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.

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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.

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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

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Radjai and Hatch (1980), HPLC offers greater efficiency, ease of use, and higher flow rates than conventional IEC methods. However, one disadvantage of using HPLC with either PTH or dansyl derivatization is that these techniques apparently lack sufficient specificity to analyze amino acids in biological fluids (Hill et al., 1979). This is reflected in the extremely small number of papers that apply HPLC techniques specifically to the analysis of amino acids in physiological fluids. Several recent reports showed that precolumn derivatization with OPTA and ethanethiol (Hill et al., 1979, 1982; Fernstrom and Fernstrom, 1981) or mercaptoethanol (Hogan et al., 1982), followed by chromatography on reverse-phase columns, could successfully be employed to analyze primary amino acids in human and rat blood plasma or serum and human and canine cerebrospinal fluid in the picomolar range. OPTA–ethanethiol has been reported to form an isoindole fluorescent adduct with primary amines (Simons and Johnson, 1977).

The purpose of the work reported herein was to extend the applicability of the methods of Hill et al. (1979) and Fernstrom and Fernstrom (1981) to the analysis of free amino acids in avian blood serum where nearly half of the non-protein nitrogen is present as amino acids (Griminger, 1976). Since the effect of both the level of protein intake and the amino acid composition of the dietary protein on plasma and tissue free amino acid concentrations in birds has been of interest to many researchers (Featherston, 1972), a simple and rapid method for the analysis of free amino acids in physiological fluids would be anticipated to have widespread application. In order to assess the accuracy of the technique, HPLC data were compared to the results obtained by conventional IEC using an amino acid analyzer.

MATERIALS AND METHODS

Equipment. *HPLC.* The HPLC system (Waters Associates, Milford, MA) consisted of two pumps (one Model 45, one Model 6000), an automatic sample injector (Model 710 B), a microprocessor system controller (Model 720), a data integrator (Model 730), a fluorescence detector (Model 420), and a μBondapak C_{18} reverse-phase column (3.9 mm × 300 mm). The column temperature was maintained by using an electronic column heater (Model LC-22/23, Bioanalytical Systems, West Lafayette, IN). The Model 420 fluorescence detector was equipped with an 8-μL quartz flow cell, and the excitation and emission filters used were 338 and 425 nm, respectively. A gain setting of 16 was selected.

IEC. The conventional analyzer used was a Model 119 CL amino acid analyzer (Beckman Instruments, Palo Alto, CA) equipped with a Model 126 data reduction system. A cation-exchange resin (type W3-P), postcolumn derivatization with ninhydrin, and dual-channel absorbance detection (440 nm, 570 nm) were employed.

Reagents and Buffers. *HPLC* HPLC-grade acetonitrile and methanol were purchased from Burdick and Jackson Labortories (Muskegon, MI). An amino acid calibration standard (catalog no. 312220) was purchased from Beckman (Palo Alto, CA) while OPTA (Fluoropa, catalog no. 26010) and additional crystalline amino acids were obtained from Pierce (Rockford, IL). All other chemicals used were reagent grade.

Two buffers were employed. Buffer A consisted of 18 mM sodium phosphate, pH 7.2, while buffer B consisted of acetonitrile–buffer A (55:45) (Fernstrom and Fernstrom, 1981). Both buffers were filtered (0.45-μm pore) and degassed prior to use. Borate buffer, pH 9.5, was prepared according to Hill et al. (1979).

Table I. Reverse-Phase HPLC Procedure[a]

time, min	% buffer A (18 mM sodium phosphate, pH 7.2)	% buffer B (acetonitrile–buffer A, 55:45)	gradient
0	90	10	
50	40	60	linear
65	10	90	linear
70	90	10	linear

[a] The flow rate was 2 mL/min with the column temperature maintained at 28 °C.

Table II. Ion-Exchange Chromatography Procedure[a]

time, min	buffer
0–71	0.2 N lithium citrate, pH 2.83
72–155	0.2 N lithium citrate, pH 3.70
156–252	1.0 N lithium citrate, pH 3.75[b]
253–257	0.3 N lithium hydroxide (regeneration)
258–272	0.2 N lithium citrate, pH 2.83 (equilibration)

[a] The flow rate was 0.73 mL/min with a column temperature gradient from 40 to 65 °C beginning at 40 min. [b] Contained 6% 2-propanol.

