Amino Acids in Sorghum Hydrolysates[1]

M. E. BRILEY,[2] L. F. TRIBBLE,[3] and W. L. STARNES[4]

ABSTRACT

Two samples of sorghum grown on the Texas High Plains were analyzed for amino acid content preliminary to experiments on the simulation of porcine digestive processes. Methods for computing the amino acid content of the sorghum samples were studied to improve the precision of the analyses and eliminate systematic error in the computation of the content of threonine, serine, isoleucine, and valine. Procedures were used to liberate amino acids that were stable to hydrolysis, and correction was made for destruction of threonine and serine or for the incomplete hydrolysis of isoleucyl or valyl peptide bonds.

Cereal Chem. 56(4):359-361

Many feeding trials have been designed to measure the degree of utilization of proteins from feed grains. Maximum weight gain in animals usually requires that the diets contain free amino acids or protein to supplement the grain being studied. Information about the content of amino acid in the protein source must be accurate. Addition of lysine to grain is required, and tryptophan, methionine, threonine, and isoleucine supplementation may be necessary, depending on the protein source and the animal being fed (Eckert and Allee 1974). Analysis of feed grains for amino acids using acid hydrolyzed samples partially destroys or incompletely hydrolyzes serine, threonine, valine, and isoleucine under normal conditions (Blackburn 1968, Needleman 1970, Robbins et al 1971).

Our objective was to determine the amino acid content of two samples of sorghum for a system simulating porcine digestion, using a refined procedure for acid hydrolysis that permitted correction of serine and threonine content to zero hydrolysis time and of valine and isoleucine content to infinite hydrolysis time.

MATERIALS AND METHODS

Protein Sources

Grain sorghum sample I was purchased at an elevator in Lubbock, TX. Grain sorghum sample II was obtained from DeKalb Seed Company and was variety CYZ-Y, a yellow endosperm type.

Estimation of Protein Content

The samples of sorghum were ground in a Wiley mill and analyzed for crude protein content by the Kjeldahl procedure (Association of Official Agricultural Chemists 1975) and for true protein by summation of the amino acid residue content after analysis. Triplicate sorghum samples of 1.12 mg of nitrogen were hydrolyzed in 6*N* HCl for each of five time intervals. Portions of sample I were hydrolyzed at 110°C for 20, 26, 44, 56, and 70 hr in thick-walled, evacuated sealed tubes, frozen in a dry ice-isopropyl alcohol bath, and kept cold during sealing. Similarly prepared portions of sample II were hydrolyzed for 20, 26, 36, 48, and 72 hr. Except for the first and last time intervals, the times were chosen for convenience. At the completion of the hydrolysis process, the samples were frozen. For analysis, samples were thawed and particulate matter was removed by a sintered glass filter. The filtrate was concentrated and the residues were dissolved in 0.2*N* citrate buffer, pH 2.2, for amino acid analysis. Single aliquots of each hydrolysis sample were analyzed for amino acid content. Nor-leucine and α-amino-βguanidino-propionic acid (AGPA) were used as an internal standard to permit analysis on different days to be related. A Beckman model 121 HP analyzer was used for a standard 4-hr procedure (Stein and Moore 1963, Pomeranz and Robbins 1972).

Estimation of Serine and Threonine Content

Because seryl and threonyl residues are unstable during acid hydrolysis, the amount of serine and threonine in the unhydrolyzed sorghum is greater than the content obtained upon hydrolysis. The modified procedure was used to correct the analyses to zero time of hydrolysis. First order (or pseudo-first order) kinetics for the decomposition were assumed,

$$\frac{dc}{dt} = -kt \qquad (1)$$

where c is the concentration of the residue at time t, dc/dt is the rate of change in concentration of the residue, and k is the first order rate constant. Integration of this differential equation and conversion of the solution to logarithmic form yielded the linear equation:

