corn [(*Zea mays* (L) (Nelson et al., 1965)] and barley (*H. vulgare*) (Eppendorfer, 1978).

Although SK 5912 was easily the highest in its concentration of essential amino acids, the other two genotypes did not clearly differ in this regard. For example, the ranking of genotypes for individual essential amino acids gave the following orders: SK 5912 > RCFA × L.187 > L.187 for lysine and leucine, while it was SK 5912 > RCFA × L.187 > L.187 for valine and SK 5912 > L.187 > RCFA × L.187 for methionine consistently in both years (unpublished data).

The negative *r* values obtained for correlations of NRA with either lysine or methionine as well as correlation of grain protein at harvest with either of these amino acids show some inverse or antagonistic relationships among these factors. Similar reports were given by Eppendorfer (1975). This negative relationship could be due to much larger increases in the prolamin and partly glutelin fractions of grain protein than the albumin and globulin fractions. This would then result in a depression in the concentrations of lysine and methionine in the total grain protein (Eppendorfer, 1975). This explanation is further supported by the general increase in total amino acids with increase in nitrogen levels in this study whereas total essential amino acids decreased (Table I).

## CONCLUSIONS

High nitrogen levels would tend to decrease the essential amino acids of sorghum grains on a grams per 16 g of N basis. Except for lysine and aspartic acid, amino acid values of sorghum grains were highest at harvest. There is, therefore, not much nutritional advantage in harvesting sorghum grains for human consumption before grain maturity. Of the three genotypes tested in this study, SK 5912 was the highest in amino acid composition. The genotype is, therefore, a possible parent line where breeding for high protein and amino acids in sorghum is the object of study.

Inverse relationships were evident between grain protein and lysine, grain protein, and methionine, between NRA and lysine, and also between NRA and methionine. The three genotypes were rich in glutamic acid, aspartic acid, leucine, alanine, and proline. They had moderate amounts of phenylalanine, arginine, histidine, tyrosine, and isoleucine but were very low in lysine and methionine. Methionine was the lowest of all the amino acids and in each of the genotypes studied.

**Registry No.** Valine, 72-18-4; methionine, 63-68-3; lysine, 56-87-1; nitrate reductase, 9013-03-0.

## LITERATURE CITED

AERLS "Recommended Practices No. 2"; Agricultural Extension and Research Liaison Services, Ahmadu Bello University: Zaria, Nigeria, 1976.
AERLS "Recommended Practices No. 2"; Agricultural Extension and Research Liaison Services, Ahmadu Bello University: Zaria, Nigeria, 1978.
Breteler, H. *J. Sci. Food Agric.* **1976**, *27*, 978–982.
Eggum, B. O. In "Nuclear techniques for seed protein improvement"; I. ALA: Vienna, 1973; pp 39–408.
Eppendorfer, W. H. "Proceedings of the 11th Colloquium of the International Potash Institute, Ronne, Bornholm, Denmark"; International Potash Institute, CH-3048: Worblanfen-Berne, Switzerland, 1975; pp 249–260.
Eppendorfer, W. H. *J. Sci. Food Agric.* **1978**, *29*, 995–1001.
Frey, K. J. *Iowa, Agric. Home Econ. Exp. Stn., Ames Proj.* **1973**, *No. J.7322*, 1752.
Klepper, L.; Flesher, D.; Hageman, R. H. *Plant Physiol.* **1971**, *48*, 580–590.
Mitchell, G. A.; Bingham, F. T.; Labanauskas, C. K.; Yermanos, D. M. *Soil Sci. Soc. Am. J.* **1976**, *40*, 64–68.
Munck, L., FAO/IAEA/CST Third Research Co-ordination Meetings, Hahnenklee, Federal Republic of Germany, 1975.
Nelson, O. E.; Mertz, E. T.; Bates, L. S. *Science (Washington, D.C.)* **1965**, *150*, 1469–1470.
Radin, J. W. *Plant Physiol.* **1973**, *51*, 332–336.
Rhodes, D. A.; Mathers, J. C. *J. Sci. Food Agric.* **1974**, *25*, 963–972.
Sikka, K. C.; Johari, R. P. *J. Agric. Food Chem.* **1979**, *27*, 12.