IEC. Amino acid calibration standards (Hamilton Types P-B and P-AN, catalog no. 878290 and 878291) and lithium buffers were purchased from Beckman (Palo Alto, CA). Ninhydrin was obtained from Pierce (Rockford, IL). Ethylene glycol monomethyl ether was purchased from Fisher Scientific (Fair Lawn, NJ).

Sample and Standard Preparation. *HPLC.* Serum samples were diluted with 9 volumes of methanol, mixed, and centrifuged at 4 °C at 30000*g* for 15 min. The clear supernates were then decanted. An aliquot of the calibration standard solution was diluted with 99 volumes of methanol prior to use. Both serum and standard samples were derivatized according to the procedure of Hill et al. (1979) with the modification that 0.2 mL of borate buffer containing asparagine, glutamine, taurine, and tryptophan was added to the standard reaction flask at the expense of methanol, while 0.2 mL of borate buffer without amino acids replaced 0.2 mL of methanol in the serum reaction flasks.

IEC. Serum samples were mixed with cold 15% sulfosalicylic acid (1 mL of serum–0.25 mL of sulfosalicylic acid) and centrifuged as described above. A physiological calibration standard solution, containing 40 amino acids and related compounds, was diluted with 9 volumes of 0.2 N lithium citrate buffer, pH 2.2, prior to use.

Chromatographic Procedures. *HPLC.* The method of Fernstrom and Fernstrom (1981) was used with the exception that buffer A contained 18 mM, rather than 15 mM, sodium phosphate. The procedure is summarized in Table I. Forty-microliter aliquots of derivatized serum and standard solutions were injected onto the column.

IEC. The method described in the Beckman 118/119 CL Application Notes was employed and is shown in Table II. One hundred microliter aliquots of deproteinized serum or diluted standard solutions were injected onto the column.

Animals. Four individual serum samples were obtained according to de Andrade et al. (1977) from each of the following groups of birds: 3-week-old male Hubbard chicks, 12-week-old female Nicholas turkeys, and 24-week-old male White Pekin ducks. The sera were stored at −20 °C until subsequent use. Triplicate analyses of the four individual serum samples from each species were performed.

Statistics. Student's *t* tests were performed to compare HPLC and IEC data (Steel and Torrie, 1980).

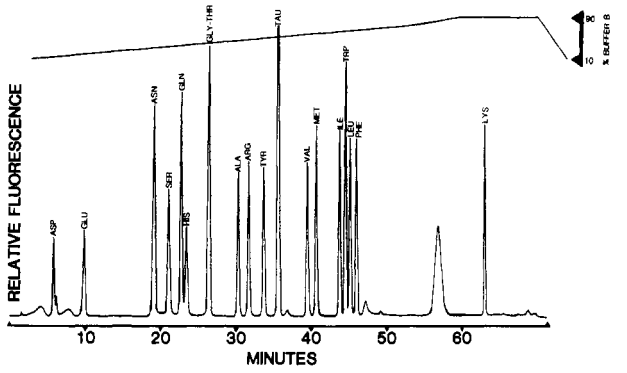

Figure 1. Reverse-phase HPLC elution profile of amino acid standards derivatized by reaction with OPTA and ethanethiol. Each peak represents 200 pmol except for Asn (369 pmol), Gln (322 pmol), Tau (352 pmol), and Trp (335 pmol). A gradient trace appears at the top of the chromatogram.

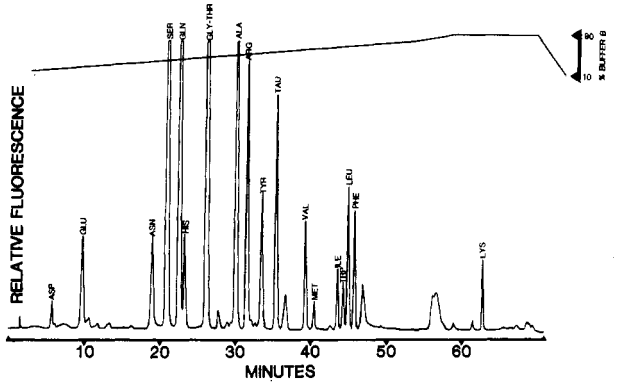

Figure 2. Reverse-phase HPLC elution profile of a representative chick serum sample derivatized by reaction with OPTA and ethanethiol. The amount of serum analyzed per injection was 0.8 μL.

