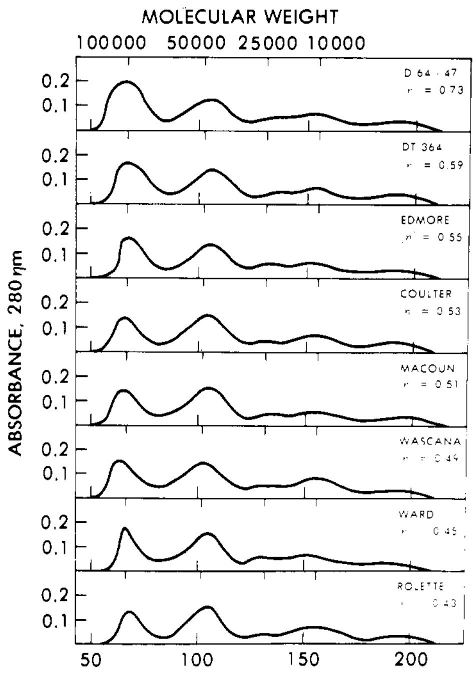

Figure 1. Representative gel filtration profiles of AUC extracts of lactic acid soluble gluten protein on Sephadex G-150.

that qualitative differences within the protein fractions may also play a role in determining gluten properties. No relationship was found between lactic acid soluble protein intrinsic viscosity and cooking quality, suggesting that variations in the nature of the soluble gluten proteins are not likely an important cooking quality factor.

ACKNOWLEDGMENT

We thank T. F. Townley-Smith, Agriculture Canada, Swift Current, E. D. Mallough, Agriculture Canada, Regina, and D. Leisle, Agriculture Canada, Winnipeg, for providing the plant material used in this study. Amino acid analyses were performed by K. R. Preston. The technical assistance of B. C. Morgan, S. Loutas, R. W. Daniel, and J. W. Bradley is gratefully acknowledged.

LITERATURE CITED

Axford, D. W. E., McDermott, E. E., Redman, D. G., *Milling Feed Fert.* **66**(5), 18 (1978).
Bushuk, W., Wrigley, C. W., *Cereal Chem.* **48**, 448 (1971).
Chen, C. H., Bushuk, W., *Can. J. Plant Sci.* **50**, 9 (1970).
Danno, G., Kanazawa, K., Natake, M., *Agric. Biol. Chem.* **38**, 1947 (1974).
Dexter, J. E., Matsuo, R. R., *Can. J. Plant Sci.* **57**, 7 (1977a).
Dexter, J. E., Matsuo, R. R., *Can. J. Plant Sci.* **57**, 717 (1977b).
Dexter, J. E., Matsuo, R. R., *Cereal Chem.* **54**, 882 (1977c).
Dexter, J. E., Matsuo, R. R., *Cereal Chem.* **55**, 44 (1978a).
Dexter, J. E., Matsuo, R. R., *Cereal Chem.* **55**, 841 (1978b).
Dexter, J. E., Matsuo, R. R., Kosmolak, F. G., Leisle, D., Marchylo, B. A., *Can. J. Plant Sci.* **60**, 25 (1980).
Grzybowski, R. A., Donnelly, B. J., *J. Agric. Food Chem.* **27**, 380 (1979).
Irvine, G. N., Bradley, J. W., Martin, G. C., *Cereal Chem.* **38**, 153 (1961).
Khan, K., Bushuk, W., *Cereal Chem.* **56**, 63 (1979).
Matsuo, R. R., *Cereal Chem.* **55**, 259 (1978).
Matsuo, R. R., Bradley, J. W., Irvine, G. N., *Cereal Chem.* **49**, 707 (1972).
Matsuo, R. R., Irvine, G. N., *Cereal Chem.* **46**, 1 (1969).
Matsuo, R. R., Irvine, G. N., *Cereal Chem.* **47**, 173 (1970).
Matsuo, R. R., Irvine, G. N., *Cereal Chem.* **48**, 554 (1971).
Meredith, O. B., Wren, J. J., *Cereal Chem.* **43**, 169 (1966).
Mitcheson, R. C., Stowell, K. C., *J. Inst. Brew.* **76**, 335 (1970).
Orth, R. A., Baker, R. J., Bushuk, W., *Can. J. Plant Sci.* **52**, 139 (1972).
Orth, R. A., Dronzek, B. L., Bushuk, W., *Cereal Chem.* **51**, 281 (1974).
Orth, R. A., O'Brien, L., Jardine, R., *Aust. J. Agric. Res.* **27**, 575 (1976).
Payne, P. I., Corfield, K. G., *Planta* **145**, 83 (1979).
Pomeranz, Y., *J. Sci. Food Agric.* **16**, 586 (1965).
Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics", McGraw-Hill, New York, 1960.
Tkachuk, R., *Cereal Chem.* **43**, 207 (1966).
Walsh, D. E., Gilles, K. A., *Cereal Chem.* **48**, 544 (1971).
Wasik, R. J., Bushuk, W., *Cereal Chem.* **52**, 322 (1975).
Williams, P. C., *J. Sci. Food Agric.* **24**, 343 (1973).

