Amino Acid Analysis of Feedstuff Hydrolysates by Precolumn Derivatization with Phenylisothiocyanate and Reversed-Phase High-Performance Liquid Chromatography[1]

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Cereal Chem. 64(4):226–229

ABSTRACT

Corn, peanut meal, two sorghum varieties, and a corn-soybean meal-based complete mixed feed were either untreated or oxidized with performic acid before hydrolysis with hydrochloric acid. Hydrolysate amino acids were derivatized with phenylisothiocyanate (PITC), and the resulting phenylthiocarbamyl (PTC) derivatives were separated and quantitated by reversed-phase high-performance liquid chromatography (HPLC). Aliquots of each hydrolysate were also analyzed for amino acids using cation-exchange chromatography (CXC) and postcolumn derivatization with ninhydrin. In general, amino acid values determined by reversed-phase HPLC compared favorably with those obtained by CXC. PITC reacts with both primary and secondary amino acids, and the PTC-amino acid derivatives formed are stable enough for automated analysis without on-line derivatization. In addition, noncorrosive buffers were used to elute the PTC-amino acids from the reversed-phase column, and a complete separation of 17 hydrolysate amino acids plus methionine dioxide and cysteic acid was achieved in 25 min (injection-to-injection time). A PITC-based amino acid analytical system composed of modular HPLC equipment provides a viable alternative to amino acid analyzers at approximately one-half of the cost, is generally easy to operate and maintain, and is readily adaptable to the analysis of compounds other than amino acids.

The scientific feeding of nonruminant animals involves utilizing feedstuffs in combinations that provide adequate dietary levels of available nitrogen and meet the animal's indispensable amino acid requirements. Determination of the amino acid content of feedstuffs has classically been accomplished by hydrochloric acid (HCl) hydrolysis followed by cation-exchange chromatography (CXC) with postcolumn ninhydrin derivatization using an amino acid analyzer (AAA).

The first automated AAA was developed by Spackman and co-workers (1958), and, although refinements in columns and instruments have occurred since, their CXC procedure has remained essentially unchanged (Heinrikson and Meredith 1984). Today's AAA are complex, expensive, and dedicated to performing only amino acid analyses.

Alternate approaches for the analysis of amino acids in feedstuff hydrolysates include gas-liquid chromatography (Moodie et al 1982, Gehrke et al 1987) and reversed-phase high-performance liquid chromatography (HPLC) (Kan and Shipe 1981, Khayat et al 1982, Elkin 1984, Krishnamurti et al 1984). Both methods require that sample amino acids be chemically derivatized prior to injection and, regardless of the derivative employed, these techniques have generally not achieved the precision and accuracy of CXC, the standard by which any alternative procedure must be judged (Heinrikson and Meredith 1984).

Several amino acids present various difficulties during their derivatization and subsequent determination by gas-liquid chromatography (Moodie et al 1985), whereas reversed-phase HPLC precolumn derivatization techniques have suffered from one or more of the following drawbacks: poor derivative stability, less than quantitative reaction yields, interference from reagent peaks, and a lack of reactivity with secondary amino acids (Cohen et al 1986).

Successful amino acid analyses of feedstuff hydrolysates have been achieved by CXC and postcolumn derivatization with ninhydrin (Elkin and Griffith 1985b) or orthophthalaldehyde (Ashworth 1987) using modular HPLC equipment. However, in both instances, corrosion damage to the instruments by citrate or halide buffers brought about a search for an alternative methodology.

Although phenylisothiocyanate (PITC) has been used for over 30 years as the primary reagent in the Edman degradation method for sequencing proteins, it has only recently been utilized as a precolumn derivatization reagent for the amino acid analysis of purified proteins (Koop et al 1982, Tarr et al 1983, Bidlingmeyer et al 1984, Granberg 1984, Heinrikson and Meredith 1984, Scholze 1985, Yang and Sepulveda 1985, Ebert 1986, Shoji et al 1986). The various derivatization procedures employed are rapid, efficient, sensitive, and applicable to both primary and secondary amino acids. In addition, the phenylthiocarbamyl (PTC)-amino acids formed are sufficiently stable to eliminate any need for on-line derivatization (Heinrikson and Meredith 1984).