RESULTS AND DISCUSSION

A representative reverse-phase HPLC standard chromatogram is shown in Figure 1. Seventeen amino acids were individually resolved with glycine and threonine eluting as a single peak. Proline and cyst(e)ine were not detected. OPTA does not react with secondary amines such as proline (Roth, 1971; Lee and Drescher, 1978), while cyst(e)ine, for some unexplained reason, does not form a fluorescent derivative with OPTA (Roth, 1971; Larsen and West, 1981). Nevertheless, both cyst(e)ine and proline can be oxidized to yield highly fluorescent adducts with OPTA (Lee and Drescher, 1978). The lack of resolution of glycine and threonine can reportedly be overcome by employing a tetrahydrofuran-containing buffer (Fernstrom and Fernstrom, 1981; Hill et al., 1982). The elution order obtained was identical with that of Fernstrom and Fernstrom (1981) and Hill et al. (1982). Confirmation of peak identities was obtained by individually adding each amino acid, one at a time, to the calibration standard solution. Although Hill et al. (1979) observed a peak for ammonia, an attempt in this laboratory to derivatize ammonia was unsuccessful. Larsen and West (1981) reported a similar difficulty. Fernstrom and Fernstrom (1981) did not address this issue.

A reverse-phase HPLC elution profile of a representative chick serum sample appears in Figure 2. A consistent and low base line was observed, and all compounds identified in the standard chromatogram were observed in the serum sample. A comparison of the mean amino acid concentrations for the four chick samples as determined by reverse-phase HPLC and IEC showed that, for most amino acids, the absolute values obtained were similar for the two methods (Table III). The notable exception was asparagine. A probable explanation for this is that asparagine quantitation by the IEC method employed was extremely difficult because the peak was both small and poorly resolved. Although it was originally felt that integrator error may have been the cause for this discrepancy (Griffith, 1982), manual calculations did not support this suggestion. Albeit there were significant differences for taurine, methionine, tryptophan, and lysine, the numerical differences were small and only the latter two were consistently different for each species, with tryptophan values being greater when analyzed by HPLC and the opposite being true for lysine. Fernstrom and Fernstrom (1981) also observed lower lysine concentrations with HPLC as compared to IEC. Although they eliminated integrator error and incomplete derivatization with OPTA–ethanethiol as possible causes for this discrepancy, they were unable to account for this difference. A possible explanation for the higher tryptophan values as determined by HPLC is that, during the analysis, some tryptophan may have been destroyed by the rather acidic buffers used in IEC.

A reverse-phase HPLC elution profile of a representative turkey serum sample appears in Figure 3. Not unexpectedly, it was similar to the chick serum chromatogram, with a low and consistent base line and 17 amino acids resolved. With regard to the circulating amino acid levels,

Table III. Chick Serum Amino Acid Concentrations: Reverse-Phase HPLC and Ion-Exchange Chromatography (IEC) Comparison

amino acid	HPLC[a]	IEC[a]
Asp	63 ± 14	86 ± 34
Glu	197 ± 25	220 ± 52
Asn[b]	197 ± 43	644 ± 138
Ser	855 ± 156	963 ± 151
Gln	1094 ± 76	1015 ± 112
His	200 ± 28	192 ± 24
Ala	685 ± 85	729 ± 80
Arg	469 ± 73	507 ± 74
Tyr	262 ± 27	241 ± 23
Tau[b]	310 ± 20	345 ± 33
Val	220 ± 33	236 ± 43
Met[b]	62 ± 13	40 ± 10
Ile	102 ± 17	105 ± 17
Trp[b]	107 ± 12	65 ± 7
Leu	256 ± 54	260 ± 48
Phe	169 ± 24	169 ± 21
Lys[b]	74 ± 16	112 ± 28

[a] Values are presented as means ± SD in nmol/mL for triplicate analyses of four individual samples (thus $n = 12$). [b] $P \leq 0.05$ for HPLC vs. IEC.

