some loss of theoretical plates within 6 weeks. However, the useful life of a column was at least 4 months, if the upper 1 cm of deteriorated and irreversibly loaded resin in both precolumn and separation column was replaced whenever a decrease in resolution of the standard mixture was apparent.

In preliminary studies, excellent separation of the predominant organic acids in a series of beverages, fruit juices, cider and wine vinegar, and sauerkraut juice was obtained. The acid peaks were sharp, the baseline was stable, sensitivity was more than adequate (often requiring 5- to 20-fold dilution to bring samples into the normal working range), and the identity and quantity of the predominant acids found in the samples were consistent with data in the literature or on the labels. Figure 3 shows typical separations of two juice samples.

Preliminary studies of the chromatographic method (Table II) indicate excellent precision. For complex mixtures of standard acids (as in Figure 2) the standard deviation for repeated injections did not exceed ±3%, except for citric and malic acids. For this incompletely resolved pair (1.0 N sodium formate eluant), the standard deviation approached ±15%, when the concentration of one of the acids was four times the other. Further studies are underway to establish the precision and accuracy of the method for determining the predominant acids in a wide variety of liquid and solid food samples, especially in comparison with "official" methods. The anion exchange method will also be tested as a means for determining the complete profile of acids, including trace components, in various plant and animal tissues, since the method appears to have potential for application in metabolic studies.

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Method of Extrapolation for Yield-Decay-Type Data

Edward J. Robel

A method employing the use of polynomials has been devised for the extrapolation of yield-decay data to obtain original or true yield values (A_0). The method was applied to amino acid data and performs effectively with hydrolysis and loss rates that cause inaccuracy with conventional methods of extrapolation because of significant yield losses, which occur before the entire amount (A_0) is hydrolyzed. Once the method is programmed, data can be handled simply on a small desk computer.

The limited accuracy of amino acid analysis was established prior to Robel and Crane (1972) for correcting amino acid losses during hydrolysis (Hirs *et al.*, 1954; Mahowald *et al.*, 1962; Noltmann *et al.*, 1962; Smith and Stockell, 1954; Smith *et al.*, 1954). A recent report (Robel and Crane, 1972) presents a method of extrapolation for determining true or original amino acid amounts at zero hydrolysis time (A_0) and compares its usefulness over established methods of calculation—the logarithmic method with least squares linear regression analysis or semilogarithmic plotting to zero time. However, the new method (Robel and Crane, 1972) requires a large computer to correct to zero hydrolysis time. In some locations, processing data in this manner may not be possible. This report presents a method of extrapolation requiring only a desk computer and illustrates the applicability and accuracy of this method using practical hydrolysis rates and loss rates encountered with protein hydrolysates.

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METHODS AND MATERIALS

Method Application. The method for estimating A_0 values was applied to artificial error-free "data" and run on an Olivetti Underwood Programma 101 electronic desk computer. The "data" were generated using eq 4 (Robel and Crane, 1972):

$$B(t) = \frac{A_0 h}{h - l}(e^{-lt} - e^{-ht})$$

After the program equations were formed, time and corresponding yield data were entered in the computer and true yield values (A_0) were obtained.

Derivation of Equations. The polynomial method for estimating the original amino acid content of protein utilizes data to include a hydrolysis range spanned by two curvilineal fits. The residue yield plus destruction is the resultant curve from zero to maximal yield time (first curvilineal fit) and the resultant curve from maximal yield time to infinite time (second curvilineal fit). The calculations, therefore, involve a hydrolysis range spanned by two curvilineal fits. The maximal yield values determined from the first curvilineal fit, added to the amounts of de-