This report describes the application of reversed-phase HPLC with precolumn PITC derivatization for the analysis of amino acids in feedstuff hydrolysates. The accuracy of the technique was assessed by comparing reversed-phase HPLC data to results obtained either by conventional CXC using an AAA or high-performance CXC using modular HPLC equipment. Because of the lability of cystine (Cys) (and possibly methionine [Met]) during acid hydrolysis, feedstuffs were oxidized with performic acid before refluxing with 6*N* HCl. Unoxidized hydrolysates were also prepared.

MATERIALS AND METHODS

Reagents and Apparatus

Reversed-phase HPLC system. The following Waters Chromatography Division, Millipore Corporation (Milford, MA) equipment was employed: two model 6000A solvent delivery systems, 2 μm in-line precolumn filter, model 710B sample injector, 3.9 mm × 15 cm Pico-Tag column, model 720 system controller, model 730 data module, model 440 absorbance detector with a 254-nm filter and aperture plate, and an electronic column heater maintained at 33° C. Samples were prepared using a Pico-Tag workstation and a model 1376 Duo-Seal vacuum pump (Sargent-Welch, Skokie, IL).

The Pico-Tag column is a C-18 column selected and tested for PTC-amino acid analysis. Other brands of C-18 columns may be used but conditions may have to be modified. The Pico-Tag workstation, which has a vacuum/nitrogen manifold for sample drying, reagent removal, and vacuum sealing was described in detail by Bidlingmeyer et al (1984).

CXC systems. Descriptions of the equipment and methodology employed for both high-performance CXC and conventional CXC using a Beckman (Palo Alto, CA) model 119 CL AAA appear in a previous report (Elkin and Griffith 1985b).

Chemicals. HPLC grade acetonitrile and methanol were purchased from Burdick and Jackson (Muskegon, MI). Sodium acetate trihydrate (HPLC grade) and reagent grade glacial acetic acid were obtained from Fisher Scientific (Fair Lawn, NJ). Triethylamine (TEA), PITC, and amino acid standard H were

[1]Journal Paper no. 10,836, Purdue University Agricultural Experiment Station.
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purchased from Pierce (Rockford, IL). All other chemicals used were reagent grade. Water was supplied by a Milli-R/Q purification system (Millipore, Bedford, MA) that was fed with a supply of in-house deionized water. The Milli-R/Q water purifier produces CAP/ASTM type II water with 2–3 megohm-cm resistivity at 25° C.

Standard Preparation

Fifty microliters of amino acid standard H (Pierce) were mixed with 50 μl of 0.1 *N* HCl containing 2.5 μmol/ml each of cysteic acid (Cya), methionine dioxide (Met-O_2) and aminohexanoic acid (Ahx). The calibration standard solution thus contained 0.625 μmol/ml of L-Cys and 1.25 μmol/ml of each of the following: L-Ahx, L-alanine, L-arginine, L-aspartic acid, L-Cya, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-Met, L-Met-O_2, NH_3, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine.

Sample Preparation

Corn, peanut meal, sorghum RS610 (a low tannin variety), sorghum BR64 (a high tannin variety), and a complete mixed feed (a pelleted corn-soybean meal-based duck starter ration) were ground and dried as previously described (Elkin and Griffith 1985a). All samples were assayed for crude protein (AOAC 1984), but only the sorghum samples were defatted prior to hydrolysis. Dried feedstuff samples containing approximately 10 mg of nitrogen each (corn, 800 mg; peanut meal, 125 mg; sorghum RS610, 800 mg; sorghum BR64, 800 mg; and complete mixed feed, 300 mg) were oxidized with performic acid and hydrolyzed in 6*N* HCl as described elsewhere (Elkin and Griffith 1985a). Additional dried feedstuff samples were hydrolyzed in 6*N* HCl without oxidative pretreatment (Elkin and Griffith 1985a). All feedstuff hydrolysates were originally prepared for analysis by CXC and were made up in 0.2*N* sodium citrate buffer, pH 2.2, and contained L-Ahx.