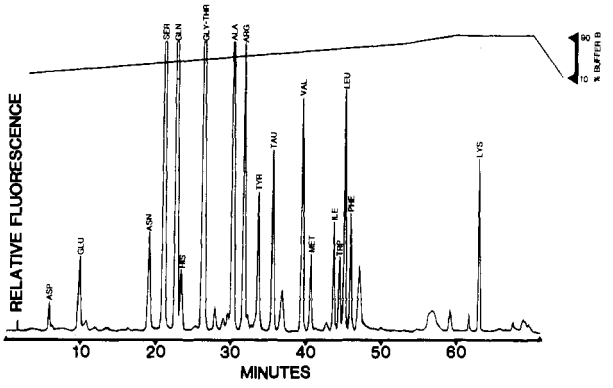

Figure 3. Reverse-phase HPLC elution profile of a representative turkey serum sample derivatized by reaction with OPTA and ethanethiol. The amount of serum analyzed per injection was 0.8 μL.

Table IV. Turkey Serum Amino Acid Concentrations: Reverse-Phase HPLC and Ion-Exchange Chromatography (IEC) Comparison

amino acid	HPLC[a]	IEC[a]
Asp	58 ± 11	59 ± 33
Glu[b]	165 ± 7	190 ± 25
Asn[b]	129 ± 56	440 ± 172
Ser	668 ± 161	760 ± 158
Gln	1119 ± 166	1075 ± 156
His	122 ± 29	123 ± 19
Ala	603 ± 129	649 ± 156
Arg	322 ± 91	356 ± 93
Tyr	267 ± 25	278 ± 42
Tau	171 ± 42	165 ± 44
Val	286 ± 68	299 ± 74
Met	91 ± 24	78 ± 8
Ile	112 ± 26	114 ± 28
Trp[b]	107 ± 20	82 ± 18
Leu	266 ± 58	283 ± 60
Phe	134 ± 21	138 ± 24
Lys[b]	155 ± 42	210 ± 51

[a] Values are presented as means ± SD in nmol/mL for triplicate analyses of four individual samples (thus $n = 12$). [b] $P \leq 0.05$ for HPLC vs. IEC.

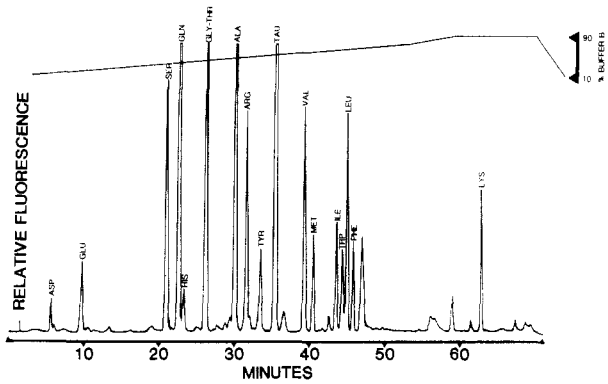

Figure 4. Reverse-phase HPLC elution profile of a representative duck serum sample derivatized by reaction with OPTA and ethanethiol. The amount of serum analyzed per injection was 0.8 μL.

both methods were comparable when averaged over the four individual samples, except for glutamic acid, asparagine, tryptophan, and lysine concentrations being significantly different (Table IV). Taurine and methionine levels were not different in this instance as compared to the chick data.

A reverse-phase HPLC elution profile of a representative duck serum sample appears in Figure 4. Two differences from the previous two species were the apparent lack of asparagine and the large amount of taurine present. In considering circulating amino acid levels, once again excellent agreement between the two methods was observed with only serine, tryptophan, and lysine concentrations being significantly different (Table V).

Overall, the reproducibility of each method was very similar across the four individual samples within a species as evidenced by similar standard deviations. Although not shown, for both methods the within assay coefficients of variation (Steel and Torrie, 1980) for each amino acid within individual serum sample analyses tended to be low, that is, less than 5%.

Taken together, these results demonstrate a simple, rapid, and accurate reverse-phase HPLC procedure for determining picomolar amounts of amino acids in avian serum. However, one potential problem with the method is that, within hours, a significant loss of fluorescence occurs for some amino acids. This is illustrated in Table