$$\ln c = -kt + \ln c_o \qquad (2)$$

In equation (2), c, t, and k have the same meaning and c_o is the concentration of the residue at zero time, t_o. An unweighted least squares analysis of a plot of ln c vs t yielded the slope as the first order rate constant and as the intercept on the ordinate, ln c_o, the antilog of which is the concentration of the amino acid residue before hydrolysis. In practice, any value proportional to concentration may be plotted without changing the linear nature of the plot. The proportionality can be used to calculate the zero time concentration of the amino acid from the intercept on the ordinate. Values called Ratio plotted in Fig. 1A and B (and in Fig. 2A and B with reference to valyl residues) were computed from the concentration of threonine or serine divided by the average yield in each sample of four amino acids.

Extrapolation of Isoleucine and Valine Content

Free isoleucine and valine content increased to a limit with hydrolysis time. The cubic hyperbola obtained as a function of time of hydrolysis increased in a linear fashion with time at the beginning and later did not vary with time (Fig. 2A). The form of the curve suggested a plot of 1/c vs 1/t and permitted extrapolation to obtain the theoretical asymptote at very large times of hydrolysis without actually requiring complete hydrolysis. The term "Ratio" was used for computation and was defined in the same way as for threonine and serine. The result was the concentration of isoleucine or valine (c) in the hydrolysis mixture at time t. The extrapolation was based on the curve properties for residue content as a function of time of hydrolysis.

Computation of the Ratio and Amino Acid Content

In practice, to permit simplification of the computer program, the Ratio was computed for each amino acid residue in each separately hydrolyzed sample. The formula for Ratio used in this work was:

$$\text{Ratio} = \frac{\text{moles X}}{\text{moles glycine + moles alanine + moles leucine + moles phenylalanine}} \qquad (3)$$

where X is any amino acid residue. The average content was computed and back calculated to percent of content. The selection of

[1]Article T-5-136, approved by the Dean of College Agriculture, Texas Tech University.
[2]Division of Nutrition and Food, University of Texas at Austin, Austin, TX 78712.
[3]Department of Animal Science, Texas Tech University, Lubbock, TX 79409.
[4]Department of Biochemistry, Texas Tech University, School of Medicine, Lubbock, TX 79409. Present address: University of Texas Medical Branch, Galveston, TX 77550.

0009-0352/79/000055$3.00/0

the residue for the denominator was based on a preliminary determination of the fractional content in a sample computed in the normal fashion. Chemically stable amino acids were used after inspection. Half-cystine was determined only once in sample II. Only the first hydrolysis mixture for this sample showed significant half-cystine. Remaining samples were presumed to have oxidized.

TABLE I
Protein and Amino Acid Residue Content in Sorghum Samples I and II[a]

Amino Acid	Sample I (%)	Sample II (%)
Protein Kjeldahl	9.45	8.17
amino acid analysis	7.86	5.86
Lysine	0.15 ± .05	0.14 ± .02
Histidine	0.19 ± .05	0.15 ± .02
Ammonia	0.28 ± .09	0.19 ± .02
Arginine	0.27 ± .08	0.19 ± .02
Aspartic acid	0.72 ± .11	0.48 ± .07
Threonine[b]	0.27 ± .05	0.21 ± .03
Serine[b]	0.28 ± .04	0.23 ± .03
Glutamic acid	1.85 ± .48	1.26 ± .06
Proline	0.50 ± .21	0.50 ± .04
Glycine	0.26 ± .06	0.28 ± .03
Alanine	0.78 ± .14	0.54 ± .04
Half-cystine	0.07 ± .01	0.05[c] ± ...
Valine[b]	0.38 ± .12	0.30 ± .03
Methionine	0.10 ± .01	0.07 ± .01
Isoleucine[b]	0.27 ± .09	0.28 ± .03
Leucine	1.12 ± .22	0.75 ± .08
Tyrosine	0.22 ± .03	0.13 ± .01
Phenylalanine	0.43 ± .09	0.30 ± .04

[a]Values are means ± standard deviations for 15 subsamples, with one analysis per subsample.
[b]Corrected for time of hydrolysis.
[c]n=1.