Received for review March 6, 1980. Accepted June 16, 1980. Contribution No. 442 of the Canadian Grain Commission, Grain Research Laboratory.

Nitrogen Fertilization Effects on Amino Acid Composition of Pecan (*Carya illinoensis*) Nutmeats

Carroll D. Elmore* and Samuel G. Polles

"Desirable" pecan trees (*Carya illinoensis*) were fertilized with ammonium nitrate or nitroform at five rates up to 6.8 kg of N/tree. Effects of each N fertilizer form on amino acid composition were determined. Both forms affected amino acid composition, but in different ways. Ammonium nitrate increased the concentration of glutamate and proline relative to the nitroform fertilized samples. Regression analyses also revealed differences between the two fertilizers for lysine, arginine, aspartate, serine, glutamate, alanine, cysteine, valine, isoleucine, leucine, tyrosine and phenylalanine. These differences, though small, probably resulted from the slow release nature of nitroform that provides a more constant N supply to the developing nut than ammonium nitrate does. In addition, the free amino acid profile is presented and the prevalence of the urea cycle amino acids is shown. These data are of importance from a human nutritional viewpoint.

Pecans are a prominent nut crop in the United States with a current annual production of 250 million pounds, which is increasing yearly (Livingston, 1976). The composition of pecan nutmeats has been fairly well characterized. The average oil and protein contents are known to be 70 and 10%, respectively (Meredith, 1974). The fatty acid and mineral compositions are also known (Senter,

Delta State Agricultural Research Center, Stoneville, Mississippi 38776 (Plant Physiologist and Entomologist).

Table I. Percent Composition of Amino Acids in Pecan Nutmeats As Affected by Rates and Type of N Fertilization[a]