Table V. Duck Serum Amino Acid Concentrations: Reverse-Phase HPLC and Ion-Exchange Chromatography (IEC) Comparison

amino acid	HPLC[a]	IEC[a]
Asp	94 ± 29	109 ± 44
Glu	194 ± 28	213 ± 24
Asn	18 ± 5	97 ± 77
Ser[b]	559 ± 63	660 ± 92
Gln	1062 ± 107	1011 ± 95
His	118 ± 27	117 ± 30
Ala	1047 ± 124	1142 ± 174
Arg	356 ± 50	410 ± 76
Tyr	191 ± 54	186 ± 67
Tau	494 ± 266	552 ± 309
Val	366 ± 56	382 ± 83
Met	143 ± 19	131 ± 16
Ile	145 ± 20	151 ± 27
Trp[b]	149 ± 35	84 ± 14
Leu	304 ± 45	318 ± 55
Phe	141 ± 18	144 ± 25
Lys[b]	297 ± 78	433 ± 106

[a] Values are presented as means ± SD in nmol/mL for triplicate analyses of four individual samples (thus $n = 12$). [b] $P \leq 0.05$ for HPLC vs. IEC.

Table VI. Calibration Standard Amino Acid Fluorescence Changes during an 11-h Period

amino acid	% change
Lys	−24
His	−24
Ser	−21
Tau	−21
Ala	−9
Gln	−7
Glu	−7
Asn	−7
Arg	−7
Tyr	−6
Asp	−6
Val	−6
Ile	−5
Leu	−4
Phe	−4
Met	−2
Trp	+6

VI where, over an 11-h period, large fluorescence losses occurred for lysine, histidine, serine, and taurine. For some unknown reason, tryptophan fluorescence actually increased slightly. Fernstrom and Fernstrom (1981) also noted fluorescence losses occurring over a 10-h period, particularly for serine, histidine, and lysine. The loss of OPTA adduct fluorescence has been attributed to the decomposition of the derivative (Simons and Johnson, 1977).

Rather than preparing and running samples in groups of three to four to avoid fluorescence losses as recommended by Fernstrom and Fernstrom (1981), and alternate procedure is suggested. The problem of fluorescence loss can be overcome simply by running a calibration standard solution, which had been derivatized at the same time as the samples, immediately following each group of three serum samples. In this manner, 12–15 samples could be prepared at once and chromatographed over a 20-h period. In comparison, only four physiological sample analyses could be performed by standard IEC in the same amount of time.

The results of the present study both confirm the method of Fernstrom and Fernstrom (1981) and extend its applicability to the analysis of free amino acids in avian blood serum. According to Lee and Drescher (1979), HPLC with OPTA is probably the most sensitive liquid

chromatographic method yet devised for studies of amino acid composition of proteins and peptides. In all likelihood, HPLC techniques will continue to gain popularity for the routine analysis of amino acids.

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Registry No. Asp, 56-84-8; Glu, 56-86-0; Asn, 70-47-3; Ser, 56-45-1; Gln, 56-85-9; His, 71-00-1; Ala, 56-41-7; Arg, 74-79-3; Tyr, 60-18-4; Tau, 107-35-7; Val, 72-18-4; Met, 63-68-3; Ile, 73-32-5; Trp, 73-22-3; Leu, 61-90-5; Phe, 63-91-2; Lys, 56-87-1.