Received for review September 10, 1982. Revised manuscript received March 24, 1983. Accepted August 29, 1983.

# Fatty Acids of Soybean Seeds Harvested from Plants Exposed to Air Pollutants

Claus Grunwald* and Anton G. Endress

The neutral ester, free, and polar fatty acid fractions, obtained from soybean seeds harvested from field-grown plants exposed to $SO_2$ or $O_3$, were analyzed in both qualitative and quantitative terms. Plant exposure to $SO_2$ or $O_3$ produced seeds of lower oil:protein ratio, but no significant changes in any of the fatty acids were observed. It is suggested that the soybean pod protects the seed from the pollutant and that $SO_2$ or $O_3$ exposure of plants within federal guidelines should not affect oil quality.

Several investigators have examined the consequences of chronic air pollutant exposure on the physiology and biochemistry of the soybean [*Glycine max* (L.) Merr.] plant (Heath, 1980; Ziegler, 1975), but relatively few have examined air pollutant effects on seed quality.

The soybean seed consists of protein (30–46%), lipids (12–24%), carbohydrates (33–37%), and minerals (5%). The exact ratio of these constituents depends on the cultivar and environmental conditions under which the plant is grown. The oils and proteins are the commercially important seed constituents (Smith and Circle, 1972). Generally, there is an inverse relationship between seed oil (lipid) and protein content, and if environmental factors alter the seed oil:protein ratio, changes in other components may also occur.

Frey (1972) reported that seeds from soybean plants grown under 980 $\mu$g of $O_3/m^3$ (1.96 mg/m$^3$ = 1 ppm of $O_3$) had 4% less oil and 21% more amino acid (protein) than the seeds from control plants. This $O_3$ concentration is significantly above the national secondary $O_3$ standard (235

Botany and Plant Pathology, Illinois Natural History Survey, Champaign, Illinois 61820 (C.G. and A.G.E.), and Department of Plant Biology, University of Illinois, Urbana, Illinois 61801 (C.G.).

0021-8561/84/1432-0050$01.50/0 

$\mu g/m^3$). Howell and Rose (1980), using open-top field chambers, found no consistent statistical difference in percent of oil or protein in seeds from plants grown either in activated carbon-filtered air or in nonfiltered air. In their experiments over a 3-year period, the highest $O_3$ level in the nonfiltered air chamber was generally 196 $\mu g/m^3$. Exposure of harvested, mature soybean seeds to very high levels of $O_3$ (2.94 $mg/m^3$) did not alter the oil content but did lower the level of fatty acid saturation in some bean cultivars (Brooks and Csallany, 1978). Beans produced in an open-air $SO_2$ fumigation system generally did not show a difference in oil content but showed a slightly reduced protein level at pollutant concentrations above 800 $\mu g$ of $SO_2/m^3$ (2.7 $mg/m^3$ = 1 ppm of $SO_2$) (Sprugel et al., 1980).

Any reduction in the oil or protein content of the bean is of importance to the producer since changes in fatty acid composition may also affect product quality (Sheppard et al., 1978). The level of fatty acid saturation is important in determining product odor and flavor. A shift to higher unsaturation generally makes the oil less desirable because of fatty acid autoxidation and the development of rancidity. Thus, oils with highly unsaturated fatty acids, particularly linolenic acid, could increase the processing cost. The purpose of the present study was to examine the oil fraction of seeds harvested from field-grown soybean plants exposed to chronic levels of $SO_2$ and $O_3$ for qualitative and quantitative changes in lipid fractions and fatty acids. The oil fraction was divided into neutral esters, free fatty acids, and polar lipids.