amino acid	check, 0 kg of N/tree	ammonium nitrate, kg of N/tree				nitroform, kg of N/tree			
		3.4	4.5	5.7	6.8	3.4	4.5	5.7	6.8
lysine	3.34^{abc}	3.21^{abc}	3.33^{abc}	3.18^{abc}	3.10^{c}	3.38^{ab}	3.21^{abc}	3.31^{abc}	3.39^{a}
histidine	2.56^{ab}	2.48^{b}	2.59^{ab}	2.46^{b}	2.53^{ab}	2.63^{a}	2.52^{ab}	2.58^{ab}	2.57^{ab}
ammonia	1.84^{bc}	1.81^{c}	1.88^{abc}	1.83^{bc}	1.87^{abc}	1.90^{ab}	1.81^{c}	1.86^{abc}	1.94^{a}
arginine	14.58	14.31	13.81	13.58	14.39	14.13	13.85	14.05	14.28
aspartate	9.31^{b}	9.36^{ab}	9.00^{c}	9.42^{ab}	9.36^{ab}	9.36^{ab}	9.55^{a}	9.58^{a}	9.62^{a}
threonine	3.12^{ab}	3.09^{ab}	2.87^{c}	3.18^{ab}	3.10^{ab}	3.00^{bc}	3.26^{a}	3.20^{ab}	3.25^{a}
serine	5.06^{abc}	5.01^{abc}	4.62^{d}	4.92^{bc}	4.84^{c}	4.86^{bc}	5.07^{ab}	5.06^{abc}	5.21^{a}
glutamate	20.14^{abc}	20.45^{ab}	20.84^{a}	20.73^{ab}	20.59^{ab}	20.17^{abc}	20.40^{ab}	20.38^{ab}	19.36^{c}
proline	4.46^{a}	4.69^{a}	4.58^{a}	4.93^{a}	4.74^{a}	3.91^{b}	3.85^{b}	3.77^{b}	3.87^{b}
glycine	5.21^{cd}	5.19^{cd}	5.47^{a}	5.26^{bc}	5.23^{cd}	5.27^{abc}	5.12^{cd}	5.03^{d}	5.15^{cd}
alanine	4.78^{cd}	4.94^{bc}	5.22^{a}	5.08^{ab}	5.03^{ab}	5.01^{ab}	4.71^{d}	4.67^{d}	4.75^{cd}
cysteine	1.06^{ab}	1.02^{b}	1.09^{ab}	1.03^{b}	1.06^{ab}	1.06^{ab}	1.13^{ab}	1.18^{a}	1.18^{a}
valine	4.48^{abcd}	4.46^{bcd}	4.54^{abcd}	4.41^{cd}	4.40^{d}	4.66^{ab}	4.62^{abc}	4.65^{ab}	4.70^{a}
methionine	1.62	1.59	1.41	1.72	1.59	1.44	1.65	1.51	1.64
isoleucine	3.74^{ab}	3.71^{ab}	3.76^{ab}	3.75^{ab}	3.67^{b}	3.90^{a}	3.91^{a}	3.86^{ab}	3.85^{ab}
leucine	6.85	6.86	7.02	6.82	6.80	7.09	7.05	7.04	7.07
tyrosine	3.12^{ab}	3.10^{ab}	3.15^{ab}	3.05^{b}	3.03^{b}	3.22^{a}	3.26^{a}	3.25^{a}	3.23^{a}
phenylalanine	4.76^{cd}	4.72^{cd}	4.81^{bcd}	4.65^{d}	4.66^{d}	5.02^{ab}	5.05^{a}	5.03^{ab}	4.94^{abc}

[a] Amino acid data are relative data assuming 100% recovery from the column. Duncan's multiple range is given for differences among the rates and between types of N. Entries followed by the same letter(s) are not different.

1976; Senter and Horvat, 1976), but little information is available regarding amino acid composition (Food Policy and Food Science Service, 1970). Meredith (1974) summarized the available literature on amino acid composition and reported on varietal effects. The present study reports the effects of two types of nitrogen fertilizer on the total and free amino acid composition of pecan nutmeats. The free amino acid composition of pecan has not been previously reported.

EXPERIMENTAL SECTION

Pecan Fertilization. Individual Desirable trees in a pecan orchard were fertilized with ammonium nitrate (33.5% N) or nitroform (38% N) (a slow release formulation) at rates of 0, 3.4, 4.5, 5.7, or 6.8 kg of N/tree during dormancy in the winter of 1978 in a recommended fashion. The trees were 18 years old with an average dbh of 12 in. The plots were arranged in a Randomized Complete Block design and replicated 3 times. Nuts were harvested on Nov 1, 1978, shelled, and stored at 0 °C until analyzed.