RESULTS AND DISCUSSION

According to equation (2) and the explanations for correction of isoleucine and valine, the logarithm or the inverse of the concentration of the amino acid being destroyed or liberated should be plotted as the function of time. Due to the construction of the amino acid analyzer with the two-column program for analysis and the inherent problems of comparing independent hydrolysates, direct plotting of the concentrations of threonine and serine is inherently imprecise. Laboratories use internal standards such as nor-leucine to test the mechanics of a system and to correct yields of amino acids. Nor-leucine and AGPA are added to the sample with the 0.2*N* citrate buffer before analysis, but this does not provide for correction of yields from sample to sample during hydrolysis. In principle, the internal standards could be added before hydrolysis, but they will not provide a realistic picture of events in a heterogeneous mixture such as sorghum. The unusual nature of sorghum hydrolysis mixture permits a modification of the internal standard, AGPA. A procedure was developed to provide for correction of yields during hydrolysis and to eliminate differences inherent in the mechanical operations.

The Ratio (equation 3) used for computing the content of amino acids from the raw data aptly provided for the internal standardization of the hydrolysis process. The Ratio was insensitive to minor differences in the original weights of the samples and to differences in environment during the hydrolysis process. Three conditions were critical for high precision of the Ratio and for most accurate results: constant sorghum-to-acid ratio in the tubes, complete evacuation of the hydrolysis tubes, and constant temperature for hydrolysis. A small variation in temperature affected the accuracy of the early measurements of the unstable residues and the rate of appearance of free isoleucine and valine in the early stages of hydrolysis.

The method used to correct the content of threonine and serine as in Fig. 1A and B can be used to correct for any residue that decomposes in the reaction mixture. Extreme conditions of hydrolysis caused other residues to decompose. The assumptions in the

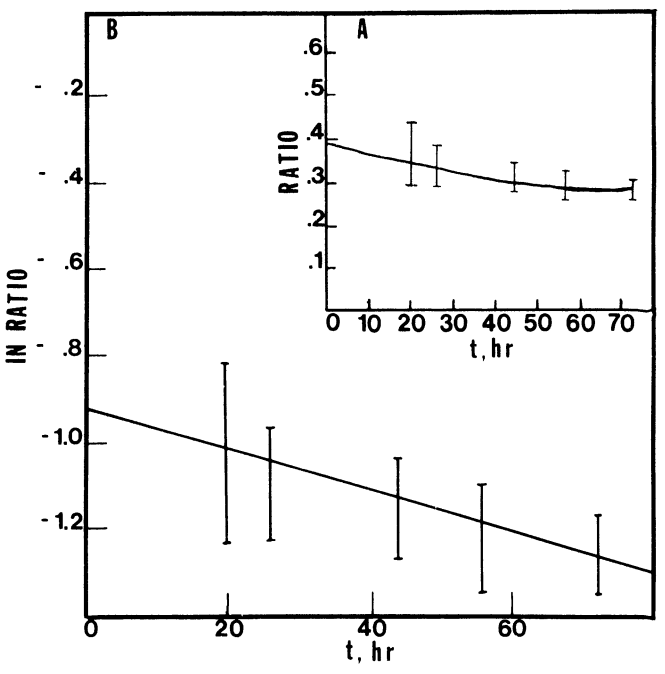

Fig. 1. A, Variation of the serine content in sorghum sample I vs time of hydrolysis. The line was computed from the line obtained in Fig. 1B. **B,** Correction of serine content of sorghum sample I for time of hydrolysis. The plot shown assumes first order or pseudo-first order kinetics. The line was obtained by an unweighted least squares analysis. The term "Ratio" was proportional to the molar content of serine in the sample. The error bars refer to the range of the value in the Ratio.