## EXPERIMENTAL SECTION

The soybean seeds [*Glycine max* (L.) Merr. cv. Corsoy] used in this investigation were field grown at the Argonne National Laboratory, Argonne, IL. For $O_3$ treatments, the plants were grown in open-top chambers in charcoal-filtered air at a seasonal 7-h average of 43.1 $\mu g$ of $O_3/m^3$ or in unfiltered air at 225.4 $\mu g$ of $O_3/m^3$, respectively. The growing period was July 1–Oct 1, 1980. The experimental treatment began on Aug 6 (at time of flowering) and continued through Sept 30. $O_3$ was added daily to the chambers from 0900 to 1600 Central Standard Time, except on rainy days. For a complete description of the experimental protocol, see Kress and Miller (1983).

The $SO_2$-treated seeds were produced in a separate field experiment during 1980. An open-air fumigation system (ZAPS type), as described by Sprugel et al. (1980), was employed to grow the plants. Seeds for analysis were taken from plants that received ambient air or ambient air spiked with additional $SO_2$ with an arithmetic mean $SO_2$ concentration of <27 $\mu g/m^3$ or 101.3 $\mu g$ of $SO_2/m^3$, respectively. Both $SO_2$ concentrations were the mean concentrations of sixteen 3-h exposures (Miller et al., 1981).

For chemical analysis, the beans were ground in a Wiley mill to pass through a 40-mesh screen. Total oil content was determined gravimetrically through exhaustive hexane extraction of ca. 3 g of ground soybean meal in a Soxhlet apparatus. For analysis of fatty acids, ground soybean seeds (~100 mg) were homogenized in a glass grinder by using chloroform–methanol (2:1 v/v) as the solvent. A known amount of heptadecanoic acid, or its methyl ester, was added as internal standard to the homogenate. The mixture was washed 2× with 0.2 volume of 3 mM $CaCl_2$. The organic phase, which contained the lipids, was filtered, taken to dryness, and redissolved in a known volume of methanol. One-fourth of the fraction was removed for total fatty acid analysis, while the remaining portion was made alkaline (pH 9–10) and washed 3× with hexane for neutral ester extraction. If two phases did not form, a small amount of water was added. The basic alcoholic mixture was acidified (pH 2–3) and again extracted 3× with hexane to remove the free fatty acids. The remaining acidic alcohol fraction, to which a known quantity of heptadecanoic was added, contained the polar lipids, mainly phospho- and glycolipids (Grunwald, 1981). The total fatty acid, neutral ester, and polar lipid fractions were saponified in 5% KOH in 95% aqueous methanol (w/v) for 30 min. The cooled mixtures were acidified and the fatty acids extracted 3× with hexane. Three replicate analyses were made.

Table I. Total Oil and Protein Content of Soybean Seeds Harvested from Field-Grown Plants Exposed to $SO_2$ and $O_3$

| experiment | treatment | yield, % of control | mg of oil/g of seed | mg of protein/g of seed | ratio oil:protein |
|---|---|---|---|---|---|
| I | $SO_2$, <27 $\mu g/m^3$ | | 228 | 279 | 0.82 |
| | $SO_2$, 101.3 $\mu g/m^3$ | 87 | 223 | 301[a] | 0.72 |
| II | $O_3$, 43.1 $\mu g/m^3$ | | 253 | 282 | 0.90 |
| | $O_3$, 225.4 $\mu g/m^3$ | 61 | 221[b] | 290 | 0.76 |

[a] Within the experiment significant at the 5% level. [b] Within the experiment, significant at the 1% level.

The fatty acids were analyzed by gas–liquid chromatography (GLC) as methyl ester derivatives (Morrison and Smith, 1964). The methyl esters were formed by adding 4 mL of 10% boron trichloride in anhydrous methanol to the dried sample, placing the Teflon-sealed vials into boiling water for 10 min, and extracting the fatty acid esters with hexane. The GLC column used was a 1.3 m long, 4 mm i.d., glass column packed with 10% SP-2330 on Chromosorb WAW, 100–120 mesh. The column temperature was programmed from 164 to 210 °C, with an initial hold of 4 min, followed by a temperature rise of 4 °C/min, with a final hold for 6 min. Injector and flame ionization detector temperatures were 250 °C. Nitrogen was the carrier gas at 40 mL/min. The various fatty acid methyl esters were corrected for differences in detector response.

