Comparison between the Microbiological and the Ion Exchange Chromatographic Analyses of Foods for Amino Acid Content

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Triplicate hydrolysates of ten foods were analyzed for content of 16 amino acids by both microbiological and ion exchange column chromatographic methods. Values obtained by the two methods were in general agreement; however statistically significant differences were encountered for several amino acids in each food. No indication was evident that the methods consistently differed in determining any single amino acid.

Following the development of the ion exchange column chromatographic method of determining amino acids (Moore and Stein, 1951), the other analytical methods for amino acids were virtually replaced by the newer method. This was particularly so upon publication of the automated version of this method (Spackman *et al.*, 1955) and after instruments for its application became commercially available. Previously, a sizable mass of microbiological assay data on the amino acid content of foods had been published. Parallel data obtained by the newer chromatographic method have steadily accumulated. Values for individual amino acids in foods yielded by the two methods disagree in many instances; yet no thoroughgoing study has been made to evaluate the differences. There have been reported comparisons of microbiological assay values with those of paper chromatography (Bolinder, 1968a,b; Kovacs and Toth, 1966; Nehring and Wünsche, 1964; Schroeder and Bock, 1963), the enzymatic method (Pomeranz and Miller, 1963), and starch column chromatography (Miller *et al.*, 1952), as well as ion exchange column chromatography (Bolinder, 1968a,b; Dunn *et al.*, 1962; Evans *et al.*, 1960, 1959; Pomeranz and Miller, 1963; Schroeder and Bock, 1963; Williams, 1955). Most of the latter studies involved work on only a single amino acid (Bolinder, 1968a,b; Evans *et al.*, 1960; Pomeranz and Miller, 1963), others on several amino acids in casein and soybean oil meal (Williams, 1955), in rat and mouse tumors (Dunn *et al.*, 1962), and in several animal feeds (Schroeder and Bock, 1963).

The Food and Agriculture Organization of the United Nations published an extensive compilation (Food Policy and Food Science Service, Nutrition Division, F.A.O., 1970) of amino acid values in foods, both chromatographic and microbiological. Among these can be found numerous instances of wide differences between values obtained by the two methods. For example, the tabulated values obtained by the two methods differ by 22, 49, and 27% for glycine in milk powder, tyrosine in milled polished rice, and histidine in whole grain wheat, respectively.

The differences might be accounted for to some extent by variation among laboratories, since the compilation comprises data from many laboratories. However, similar differences were obtained where both methods were used in the same laboratory or where the same hydrolysis technique was used in both. In one study (Williams, 1955), in which the two methods were used in the same laboratory but with different techniques of hydrolysis, differences in values for some amino acids were found to be in the order of 20 to 30%. In others (Evans *et al.*, 1959; Pomeranz and Miller, 1963) in which the work was done in a single laboratory with the same hydrolytic method being used for both types of analyses, some values yielded by the two methods again differed markedly.

Past work has thus involved uncontrolled sources of variation, some of which are susceptible to control. In order to minimize such variation in the present study, amino acids were determined microbiologically and chromatographically on the same food hydrolysates.

EXPERIMENTAL

Ten samples were purchased: dried skim milk, cottage cheese, lean beef round, beef liver, gelatin, whole wheat flour, soy flour, rice, split peas, and lettuce. Of these, cottage cheese, the meats, and lettuce were freeze-dried for ease in subsequent handling. All were then ground to pass through a 20-mesh screen and were stored at $-10°$ C until used.

The samples were analyzed for nitrogen content in triplicate by the Kjeldahl method (Association of Official Agricultural Chemists, 1965). For hydrolysis, 1 g of sample was heated at 110° C for 24 hr in an atmosphere of nitrogen in a sealed container with 100 ml of 6 *N* hydrochloric acid. The hydrolysate was cooled and filtered quantitatively through a Millipore filter. Excess hydrochloric acid was removed by three successive evaporations (to dryness) using a rotary evaporator with addition of water. The residue was taken up in water and aliquots of the resulting solution were adjusted to pH 2.2 for chromatographic analysis and 6.8 for microbiological assay.