Derivatization Procedure

Samples were derivatized based on the procedure of Bidlingmeyer et al (1984) with modifications. Ten microliters of calibration standard solution, 25 μl of water (blank), or 25 μl of each feedstuff hydrolysate solution were pipetted into individual 6 × 50 mm sample tubes and dried in vacuo. Fifty microliters of a redrying solution containing methanol, water, and TEA (2:2:1, v/v) was added to each tube, mixed, and dried in vacuo. A second 50-μl aliquot of the above solution was added to each tube, mixed, and dried in vacuo. Next, 50-μl of derivatizing reagent containing methanol, TEA, water, and PITC (7:1:1:1, v/v) was added to each tube, mixed, and incubated at room temperature for 20 min. Samples were then dried in vacuo for approximately 10 min, and 50 μl of methanol was then added to each tube. The methanol was dried in vacuo, and a second 50-μl aliquot of methanol was added to each tube and subsequently dried in vacuo for approximately 60 min. Following this, sample residues were dissolved in 200 μl of a solution containing 5 m*M* disodium hydrogen phosphate (pH 7.4) and acetonitrile (95:5, v/v). The total time required for sample derivatization was approximately 4 hr.

TABLE I
Gradient Program Employed for the Separation of Phenylthiocarbamyl-Amino Acids[a]

Time[b] (min)	Flow Rate (ml/min)	% Buffer A (0.14*M* sodium acetate with 0.7 ml/L TEA,[c] pH 5.70)	% Buffer B (60% acetonitrile in water)	Gradient Curve[d]
0	1.0	90	10	
5.0	1.0	70	30	6
13.0	1.0	52	48	6
13.5	1.0	0	100	6
16.0	1.5	0	100	11
16.9	1.5	0	100	6
17.0	1.5	90	10	6
24.5	1.5	90	10	6
25.0	1.0	90	10	6

[a] Column temperature maintained at 33° C.
[b] Run time was 16 min plus 9 min column regeneration time. Therefore, injection-to-injection time was 25 min.
[c] TEA = Triethylamine.
[d] Curve 6 is linear; curve 11 is a concave gradient hold.

Chromatographic Procedures

Reversed-phase HPLC. Eight-microliter aliquots of standard and feedstuff hydrolysates were injected and chromatographed (injection-to-injection time of 25 min) according to the procedure shown in Table I. Eight microliters of the calibration standard solution contained 500 pmol of each amino acid except Cys (250 pmol). The actual amounts of corn, peanut meal, sorghum RS610, sorghum BR64, and complete mixed feed analyzed per 8-μl injection were 11.75, 1.70, 11.44, 11.77, and 4.20 μg, respectively.

Solvent A consisted of 0.14*M* sodium acetate containing 0.7 ml/L of TEA and was titrated to pH 5.70 with glacial acetic acid. Solvent B consisted of acetonitrile and water (60:40, v/v). Eluants were filtered, degassed, and kept under room atmosphere.

CXC. The chromatographic procedures for high-performance CXC and conventional CXC using an AAA have been reported previously (Elkin and Griffith 1985b).

RESULTS AND DISCUSSION

A representative PTC-amino acid calibration standard chromatogram appears in Figure 1. Each peak represents 500 pmol except for Cys (250 pmol). Modification of the gradient program reported by Bidlingmeyer et al (1984) was necessary in order to completely resolve Met-O_2 from proline. Ammonia, which is present in feedstuff hydrolysates in considerable amounts and readily reacts with ninhydrin (Elkin and Griffith 1985a,b), did not yield a detectable peak because it was presumably displaced from the sample by TEA during the redrying step of the derivatization procedure.