Amino Acid Determination. Hydrolysis of amino acids and amino acid determinations were as reported previously (Elmore and Leffler, 1976). Free amino acids were extracted and analyzed as described by Elmore and King (1978) on trees receiving no additional N fertilization. Amino acid composition data were analyzed and regression equations computed for linear and quadratic models by SAS Washington Computer Center.

RESULTS

Nitrogen fertilization affected the amino acid composition of pecan nutmeats (Table I). Only arginine, methionine, and leucine were unaffected by the rate of N. Ammonium nitrate differed from nitroform for 12 of the 17 reported amino acids, chiefly because the concentrations of glutamate, proline, and alanine were highest in the ammonium nitrate fertilized nutmeats. Concentrations of all of the other affected amino acids were lower in the ammonium nitrate treated tissue. Another indication that the forms of nitrogen differed was the rate × treatment interactions, which were significant for all amino acids except arginine, glutamate, methionine, leucine and isoleucine. Reactions to rates of fertilizer were best depicted by regression equations. In Table II is shown the effects of rates of each N form on amino acid concentration. In the cases where the regression equations were significant,

Table II. Regression Equations of Percent Amino Acid Composition of Pecan Nutmeats As Affected by Rate and Kind of N Fertilization

	ammonia nitrate	nitroform
lysine	$3.35 - 0.01x$	ns
histidine	ns[a]	ns
ammonia	ns	$1.83 + 0.004x$
arginine	ns	$14.58 - 0.13x + 0.007x^2$
aspartate	ns	$9.29 + 0.02x$
threonine	ns	ns
serine	$5.03 - 0.02x$	$5.04 - 0.038x + 0.003x^2$
glutamate	$20.13 + 0.095x - 0.004x^2$	ns
proline	$4.46 + 0.02x$	$4.40 - 0.05x$
glycine	ns	ns
alanine	$4.78 + 0.06x - 0.003x^2$	ns
cysteine	ns	$1.04 + 0.008x$
valine	ns	$4.48 + 0.01x$
methionine	ns	ns
isoleucine	ns	$3.76 + 0.01x$
leucine	$6.84 + 0.027x - 0.002x^2$	$6.88 + 0.02x$
tyrosine	ns	$3.13 + 0.01x$
phenylalanine	ns	$4.75 + 0.06x - 0.003x^2$

[a] ns = not significant.

they are not the same for each form. For example, serine is fitted to a quadratic equation for nitroform while a linear equation works for ammonium nitrate. This is a further demonstration that N forms affected amino acids differently.

Free amino acids in the unfertilized pecan nutmeats were generally present in very low amounts; a variety of amino acids and amino-containing compounds were also present that contributed to the overall profile (Table III). Aspartate and glutamate, rather than their amides, were the most prominent free amino acids. Citrulline was the next most common amino acid.

DISCUSSION

The data on amino acid response to N rates indicated that pecans react very much like other agronomic crops in which amino acid profiles change markedly in response to N fertilization (Elmore et al., 1979). In this study the addition of ammonium nitrate decreased the concentration

Table III. Free Amino Acid Content of Pecan Nutmeats[a]

amino acid	μmol/g dry wt (SEM)
aspartate	1.7 (0.37)
threonine	0.1 (0.04)
serine	0.4 (0.09)
asparagine	0.2 (0.09)
glutamate	1.6 (0.37)
glutamine	0.4 (0.06)
proline	0.5 (0.04)
glycine	0.2 (<0.01)
alanine	0.5 (0.07)
valine	0.4 (0.25)
methionine	0.1 (0.02)
isoleucine	0.1 (0.02)
leucine	0.1 (0.01)
tyrosine	0.1 (0.01)
phenylalanine	0.1 (0.01)
γ-aminobutyrate	0.1 (0.02)
lysine	0.2 (0.01)
histidine	0.1 (0.01)
arginine	0.4 (0.10)
citrulline	1.4 (0.51)
ornithine	0.1 (0.04)
3-methylhistidine	<0.1
cysteine	Tr
cystathionine	<0.1
β-alanine	Tr
ethanolamine	Tr
ammonia	3.0 (0.55)

[a] Mean content of check (0 N) samples only is presented with standard error of the mean. Tr = trace.

of lysine in the nutmeat, a response similar to those seen in other crops (Elmore et al., 1979). Nitroform, on the other hand, did not affect the lysine concentration. This is probably a consequence of the slow release character of nitroform which provides continued availability of N during the growing season, especially during the nut-filling period. These effects are very important and should be studied further.