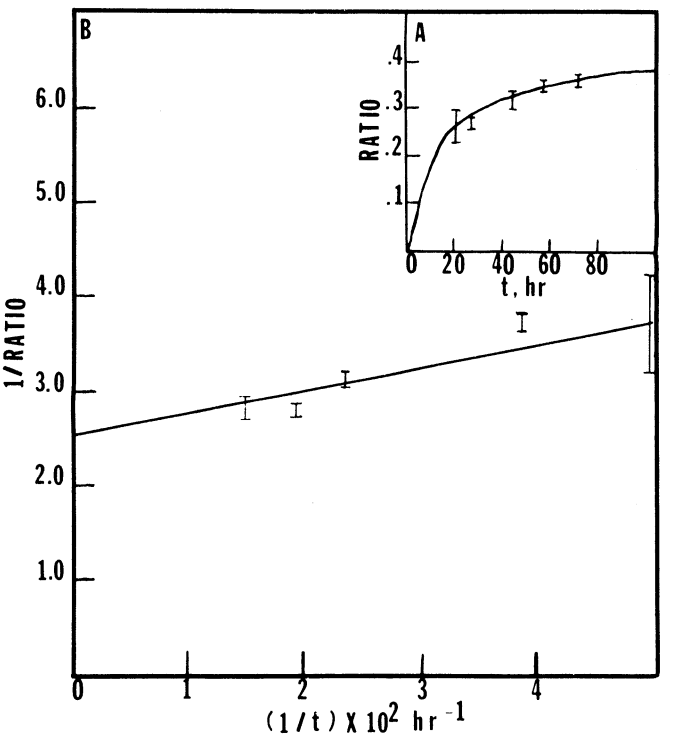

Fig. 2. A, Variation of the valine content in sorghum sample I vs time of hydrolysis. The line was computed from the line determined in Fig. 2B. **B,** Correction of valine content of sorghum sample I for time of hydrolysis. The term "Ratio" is proportional to the molar concentration of valine. The error bars refer to the range in the value of the Ratio. The line drawn was obtained by an unweighted least squares analysis.

treatment of the results were that: all seryl and threonyl residues decomposed at the same rate whether free or in peptide form; all peptide bonds in which glycine, alanine, leucine, and phenylalanine participate were hydrolyzed early in the process; and concentrations of the stable residues were invariant during the times indicated.

Correction of valine and isoleucine content (Fig. 2A and B) were based on the assumptions that: neither residue decomposed during times of hydrolysis and that the stability and rates of appearance of the four amino acid residues used for comparison were constant. The appropriate extrapolation of serine, threonine, valine, and isoleucine content to correct for time of hydrolysis showed that as much as 66% error could be introduced into the content of a specific residue (ie, isoleucine sample I) if the extrapolation was ignored. Figure 2A shows that the smallest number obtained for the content of valine at 20 hr was 58% of the corrected valine content. The correction for serine and threonine was not as great. The maximum correction was 30% for serine in sample I.

The average content of serine at 20–24 hr hydrolysis time was 93% of the value obtained by extrapolation (Fig. 1A and B). Data from Fig. 2A and B indicated that the average content of valine at 20–24 hrs was 65% of the extrapolated value. The corrections for valine and for isoleucine were of consequence. The correction successfully eliminated sources of systematic and experimental error because the true value for the content of threonine and serine was greater than the value obtained at 20–24 hr of hydrolysis time.

Amino acid content of sorghum samples I and II are shown in Table I. Sample I contained 9.45% protein by Kjeldahl and 7.85% protein by amino acid analysis. Sorghum sample II contained 8.17% protein by Kjeldahl and 5.86% protein by amino acid analysis. Comparison of these results with a sample of those of other researchers (Waggle et al 1967, Bressani and Rios 1962) shows that the sorghums analyzed were not unusual in protein or in amino acid content. A total of 15 analyses were made of the amino acid content of each sorghum sample. The coefficient of variation for sample II was smaller and more consistent than that for sample I. The variation was most likely due to inherent chemical differences between the sorghum samples, since grain sorghum sample I was an elevator run of mixed varieties typical of agronomic practices.

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[Received October 10, 1978. Accepted February 8, 1979]