Representative chromatograms of unoxidized and oxidized hydrolysates of a complete mixed feed appear in Figures 2 and 3, respectively. Because the feedstuff hydrolysates were originally prepared for and run by CXC, the large peak eluting in the column void volume was presumably sodium citrate, a component of the

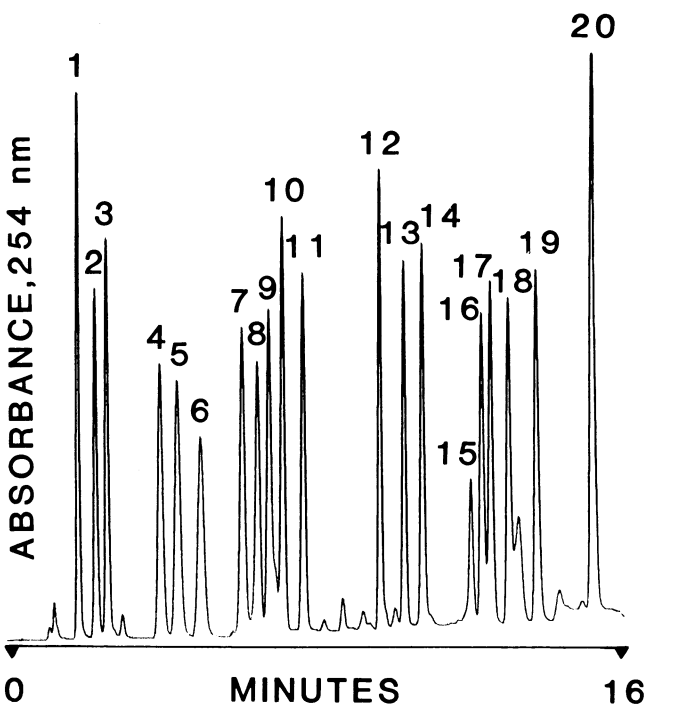

Fig. 1. Calibration standard chromatogram of phenylthiocarbamyl-amino acids. Each peak represents 500 pmol (Cys, 250 pmol). Peaks: 1 = Cya; 2 = Asp; 3 = Glu; 4 = Ser; 5 = Gly; 6 = His; 7 = Arg; 8 = Thr; 9 = Ala; 10 = Pro; 11 = Met-O_2; 12 = Tyr; 13 = Val; 14 = Met; 15 = Cys; 16 = Ile; 17 = Leu; 18 = Ahx; 19 = Phe; 20 = Lys. Absorbance: 254 nm, 0.1 AUFS.

sample diluent solution.

Although the presence of salts during sample derivatization can reduce the yield of PTC-aspartic acid (Ebert 1986) and possibly other acidic and neutral amino acids, the values determined by reversed-phase HPLC compared favorably with those obtained by CXC (Table II). Ahx, which served as an internal standard in the CXC analyses, partially coeluted with a reagent peak and was not used as an internal standard in the PTC-amino acid analyses. Thus, the reversed-phase HPLC values were not compensated for small variations in sample preparation, injection volume, and/or chromatography. The mean coefficients of variation for all amino acids averaged across all feedstuffs were 3.33 and 1.26% for reversed-phase HPLC and CXC data, respectively. Use of an acceptable internal standard would be expected to increase reproducibility of the PITC-based method.

Much of the variability of the PTC-amino acid analyses probably resulted from the absence of an internal standard, the presence of citrate in the hydrolysates, and the use of different amino acid calibration standard solutions for each method (citrate-based solution for CXC analyses versus 0.1*N* HCl for PTC-amino acid analyses). In addition, the hydrolysates were originally prepared for and assayed by CXC and were then stored frozen (−20°C) for 6 to 12 months before analysis by precolumn PITC derivatization and reversed-phase HPLC. Nevertheless, it would be reasonable to conclude that the two methodologies employed in the present study agreed with each other as well as one might expect conventional CXC amino acid analyses from different laboratories to concur (Elkin and Griffith 1985a). Furthermore, on a practical basis, absolute differences between