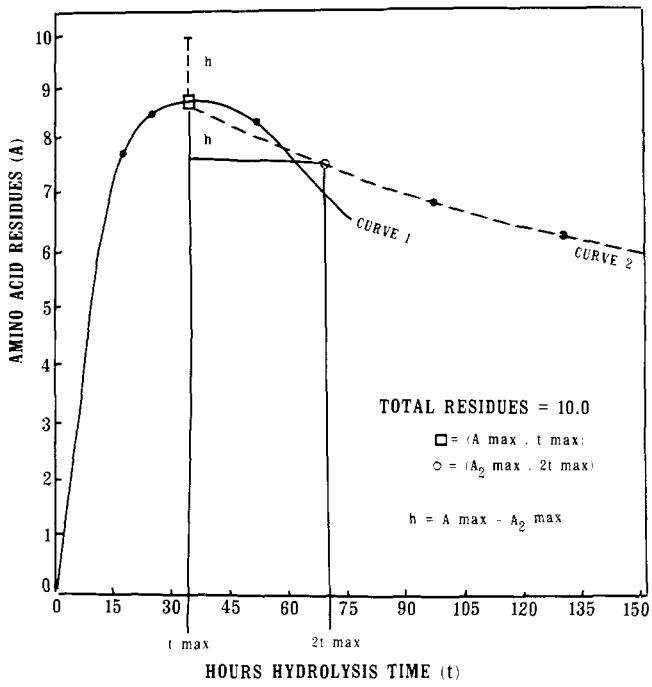


Figure 1. Two curvilineal fits for spanning five hydrolysis times. The three early hydrolysis times are used to derive A_{max},t_{max} for the first fit. A_{max},t_{max} and two later hydrolysis times are utilized for the second fit.

struction that have occurred during the time that it takes for the maximum to occur, give the amount present at zero hydrolysis time.

A general solution for the method can be described beginning with a theoretical second-order polynomial fit for an amino acid from a protein hydrolyzed at five different times (Figure 1). The hydrolysis times chosen for the error-free "data" (to be described later) used to test the accuracy and practicality of the method were 15, 21.21, 41.57, 83.57, and 141.71 hr for the curvilineal fits, corresponding to a hydrolysis rate of 0.00250 hr^{-1}, and 31.3, 50.0, 83.57, and 141.71 hr, corresponding to a hydrolysis rate of 0.00100 hr^{-1} (Figure 2). The three earlier hydrolysis times in each case were chosen for projecting the first curvilineal fit for forecasting maximum amino acid yield. The method requires the third measurement to be taken after the maximum value of the curve has been attained. With the 0.100 hydrolysis rate curves, 83.57 hr was used as a point on both the first and second curvilineal fits.

The curve shown in Figure 1 has the equation $A = at^2 + bt + c$, where a, b, and c are constants determined by requiring the curve to pass through the three selected points, with coordinates (A_1,t_1), (A_2,t_2), and (A_3,t_3). The values for the constants are given as:

$$a = \frac{A_1(t_2 - t_3) + A_2(t_3 - t_1) + A_3(t_1 - t_2)}{t_1^2(t_2 - t_3) + t_2^2(t_3 - t_1) + t_3^2(t_1 - t_2)} \quad (1)$$

$$b = \frac{A_1(t_3^2 - t_2^2) + A_2(t_1^2 - t_3^2) + A_3(t_2^2 - t_1^2)}{t_1^2(t_2 - t_3) + t_2^2(t_3 - t_1) + t_3^2(t_1 - t_2)} \quad (2)$$

$$c = \frac{A_1t_2t_3(t_2 - t_3) + A_2t_1t_3(t_3 - t_1) + A_3t_1t_2(t_1 - t_2)}{t_1^2(t_2 - t_3) + t_2^2(t_3 - t_1) + t_3^2(t_1 - t_2)} \quad (3)$$

The time (t_{max}) at which maximum amino acid yield (A_{max}) is obtained is determined by setting $dA/dt = 0$. Thus,

$$t_{max} = -\frac{b}{2a} = -\frac{A_1(t_3^2 - t_2^2) + A_2(t_1^2 - t_3^2) + A_3(t_2^2 - t_1^2)}{2[A_1(t_2 - t_3) + A_2(t_3 - t_1) + A_3(t_1 - t_2)]} \quad (4)$$