For protein analysis, the 40-mesh soybean meal (~10 mg) was further ground with distilled water in a glass grinder and assayed by the procedure of Lowry et al. (1951) using lysozyme as the protein standard.

## RESULTS AND DISCUSSION

Exposure of soybean plants to $SO_2$ significantly ($P < 0.05$) increased the protein level of the harvested seeds, but the percent oil content was not altered (Table I). Plants exposed to $O_3$, however, produced seeds that had a significantly ($P < 0.01$) lower oil content, without altering the protein level. Since the $SO_2$- and $O_3$-produced seeds were derived from two separate experiments, caution must be exercised in any direct comparison, but a generally lower oil:protein ratio was found with exposure to both $SO_2$ and $O_3$ (Table I). Howell and Rose (1980), in a 3-year field study using charcoal-filtered and nonfiltered air, found a consistently lower oil:protein ratio in the seeds harvested from soybean plants treated with nonfiltered air. Similarly, Kress and Miller (1983), in a field study using $O_3$ as the pollutant, also observed a reduction in the soybean oil:protein ratio. Conversely, Sprugel et al. (1980) reported that fumigation with $SO_2$ produced the opposite effect, that is, a general increase in the oil:protein ratio. While many of the reported absolute changes in oil and protein content of the seeds harvested from pollutant-exposed plants may be significant, no consistent picture has emerged. It is quite possible that other environmental

Table II. Total Fatty Acid Content and Its Composition in Soybean Seeds Harvested from Field-Grown Plants Exposed to $SO_2$ and $O_3$

| treatment | fatty acid, mg/g of seed | % fatty acid composition | | | | | |
|---|---|---|---|---|---|---|---|
| | | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
| $SO_2$, <27 $\mu g/m^3$ | 271 | 12.7 | tr | 3.5 | 23.2 | 52.4 | 8.2 |
| $SO_2$, 101.3 $\mu g/m^3$ | 261 | 12.0 | tr | 3.5 | 22.6 | 54.0 | 7.9 |
| $O_3$, 43.1 $\mu g/m^3$ | 232 | 12.8 | tr | 3.8 | 18.8 | 56.2 | 8.4 |
| $O_3$, 225.4 $\mu g/m^3$ | 228 | 12.5 | tr | 3.5 | 16.6 | 59.6 | 7.8 |

Table III. Neutral Ester Fatty Acid Content and Its Composition in Soybean Seeds Harvested from Field-Grown Plants Exposed to $SO_2$ and $O_3$

| treatment | fatty acid, mg/g of seed | % fatty acid composition | | | | | |
|---|---|---|---|---|---|---|---|
| | | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
| $SO_2$, <27 $\mu g/m^3$ | 217 | 12.6 | tr | 3.5 | 23.5 | 52.9 | 7.5 |
| $SO_2$, 101.3 $\mu g/m^3$ | 195 | 12.1 | tr | 3.3 | 23.0 | 53.3 | 8.3 |
| $O_3$, 43.1 $\mu g/m^3$ | 227 | 12.8 | tr | 3.5 | 20.6 | 53.7 | 9.4 |
| $O_3$, 225.4 $\mu g/m^3$ | 219 | 13.5 | tr | 3.6 | 20.7 | 53.3 | 8.9 |

factors that also play a role in determining the oil and protein content of the seed overshadow any small changes produced by exposure to pollutants.