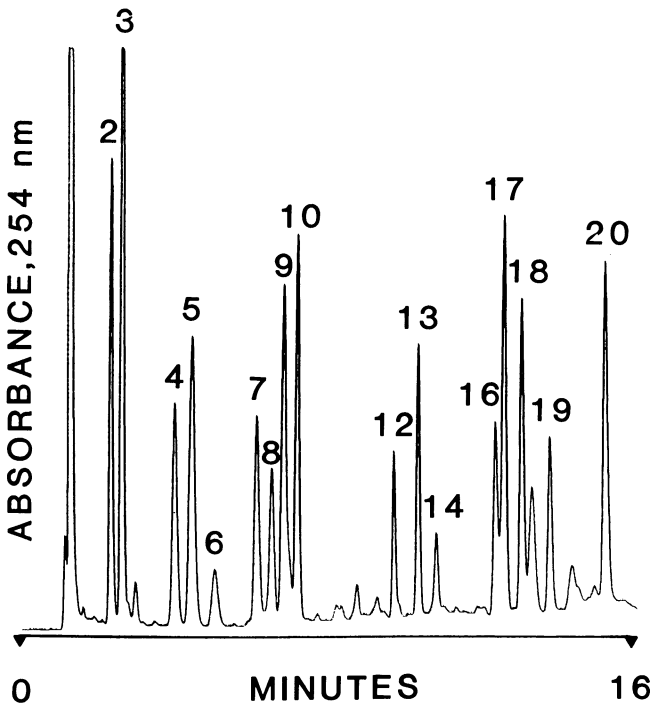

Fig. 2. Chromatogram of a complete mixed feed hydrolysate not subjected to oxidative pretreatment.

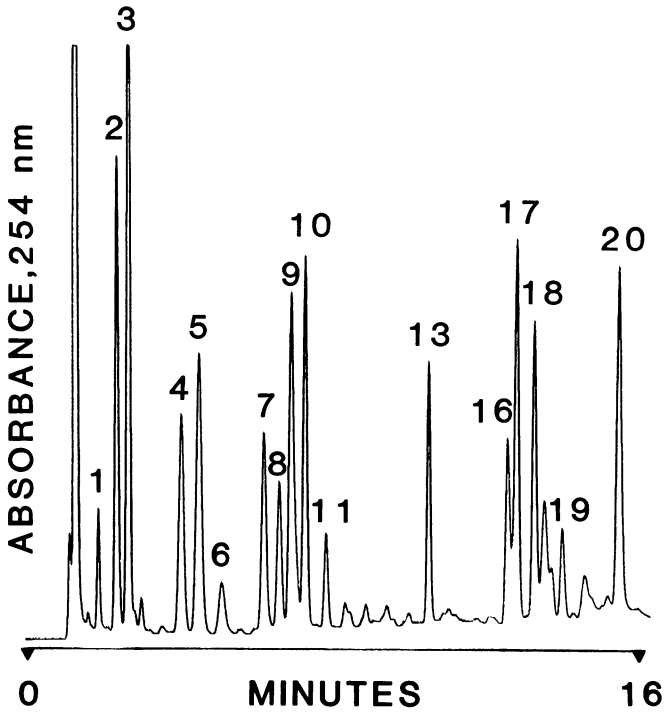

Fig. 3. Chromatogram of a complete mixed feed hydrolysate subjected to oxidative pretreatment.

TABLE II
Amino Acid Composition of Corn, Peanut Meal, a Complete Mixed Feed, and Two Sorghum Varieties as Determined by Reversed-Phase HPLC (RPLC) of Phenylthiocarbamyl Derivatives and Cation-Exchange Chromatography (CXC) with Postcolumn Ninhydrin Derivatization