Hydrolysates were prepared from each food sample on three separate days. An aliquot of each replicate hydrolysate was analyzed for 16 amino acids using both microbiological and chromatographic procedures.

The AOAC (Association of Official Agricultural Chemists, 1965) method was followed in performing the microbiological assays. The chromatographic analyses were done on a Phoenix amino acid analyzer by the Moore–Stein method (Moore and Stein, 1951; Spackman *et al.*, 1955) with norleucine as an internal standard.

The purchasing of all samples and the performances of all experimental analyses were done by the WARF Institute, Madison, Wis.

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STATISTICAL ANALYSES

As a measure of the precision with which the analyses were performed, coefficients of variation were calculated for each amino acid mean value. These were averaged to yield overall coefficients of variation for each method. The *t* test was used on paired samples to measure significance of the difference between values obtained by the two methods.

RESULTS AND DISCUSSION

Replicate nitrogen values varied from mean values by 2% or less.

The overall coefficients of variation were 2.4% for the microbiological and 4.7% for the chromatographic method. The variability of the microbiological assays was far smaller than is commonly expected for this type of analysis. Particularly surprising was the finding that the chromatographic analyses were the more variable of the two.

There was general agreement between the mean values for the two methods (Table I deposited in ACS Primary Publications Microfilm Depository). The overall difference between the means is 6.1%. Percentage differences between the means (calculated with microbiological values as base) are in most instances small, but with some large differences, such as 22.8% for histidine in cottage cheese, 20.4% for valine in gelatin, and 21.3% for methionine in whole wheat flour. Of the above differences only the values for histidine in cottage cheese are significantly different at the 5% level. For three of the amino acids tested, serine, isoleucine, and tyrosine, no significant differences were found. Significant differences at the 1% level were found for lysine, histidine, threonine, aspartic acid, glutamic acid, valine, and phenylalanine, each in only a single food (split pea, dried skim milk, beef liver, split pea, split pea, dried skim milk, and split pea, respectively). There appears, however, to be no pattern which would indicate that the two methods differ basically in determining any specific amino acid.

Although the differences are not significant, it is of interest that the microbiological values for phenylalanine are consistently lower than the chromatographic values in all of the ten foods. Microbiological values for threonine are higher than the chromatographic values in all of the foods of animal origin and lower in the vegetable foods. In lettuce the microbiological values for all 16 amino acids are consistently lower than the corresponding chromatographic values.

One could reasonably expect to find significant differences between amino acid values yielded by the microbiological and chromatographic methods. The parameters they measure are different, as are the factors influencing the measurements.

The microbiological method essentially measures the growth and metabolism of an assay microorganism in the presence of varying concentrations of amino acid. It is chemically specific in that each assay determines a single amino acid in the presence of the others. It is also biologically specific; that is, it determines the natural forms (*L* isomers) of amino acids. Being based upon the biological properties of a microorganism, it is susceptible to factors that affect these, such as stimulation or inhibition by substances which may be present in food hydrolysates. For instance, certain peptides have been reported to stimulate bacterial growth (Krehl and Fruton, 1948; Sprince and Wooley, 1944). Certain amino acids have been reported to antagonize each other, thereby inhibiting bacterial growth (Brickson *et al.*, 1948; Gladstone, 1939). These could interfere with assays.

The chromatographic method is chemically nonspecific, each amino acid being represented as a peak at a characteristic site on a chromatogram. The method makes no distinction between natural and unnatural forms of amino acids (*L* and *D* isomers); it is biologically nonspecific. While the method requires careful attention to such factors as pH and ionic strength of buffers, it is not susceptible to influence by many of the factors which affect complex biological systems.

Because of the great variability commonly associated with biological assays in general and microbiological assays in particular, doubt has existed as to the reliability of the published microbiological data. The results reported here indicate that microbiological data are probably as reliable as chromatographic data. In the instances where significantly different values were obtained for certain amino acids, there is no evidence to indicate that the chromatographic values more closely approximate true values.

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