LITERATURE CITED

Annan, W. D. "Amino Acid Analysis"; Wiley: New York, 1981; pp 66–70.
Black, S. D.; Coon, M. J. *Anal. Biochem.* **1982**, *121*, 281–285.
de Andrade, A. N.; Rogler, J. C.; Featherston, W. R.; Alliston, C. W. *Poult. Sci.* **1977**, *56*, 1178–1188.
Featherston, W. R. *Poult. Sci.* **1972**, *51*, 17–27.
Fernstrom, M. H.; Fernstrom, J. D. *Life Sci.* **1981**, *29*, 2119–2130.
Gardner, W. S.; Miller, W. H. *Anal. Biochem.* **1980**, *101*, 61–65.
Griffith, J. E., Purdue University, West Lafayette, IN, personal communication, 1982.
Griminger, P. "Avian Physiology"; Springer-Verlag: New York, 1976; pp 233–251.
Hawke, D.; Yuan, P.-M.; Shively, J. E. *Anal. Biochem.* **1982**, *120*, 302–311.
Hill, D.; Burnworth, L.; Skea, W.; Pfeifer, R. *J. Liq. Chromatogr.* **1982**, *5*, 2369–2393.
Hill, D. W.; Walters, F. H.; Wilson, T. D.; Stuart, J. D. *Anal. Chem.* **1979**, *51*, 1338–1341.
Hogan, D. L.; Kraemer, K. L.; Isenberg, J. I. *Anal. Biochem.* **1982**, *127*, 17–24.
Larsen, B. R.; West, F. G. *J. Chromatogr. Sci.* **1981**, *19*, 259–265.
Lee, K. S.; Drescher, D. G. *Int. J. Biochem.* **1978**, *9*, 457–467.
Lee, K. S.; Drescher, D. G. *J. Biol. Chem.* **1979**, *254*, 6248–6251.
Radjai, M. K.; Hatch, R. T. *J. Chromatogr.* **1980**, *196*, 319–322.
Roth, M. *Anal. Chem.* **1971**, *43*, 880–882.
Schmidt, G. J.; Olson, D. C.; Slavin, W. *J. Liq. Chromatogr.* **1979**, *2*, 1031–1045.
Simons, S. S.; Johnson, D. F. *Anal. Biochem.* **1977**, *82*, 250–254.
Steel, R. G. D.; Torrie, J. H. "Principles and Procedures of Statistics"; McGraw-Hill: New York, 1980; pp 86–121.
Tristram, G. R.; Rattenbury, J. M. "Amino Acid Analysis"; Wiley: New York, 1981; pp 16–36.
Umagat, H.; Kucera, P.; Wen, L.-F. *J. Chromatogr.* **1982**, *239*, 463–474.
Wilkinson, J. M. *J. Chromatogr. Sci.* **1978**, *16*, 547–552.
Zimmerman, C. L.; Appella, E.; Pisano, J. J. *Anal. Biochem.* **1977**, *77*, 569–573.

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Characterization of Mango-like Aroma in *Curcuma amada* Roxb.

Achyut S. Gholap and Chiranjib Bandyopadhyay*

The essential oil of the rhizomes of *Curcuma amada* Roxb. was isolated by steam distillation, distillation–extraction, and low-temperature–high-vacuum distillation techniques, and its composition with respect to mango aromatic principles was determined by gas chromatography and spectrometry. The oil was primarily composed of terpene hydrocarbons tentatively identified as α-pinene, car-3-ene, and *cis*-ocimene, where the latter two compounds contributed essentially the characteristic mango odor of this rhizome.

Curcuma amada Roxb. is a rhizome cultivated mostly in India and Malaysia, where it is known as *Curcuma mangga valet* and having vegetative characteristics similar to those of *Curcuma longa*. The rhizomes of *C. amada* are called "Amada" in Bengali because of the characteristics odor of mangoes, superimposed over the mild turmeric and ginger odor, and hence they are popularly called as "mango ginger". Besides the medicinal use of this rhizome as a healing agent for sprains and bruises, it finds an extensive application in the preparation of sweetmeats, chutneys, and pickles because of its exotic mango aroma. However, little attention has been given to studying the volatile aroma components responsible for its characteristic mango aroma. Dutt and Tayal (1941) initially examined the chemical composition of steam volatile oil of mango ginger and reported ocimene as the major constituent besides linalool, linalyl acetate, and safrol. Ahuja and Nigam (1971), on the other hand, reported curcumene as the main component of the essential oil of this rhizome and failed to detect the above constituents. Recently Govindarajan (1980) reviewed the chemistry and technology of this rhizome along with other species and indicated that terpene compounds are presumably essential components of the steam volatile oil of *C. amada*. It appears that the mango aromatic principle of this rhizome has so far not been convincingly demonstrated. The purpose of the present paper was to reevaluate the essential oil composition of *C. amada* with a view to characterize the components contributing to the mango aroma of the rhizome.

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EXPERIMENTAL SECTION

Isolation of Essential Oil. Freshly harvested rhizomes of *C. amada* procured from a local market were cut into small pieces and blended with distilled water (1:2 w/v) in a Waring blender for 2 min. The pulp (200 g each) was subjected separately to conventional steam distillation, simultaneously distillation–extraction using isopentane as the extracting solvent according to the method of Nickerson and Likens (1966), and low-temperature–high-vacuum distillation techniques as described by Bandyopadhyay et al. (1973) for the isolation of the essential oil. The distillate obtained by conventional and vacuum distillation was extracted with peroxide-free diethyl ether (3 times, 200 mL each). The solvent extract was dried over sodium sulfate and the oil was recovered after removal of solvent in a flash evaporator at room temperature.

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