Amino Acid[a]	Corn[b]		Peanut Meal[b]		Complete Mixed Feed[b]		Sorghum RS610[b]		Sorghum BR64[b]	
	RPLC[c]	CXC[d]	RPLC[c]	CXC[d]	RPLC[c]	CXC[d]	RPLC[c]	CXC[d]	RPLC[c]	CXC[d]
Ala	0.526 ± 0.044	0.575 ± 0.004	2.041 ± 0.133	2.002 ± 0.061	1.089 ± 0.077	1.145 ± 0.003	0.910 ± 0.075	1.000 ± 0.001	0.725 ± 0.042	0.762 ± 0.002
Arg	0.384 ± 0.011	0.393 ± 0.004	6.190 ± 0.197	6.518 ± 0.168	1.399 ± 0.035	1.515 ± 0.028	0.409 ± 0.012	0.412 ± 0.001	0.359 ± 0.026	0.377 ± 0.000
Asx	0.489 ± 0.019	0.510 ± 0.011	5.956 ± 0.218	5.854 ± 0.264	2.106 ± 0.060	2.170 ± 0.013	0.670 ± 0.015	0.714 ± 0.002	0.562 ± 0.015	0.561 ± 0.001
Cys[e]	0.153 ± 0.005	0.167 ± 0.004	0.549 ± 0.021	0.692 ± 0.023	0.303 ± 0.006	0.340 ± 0.006	0.153 ± 0.003	0.165 ± 0.004	0.158 ± 0.005	0.163 ± 0.001
Glx	1.324 ± 0.041	1.399 ± 0.030	9.055 ± 0.402	9.646 ± 0.496	3.574 ± 0.102	3.921 ± 0.044	2.073 ± 0.056	2.221 ± 0.003	1.657 ± 0.055	1.709 ± 0.001
Gly	0.288 ± 0.007	0.307 ± 0.005	2.831 ± 0.096	3.002 ± 0.045	1.004 ± 0.019	1.078 ± 0.003	0.321 ± 0.008	0.333 ± 0.001	0.288 ± 0.007	0.293 ± 0.000
His	0.192 ± 0.004	0.242 ± 0.006	1.049 ± 0.045	1.208 ± 0.002	0.497 ± 0.009	0.607 ± 0.007	0.211 ± 0.008	0.243 ± 0.000	0.198 ± 0.007	0.234 ± 0.000
Ile	0.288 ± 0.013	0.274 ± 0.005	1.732 ± 0.059	1.705 ± 0.040	0.925 ± 0.032	0.911 ± 0.008	0.431 ± 0.016	0.401 ± 0.001	0.357 ± 0.010	0.338 ± 0.000
Leu	0.931 ± 0.032	0.920 ± 0.016	3.218 ± 0.109	3.340 ± 0.028	1.783 ± 0.071	1.835 ± 0.008	1.379 ± 0.043	1.508 ± 0.000	1.101 ± 0.029	1.141 ± 0.001
Lys	0.201 ± 0.004	0.225 ± 0.006	1.621 ± 0.039	1.683 ± 0.057	1.009 ± 0.017	1.115 ± 0.006	0.174 ± 0.005	0.206 ± 0.000	0.164 ± 0.004	0.188 ± 0.000
Met[e]	0.170 ± 0.006	0.154 ± 0.006	0.555 ± 0.017	0.531 ± 0.011	0.445 ± 0.014	0.456 ± 0.006	0.175 ± 0.002	0.172 ± 0.001	0.167 ± 0.002	0.164 ± 0.001
Phe	0.368 ± 0.008	0.389 ± 0.008	2.390 ± 0.069	2.461 ± 0.067	0.963 ± 0.026	1.004 ± 0.028	0.547 ± 0.016	0.559 ± 0.001	0.444 ± 0.008	0.445 ± 0.001
Pro	0.644 ± 0.018	0.674 ± 0.005	2.127 ± 0.060	1.935 ± 0.083	1.250 ± 0.024	1.298 ± 0.026	0.837 ± 0.022	0.890 ± 0.005	0.710 ± 0.018	0.696 ± 0.004
Ser	0.352 ± 0.009	0.382 ± 0.006	2.326 ± 0.090	2.413 ± 0.105	0.996 ± 0.021	1.077 ± 0.006	0.446 ± 0.012	0.483 ± 0.001	0.385 ± 0.012	0.394 ± 0.002
Thr	0.242 ± 0.010	0.290 ± 0.007	1.365 ± 0.053	1.281 ± 0.054	0.782 ± 0.010	0.840 ± 0.008	0.297 ± 0.016	0.308 ± 0.002	0.252 ± 0.027	0.278 ± 0.001
Tyr	0.317 ± 0.008	0.333 ± 0.004	2.115 ± 0.074	2.068 ± 0.049	0.795 ± 0.027	0.786 ± 0.017	0.433 ± 0.016	0.452 ± 0.001	0.349 ± 0.012	0.355 ± 0.001
Val	0.346 ± 0.012	0.378 ± 0.001	1.933 ± 0.071	2.037 ± 0.001	1.003 ± 0.049	1.060 ± 0.003	0.510 ± 0.004	0.555 ± 0.001	0.411 ± 0.008	0.433 ± 0.001