Linoleic acid was the major fatty acid under all treatments and accounted for more than 50% of the total oil fatty acids (Table II). Slight, but statistically nonsignificant, changes in total fatty acids were observed. Over 90% of the soybean seed lipid fraction was in the form of neutral esters (Table III), probably mostly triacylglycerol (Gurr, 1980), and the slight decrease in lipid content with exposure of plants to air pollutants was mainly a decrease in neutral esters. The neutral esters, which accounted for most of the total oil fraction, had a fatty acid composition very similar to that of the total lipid fraction. The neutral esters did not show a major change in fatty acid profile with $SO_2$ or $O_3$ exposure.

The free fatty acids (Table IV) are a minor component of the total lipid fraction, and their composition is very similar to that of the neutral ester fatty acids. Only slight changes in free fatty acid composition were noticed with $SO_2$ and $O_3$ exposure; the most important a decrease in oleic acid and an increase in linoleic acid; however, none were statistically significant. The seeds contained small amounts of polar lipids (Table V), and $SO_2$ and $O_3$ exposure resulted in small changes in fatty acid composition. Linoleic acid, under all conditions, was the major fatty acid, and with $SO_2$ and $O_3$ exposure, this fatty acid decreased while most of the other fatty acids increased. Palmitic acid was the second most dominant fatty acid, but it did not change in concentration with exposure to air pollutants.

The results obtained in the present investigation are surprising, since a general decrease in foliar lipids had been observed with $SO_2$ exposure in long-term field (Grunwald, 1981) and greenhouse studies (Constantinidou and Kozlowski, 1979). In addition, an inhibition in the incorporation of radioactive acetate into foliar lipids in short-term experiments with $SO_2$ had also been demonstrated (Khan and Malhotra, 1977; Malhotra and Khan, 1978). Leaves

Table IV. Free Fatty Acid Content and Its Composition in Soybean Seeds Harvested from Field-Grown Plants Exposed to $SO_2$ and $O_3$

| treatment | fatty acid, mg/g of seed | % fatty acid composition | | | | |
|---|---|---|---|---|---|---|
| | | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 |
| $SO_2$, <27 $\mu g/m^3$ | 7.9 | 14.7 | 4.5 | 24.0 | 50.4 | 6.4 |
| $SO_2$, 101.3 $\mu g/m^3$ | 9.8 | 13.7 | 4.0 | 21.5 | 52.4 | 8.4 |
| $O_3$, 43.1 $\mu g/m^3$ | 8.9 | 13.7 | 3.8 | 23.8 | 49.9 | 8.8 |
| $O_3$, 225.4 $\mu g/m^3$ | 9.7 | 12.4 | 3.4 | 20.7 | 53.8 | 9.7 |

Table V. Polar Lipid Fatty Acid Content and Its Composition in Soybean Seeds Harvested from Field-Grown Plants Exposed to $SO_2$ and $O_3$

| treatment | fatty acid, mg/g of seed | % fatty acid composition | | | | | |
|---|---|---|---|---|---|---|---|
| | | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
| $SO_2$, <27 $\mu g/m^3$ | 3.7 | 35.3 | 0.5 | 9.7 | 6.6 | 42.5 | 5.4 |
| $SO_2$, 101.3 $\mu g/m^3$ | 3.0 | 36.5 | 0.3 | 11.9 | 9.0 | 37.8 | 4.5 |
| $O_3$, 43.1 $\mu g/m^3$ | 4.9 | 30.0 | 3.7 | 13.4 | 7.4 | 41.0 | 4.5 |
| $O_3$, 225.4 $\mu g/m^3$ | 4.8 | 31.8 | 4.8 | 14.0 | 8.6 | 35.2 | 5.6 |

have shown both quantitative as well as qualitative changes in lipid composition (Grunwald, 1981), yet the seeds harvested from field-exposed plants to $SO_2$ and $O_3$ showed only minor alterations in fatty acid profiles (Tables II–IV). Of the three lipid fractions, only the polar lipids showed any changes (Table V). Frey (1972) reported that high levels of $O_3$ (1470 $\mu g/m^3$) shifted the total fatty acid composition of soybean seeds toward more unsaturation. However, we did not observe this shift to greater unsaturation in fatty acids (Table II). It is unfortunate that no other studies with soybean seeds, or any other seeds, are available for comparison of fatty acid profiles.