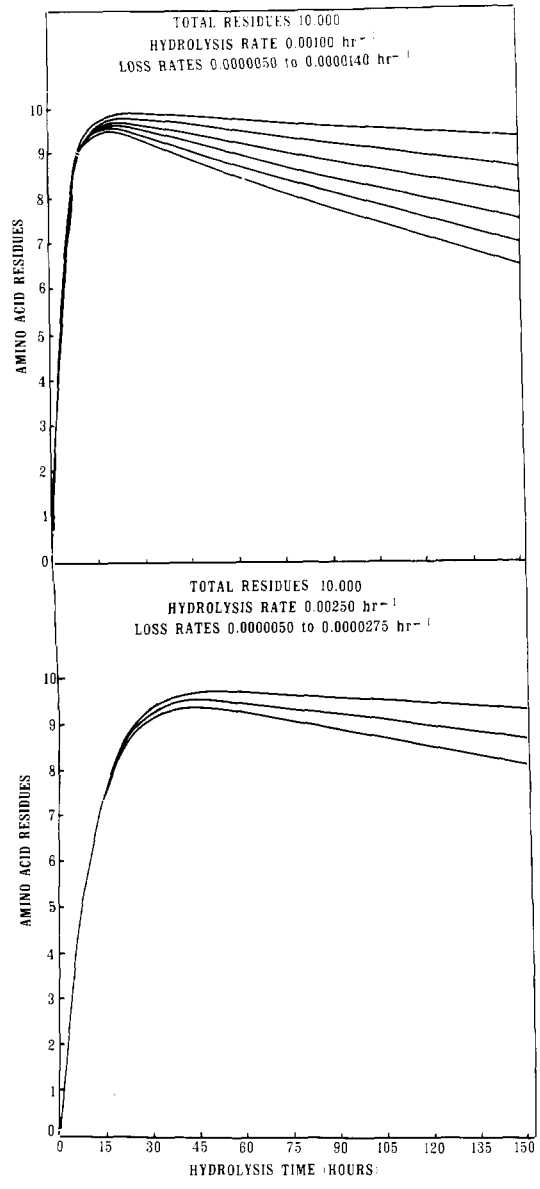


Figure 2. Curvilineal fits produced from error-free data which have a true residue value of 10.

and the maximum amino acid yield is obtained from $A_{max} = at_{max}^2 + bt_{max} + c$.

Taking the maximum position (A_{max},t_{max}) as the first point, a second curvilineal fit is formed using later error-free hydrolysis times (83.57 and 141.71 hr). Identical equations are used for the calculations of the constants when determining the second curvilineal fit as were used for the first curvilineal fit. For clarity of illustration, the letter identifying the constants will be changed. That is, the yield as obtained from the second curvilineal fit A will be given by

$$A = dt^2 + et + f$$

where

$$d = \frac{A_4(t_5 - t_6) + A_5(t_6 - t_4) + A_6(t_4 - t_5)}{t_4^2(t_5 - t_6) + t_5^2(t_6 - t_4) + t_6^2(t_4 - t_5)} \quad (5)$$

$$e = \frac{A_4(t_6^2 - t_5^2) + A_5(t_4^2 - t_6^2) + A_6(t_5^2 - t_4^2)}{t_4^2(t_5 - t_6) + t_5^2(t_6 - t_4) + t_6^2(t_4 - t_5)} \quad (6)$$

$$f = \frac{A_4t_6t_5(t_5 - t_6) + A_5t_4t_6(t_6 - t_4) + A_6t_4t_5(t_4 - t_5)}{t_4^2(t_5 - t_6) + t_5^2(t_6 - t_4) + t_6^2(t_4 - t_5)} \quad (7)$$

Table I. A Prediction of Error-Free Data Using the Polynomial Method

	A_0[a]
Yield rate, 0.250	
Loss rate 0.00050	10.18
0.00095	10.18
0.00140	10.12
0.00185	10.08
0.00230	10.12
0.00275	10.10
Yield rate, 0.100	
Loss rate 0.00050	10.32
0.00095	10.30
0.00140	10.17

[a] The true value (A_0) for each of the hydrolysis and loss rates is 10.

The amount of yield at $t = 2t_{max}$ is given as

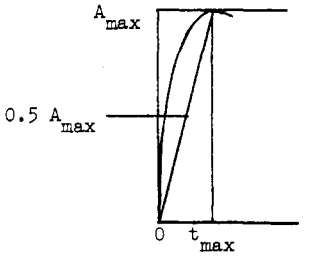
$$A_{2max} = d(2t_{max})^2 + e(2t_{max}) + f$$

and an estimate of the original amount of amino acid content of protein (A_0) is given by the maximum output $A_{max} = at_{max}^2 + bt_{max} + c$, plus the amount of destruction that occurred during that time period taken for the maximum to be reached, or $h = A_{max} - A_{2max}$ (Figure 1). That is, $A_0 = A_{max} + (A_{max} - A_{2max})$ or $A_0 = 2(at_{max}^2 + bt_{max} + c) - [d(2t_{max})^2 + e(2t_{max}) + f]$. Practical application of the method consists in the use of coordinate hydrolysis data for the solutions of t_{max}, A_{max}, A_{2max}, and A_0. With this method, hydrolysis and loss rates are not assumed to be constant nor proportional to the amount remaining but are time dependent, *i.e.*, $dA(t)/dt = 2at + b$ or $2dt + e$.