[a] Ala = alanine, Arg = arginine, Asx = Asn or Asp (asparagine or aspartic acid), Cys = cystine, Glx = Gln or Glu (glutamine or glutamic acid), Gly = glycine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Pro = proline, Ser = serine, Thr = threonine, Tyr = tyrosine, and Val = valine.
[b] Crude protein (N × 6.25) contents (%) as determined by the macro-Kjeldahl procedure (AOAC 1984): corn (8.14), peanut meal (53.99), complete mixed feed (22.27), sorghum RS610 (10.77), sorghum BR64 (8.71).
[c] Values are mean ± SD in % ("as is" basis) of five separate derivatizations, each sample run once.
[d] Values are mean ± SD in % ("as is" basis) of duplicate analyses performed by high performance cation exchange chromatography (CXC) using modular HPLC equipment (corn, peanut meal, complete mixed feed) or conventional CXC using a Beckman 119CL amino acid analyzer (sorghum RS610, sorghum BR64).
[e] Cys and Met determined from oxidized samples as cysteic acid and Met dioxide, respectively.

methods in feedstuff amino acid compositions were generally small and would be anticipated to be of minor significance with regard to diet formulation.

There are several advantages that favor the use of PITC as a precolumn derivatization reagent for reversed-phase HPLC. A linear ultraviolet absorbance response between 20 and 500 pmol of amino acid has been reported (Bidlingmeyer et al 1984), noncorrosive buffers are used to elute PTC-amino acids from the reversed-phase column, and a complete separation of 17 hydrolysate amino acids plus Cya and Met-O_2 can be achieved in 25 min.

Unlike orthophthalaldehyde, PITC reacts with both primary and secondary amino acids, and the PTC-amino acid derivatives formed are stable enough for automated analysis without on-line derivatization (Heinrikson and Meredith 1984). However, it should be noted that samples will usually remain stable after derivatization for 10 hr at room temperature or 24 hr at 4°C.

Twenty-four hydrolysates can be analyzed in a 10-hr period, whereas sample oxidation and (reflux) hydrolysis require approximately 40 hr. Because feedstuffs are routinely hydrolyzed in groups of four in our laboratory, there is little practical significance of the 10-hr stability limit of PTC-amino acids at room temperature because it takes much longer to prepare hydrolysates than to analyze them.

A PITC-based amino acid analytical system composed of modular HPLC components provides a viable alternative to AAA at approximately one-half the cost, is generally easy to operate and maintain, and is readily adaptable to the analysis of compounds other than amino acids.

ACKNOWLEDGMENTS

Appreciation is expressed to T. E. Wheat and B. A. Frost of Waters Chromatography Division, Millipore Corporation, for their expert advice and assistance in performing the PTC-amino acid analyses.

LITERATURE CITED

ASHWORTH, R. B. 1987. Ion-exchange separation of amino acids with postcolumn orthophthaldehyde detection. J. Assoc. Off. Anal. Chem. 70:248.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1984. Section 2.062. Official Methods of Analysis. The Association: Arlington, VA.

BIDLINGMEYER, B. A., COHEN, S. A., and TARVIN, T. L. 1984. Rapid analysis of amino acids using precolumn derivatization. J. Chromatogr. 336:93.

COHEN, S. A., BIDLINGMEYER, B. A., and TARVIN, T. L. 1986. PITC derivatives in amino acid analysis. Nature 320:769.

EBERT, R. F. 1986. Amino acid analysis by HPLC: Optimized conditions for chromatography of phenylthiocarbamyl derivatives. Anal. Biochem. 154:431.