The inhibitory effect of $SO_2$ and $O_3$ on fatty acid biosynthesis in foliar tissue has been documented (Mudd et al., 1971; Khan and Malhotra, 1977; Malhotra and Khan, 1978); however, the effect of these pollutants on the biosynthesis of seed lipids has not yet been examined. The biosynthesis of fatty acids in leaves is quite different from that in seeds. In leaves, the center of fatty acid biosynthesis is the chloroplast and the major fatty acid generally produced is linolenic acid, while, in seeds, the proplastids perform lipid biosynthesis (Stumpf, 1980) and their major product is linoleic acid. From all available evidence there is no movement of fatty acids from one pool to another; thus fatty acids found in the leaf have been synthesized by chloroplasts in the leaf and fatty acids found in the seed have been synthesized by proplastids. It is quite possible that proplastids are more resistant than chloroplasts to $SO_2$ and $O_3$ injury and thus never shown any inhibitory effect. However, a more likely explanation is that the soybean pod protects the seed from exposure to the pollutant and thus the proplastids are never exposed to $SO_2$ and $O_3$. For example, in the case with $SO_2$, the pollutant is absorbed by the pods and upon hydration at the cell surface forms sulfurous acid (Ziegler, 1975). The sulfite dissociation products are largely responsible for the biochemical injury, and biological mechanisms that promote sulfite oxidation would greatly detoxify the harmful agent. Sulfate is 30× less toxic to plants than sulfite (Thomas et al., 1943), and

if the soybean pod oxidizes sulfite, the proplastids may never be exposed to sulfite. It has become apparent from sulfur translocation experiments with $^{35}SO$ that sulfate, cysteine, and glutathione are the major constituents in the translocation stream. Therefore, proplastids are probably exposed to only very low levels of toxic sulfite ions, and any translocated sulfate is readily metabolized by the seed (Schiff and Hodson, 1973).

In summary, it appears that the pollutants $SO_2$ and $O_3$ have only negligible effects on soybean seed lipids and do not significantly alter the quality of the oil fraction.

ACKNOWLEDGMENT

We are indebted to J. E. Miller and L. W. Kress, of the Argonne National Laboratory, for supplying the seeds. The technical assistance of B. Kaplan is gratefully acknowledged.

**Registry No.** Ozone, 10028-15-6; sulfur dioxide, 7446-09-5.

LITERATURE CITED

Brooks, R. I.; Csallany, A. J. *J. Agric. Food Chem.* **1978**, *26*, 1203.
Constantinidou, H. A.; Kozlowski, T. T. *Can. J. Bot.* **1979**, *57*, 176.
Frey, J. E. Ph.D. Thesis, University of Northern Colorado, Greeley, CO, 1972.
Grunwald, C. *Plant Physiol.* **1981**, *68*, 868.
Gurr, M. I. In "Lipids: Structure and Function"; Stumpf, P. K., Ed.; Academic Press: New York, 1980; Vol. 4, pp 205–248.
Heath, R. L. *Annu. Rev. Plant Physiol.* **1980**, *31*, 395.
Howell, R. K.; Rose, L. P., Jr. *Plant Dis.* **1980**, *64*, 385.
Khan, A. A.; Malhotra, S. S. *Phytochemistry* **1977**, *16*, 539.
Kress, L. W.; Miller, J. E. *J. Environ. Qual.* **1983**, *12*, 276.
Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Malhotra, S. S.; Khan, A. A. *Phytochemistry* **1978**, *17*, 241.
Miller, J. E.; Smith, H. J.; Xerikos, P. B. Argonne, IL, 1981, Argonne National Laboratory Report ANL-80-115, Part III, p 15.
Morrison, W. R.; Smith, L. M. *J. Lipid Res.* **1964**, *5*, 600.
Mudd, J. B.; McManus, T. T.; Ongun, A. *Proc. Int. Clean Air Congr., 2nd, 1970* **1971**, 256.
Schiff, J. A.; Hodson, R. D. *Annu. Rev. Plant Physiol.* **1973**, *24*, 381.
Sheppard, A. J.; Iverson, J. L.; Weibrouch, J. L. In "Handbook of Lipid Research—Fatty Acids and Glycerides"; Kuksis, A., Ed.; Plenum Press: New York, 1978; pp 341–379.
Smith, A. K.; Circle, S. J. In "Soybeans: Chemistry and Technology"; Avi Publishing Co.: Westport, CT, 1972; pp 61–92.
Sprugel, D. G.; Miller, J. E.; Muller, R. N.; Smith, H. J.; Xerikos, P. B. *Phytopathology* **1980**, *70*, 1129.
Stumpf, P. K. In "The Biochemistry of Plants, Lipids: Structure and Function"; Stumpf, P. K., Ed.; Academic Press: New York, 1980; Vol. 4, pp 177–204.
Thomas, M. D.; Hendricks, R. H.; Colier, T. R.; Hill, G. R. *Plant Physiol.* **1943**, *18*, 435.
Ziegler, I. In "Residue Reviews"; Gunther, F. A.; Gunther, J. D., Eds.; Springer-Verlag: New York, 1975; pp 79–105.