Since the amount of destruction which occurs before t_{max} is considered to be less than the amount of destruction after t_{max}, and because of the amount of total hydrolysis that is present in each of the time spans, the accuracy of the correction factor ($A_{max} - A_{2max}$) used to adjust A_{max} to the true A_0 value was investigated. Considering the data and the parameters used (Robel and Crane, 1972), a final adjustment for A_0 then corresponds to the following equation.

$$A_0 = A_{max} + 0.75(A_{max} - A_{2max})$$

The error involved using the correction factor in this equation was found to be 1.3%, or less with the 0.00250 hr^{-1} hydrolysis rate curves, and 2.1%, or less with the 0.00100 hr^{-1} hydrolysis rate curves. The rationale for the choice of the coefficient 0.75 is presented with the diagram below.



Average $A_{(t)}$ for t between $0,t_{max}$ is $>0.5\ A_{(t(max))} = A_{max}$, and, of course, the average of $A_{(t)}$ for t between $0,t_{max}$ is less than A_{max}. Since $A_{max} - A_{2max}$ is small, simply splitting the difference $0.5\ A_{max} + 1.0\ A_{max}/2 = 0.75\ A_{max}$ is close enough. The maximum possible error, therefore, is $0.25\ (A_{max} - A_{2max})$.

When the data are such that t_{max} is forecast in the minus time quadrant, the calculations for determining A_0 values are simplified. In this case, only the first curvilineal fit is used and the amount of original content is taken as the extrapolated value obtained at t equal to zero, that is, $A_0 = c$. This is possible, since the amount of destruction that has occurred during the early hours is neglected in the estimate made. This situation is likely to occur for the amino acids serine and threonine.

When the calculations are applied to those amino acids which remain stable to give increased yields during later hydrolysis, the same calculations are applied. However, the hydrolysis data should be chosen to span the maximum yield position which occurs during extended hydrolysis.

RESULTS AND DISCUSSION

It has been established that current methods of extrapolation for certain amino acids are not accurate to the highest degree when significant amino acid loss occurs before the entire amount A_0 is hydrolyzed and observed by analysis (Robel and Crane, 1972). In order to test the applicability of the present method, we have employed some hydrolysis and loss rates which apply to this situation. Using eq 4 (Robel and Crane, 1972), which gives the ideal or theoretical curve under the assumption of constant hydrolysis and decay rates, the mathematical generation of error-free "data" was possible. Curves for the artificial data are shown in Figure 2 for the hydrolysis rates of 0.00250 and 0.00100 hr^{-1}, with the combination of loss rates of 0.0000050 to 0.0000275 hr^{-1} and 0.0000050 to 0.0000140 hr^{-1}, respectively, in steps of 0.0000045 hr^{-1}. The results of Table I show the polynomial method to predict A_0 values for the artificial data to be within the error of 1.8% or less with the 0.00250 hr^{-1} hydrolysis rate curves and 3.2% or less with the 0.00100 hr^{-1} hydrolysis rate curves. This suggests, therefore, that the method can be effectively applied to practical analytical data. It must be understood, however, that the precision of the A_0 values obtained with the artificial data may not become a reality with analytical data because of the extent of error limits inherent in the actual data points. The polynomial method operates effectively only within the true range of data, since it does not correct for data error. Its use becomes dangerous when data are out of the true range.

It is evident from the accuracy of the A_0 estimations of the error-free "data" that the method could be useful for handling reliable analytical data where large computer service is unavailable. Once the program for the equations is formed, all that is required is to enter the hydrolysis times and corresponding yields in the electronic desk computer and true yield values (A_0) are readily obtained.

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