ELKIN, R. G. 1984. Quantitative amino acid analysis of feedstuff hydrolysates by reverse phase liquid chromatography and conventional ion-exchange chromatography. J. Assoc. Off. Anal. Chem. 67:1024.

ELKIN, R. G., and GRIFFITH, J. E. 1985a. Hydrolysate preparation for analysis of amino acids in sorghum grains: Effect of oxidative pretreatment. J. Assoc. Off. Anal. Chem. 68:1117.

ELKIN, R. G., and GRIFFITH, J. E. 1985b. Amino acid analysis of feedstuff hydrolysates by cation exchange high performance liquid chromatography. J. Assoc. Off. Anal. Chem. 68:1028.

GEHRKE, C. W., KUO, K. C., KAISER, F. E., and ZUMWALT, R. W. 1987. Analysis of amino acids by gas chromatography as the N-trifluoroacetyl *n*-butyl esters. J. Assoc. Off. Anal. Chem. 70:160.

GRANBERG, R. R. 1984. High resolution analysis of PITC-derivatized amino acids with UV and electrochemical detection. LC 2:776.

HEINRIKSON, R. L., and MEREDITH, S. C. 1984. Amino acid analysis by reverse-phase high-performance liquid chromatography: Precolumn derivatization with phenylisothiocyanate. Anal. Biochem. 136:65.

KAN, T. A., and SHIPE, W. F. 1981. Modification and evaluation of a reverse phase high performance liquid chromatographic method for amino acid analysis. J. Food Sci. 47:338.

KHAYAT, A., REDENZ, P. K., and GORMAN, L. A. 1982. Quantitative determination of amino acids in food by high-pressure liquid chromatography. Food Technol. 36:46.

KOOP, D. R., MORGAN, E. T., TARR, G. E., and COON, M. J. 1982. Purification and characterization of a unique isozyme of cytochrome P-450 from liver microsomes of ethanol-treated rabbits. J. Biol. Chem. 257:8472.

KRISHNAMURTI, C. R., HEINDZE, A. M., and GALZY, G. 1984. Application of reversed-phase high-performance liquid chromatography using pre-column derivatization with *o*-phthaldialdehyde for the quantitative analysis of amino acids in adult and fetal sheep plasma, animal feeds, and tissues. J. Chromatogr. 315:321.

MOODIE, I. M., COLLIER, G. S., BURGER, J. A., and WERB, B. C. 1982. An improved method based on gas chromatography for determining the amino acid composition of commercially produced fish-meal. J. Sci. Food Agric. 33:345.

MOODIE, I. M., BURGER, J. A., HOUGH, B. J., SHEPHARD, G. S., and LABADARIOS, D. 1985. Gas-liquid chromatography of amino acids: Variable arginine response. J. Chromatogr. 347:179.

SCHOLZE, H. 1985. Determination of phenylthiocarbamyl amino acids by reversed-phase high-performance liquid chromatography. J. Chromatogr. 350:453.

SHOJI, S., ICHIKAWA, M., YAMAOKA, T., FUNAKOSHI, T., and KUBOTA, Y. 1986. High-sensitivity amino acid analysis of stained peptides and proteins from a sodium dodecyl sulphate-polyacrylamide slab gel. J. Chromatogr. 354:463.

SPACKMAN, D. H., STEIN, W. H., and MOORE, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30:1190.

TARR, G. E., BLACK, S. D., FUJITA, V. S., and COON, M. J. 1983. Complete amino acid sequence and predicted membrane topology of phenobarbital-induced cytochrome P-450 (isozyme 2) from rabbit liver microsomes. Proc. Natl. Acad. Sci. USA 80:6552.

YANG, C.-Y., and SEPULVEDA, F. I. 1985. Separation of phenylthiocarbamyl amino acids by high-performance liquid chromatography on spherisorb octadecylsilane columns. J. Chromatogr. 346:413.

[Received September 19, 1986. Revision received January 19, 1987. Accepted January 26, 1987.]