Received for review June 13, 1983. Accepted September 15, 1983. This research was supported in part by a grant from the Illinois Department of Energy and Natural Resources Contract No. STIL-ENR10.102-39498.

# Measurement of Free Amino Acids in Avian Blood Serum by Reverse-Phase High-Performance Liquid Chromatography As Compared to Ion-Exchange Chromatography

Robert G. Elkin

Chick, turkey, and duck serum samples were analyzed for free amino acid content by a recently developed reverse-phase high-performance liquid chromatography (HPLC) method and by conventional ion-exchange chromatography using an amino acid analyzer. The former method employed precolumn derivatization with *o*-phthalaldehyde–ethanethiol and fluorescence detection. With the HPLC procedure, 17 amino acids were separated and quantitated with a total run time of 70 min (column regeneration time included), while the ion-exchange method required a run time of approximately 270 min. In the former procedure, glycine and threonine were not resolved and cyst(e)ine and proline were not detected. Within a species, the serum amino acid concentrations obtained by the two methods were very similar with the exception of asparagine, lysine, and tryptophan. Levels of the first two were consistently higher when analyzed by ion-exchange chromatography, while tryptophan values were consistently greater when analyzed by HPLC. These results suggest that free amino acid analyses of avian serum can be both accurately and reproducibly achieved by HPLC.

Among the numerous methods developed for the separation and detection of amino acids, ion-exchange chromatography (IEC) with ninhydrin derivatization has been the most popular (Tristram and Rattenbury, 1981). To conduct such analyses, the use of amino acid analyzers has been widely advocated. However, these very expensive instruments are usually dedicated to only one type of analysis and generally suffer form such disadvantages as long analysis times and moderate sensitivity (Fernstrom and Fernstrom, 1981; Umagat et al., 1982).

Thus, alternatives to IEC methods with amino acid analyzers have been emerging. High-performance liquid chromatography (HPLC), of which IEC is strictly just one type (Tristram and Rattenbury, 1981), has continued to gain popularity for the analysis of amino acids as their phenylthiohydantoin (PTH) (Zimmerman et al., 1977; Annan, 1981; Hawke et al., 1982; Black and Coon, 1982), 5-(dimethylamino)naphthalene-1-sulfonyl (dansyl) (Wilkinson, 1978; Schmidt et al., 1979), or *o*-phthalaldehyde (OPTA) (Hill et al., 1979; Gardner and Miller, 1980; Larsen and West, 1981; Fernstrom and Fernstrom, 1981; Umagat et al., 1982) derivatives. According to Hill et al. (1979) and

Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907.

0021-8561/84/1432-0053$01.50/0 