in the cessation of aromatic amino acid synthesis, followed by reduced protein synthesis, growth, and premature cellular death (Duke, 1988). Inhibition of EPSP synthase also causes high levels of shikimic acid to accumulate (Amrhein et al., 1980; Berlin and Witte, 1980). These levels are even larger than might be expected because lowered levels of shikimic acid pathway products result in deregulation (from end product inhibition) of carbon flow into the shikimic acid pathway (Jensen, 1985).

The shikimic acid pathway is responsible for synthesis of most phenolic compounds found in higher plants. Most

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