This presentation of the free amino acid composition is the first available in the literature for pecans and shows that there are more different kinds of amino acids or amino-containing compounds in pecan than in many other seeds that are used for food. The urea cycle components were quite prominent.

Pecans were shown to be a reasonable source of protein, with acceptable levels of the sulfur amino acids; however, they are limiting in lysine and thus of limited use for monogastric animal nutrition.

ACKNOWLEDGMENT

We thank M. K. Heaton and S. D. Senter for reviewing a prior draft of this manuscript and acknowledge the excellent technical assistance of Louis Clarke and Wesley Long.

LITERATURE CITED

Elmore, C. D., King, E. E., *Plant Physiol.* **62**, 531 (1978).
Elmore, C. D., Leffler, H. R., *Crop Sci.* **16**, 867 (1976).
Elmore, C. D., Spurgeon, W. F., Thom, W. O., *Agron. J.* **71**, 713 (1979).
Food Policy and Food Science Service, *Food Agric. Organ. U.N., FAO Nutr. Studies* **24**, 74 (1970).
Livingston, R. L., *Pecan South* **3**, 531 (1976).
Meredith, Filmore I., *Fla. State Hortic. Soc. Proc.* **87**, 362 (1974).
Senter, S. D., *J. Food Sci.* **41**, 963 (1976).
Senter, S. D., Horvat, R. D., *J. Food Sci.* **41**, 1201 (1976).

Received for review January 22, 1980. Accepted May 27, 1980.

Purification and Characterization of a Corn (*Zea mays*) Protein Similar to Purothionins

Berne L. Jones* and D. Blake Cooper

Extraction of petroleum ether defatted ground corn (*Zea mays*) with dilute sulfuric acid yielded six protein fractions, each of which contained large numbers of basic amino acid and cysteine residues. All of the proteins appeared to be similar in size and amino acid composition to the purothionins found in wheat flour. One protein was almost identical in amino acid composition to the thionin recently isolated from rye flour. This protein, corn protein I, was hydrolyzed with chymotrypsin and yielded four peptides, none of which resembled the two peptides released from purothionins under identical conditions. None of the six proteins were toxic when injected into tobacco hornworm (*Manduca sexta*) larvae. Even though the corn protein I is very similar to purothionins and rye thionin in amino acid composition and in several chemical and physical properties, it is probably not homologous in primary structure.

Purothionins are a group of small proteins (or large polypeptides) 45 amino acids in length that were isolated from wheat flour (Balls and Hale, 1940). They are very basic (20% Arg + Lys) and contain large amounts of Cys (20%) but no His, Met, or Trp (Nimmo et al., 1974; Redman and Fisher, 1968). The amino acid sequences of the three purothionin species present in bread wheat (*Triticum aestivum*) have been determined (Mak and Jones, 1976a; Jones and Mak, 1977; Ohtani et al., 1975). Wheat purothionins are homologous in structure with a group of toxic mistletoe (*Viscum album*) proteins (Mak and Jones, 1976a; Samelsson et al., 1968; Samuelsson, 1974). Homologous proteins have also been found in various species of the Aegilops and Triticum groups (Carbonero and Garcia-Olmedo, 1969) and have been purified from barley (Redman and Fisher, 1969) and from rye (Hernandez-Lucas et al., 1978). As a group, these

U. S. Grain Marketing Research Laboratory, Agricultural Research, Science and Education Administration, U. S. Department of Agriculture, Manhattan, Kansas 66502.