Optimum Conditions of Hydrolysis for Microbiological Assay of Amino Acids, Methionine, and Cystine in Poultry Meat

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Acidimetric titrations are much more suitable for microbiological analyses of methionine and cystine in poultry meat than turbidimetric determinations. The use of a 4-hour sealed-tube hydrolyzate (3*N* hydrochloric acid) for cystine and an 8-hour sealed-tube hydrolyzate (3*N* hydrochloric acid) for methionine gave maximum values in microbiological analyses using titrations of the acid produced by the test organism *Leuconostoc mesenteroides*.

ALTHOUGH research has been conducted on the amino acid content of poultry meat, no reports could be found where the optimum time and method of hydrolysis of poultry meat had been studied. Studies on other products have indicated that long hydrolysis of the test sample causes some loss of cystine (*5*. A 2-hour hydrolysis was proposed fo cystine determinations. Balasubramanian and Ramachandran (*2*) recommended a hydrolysis period of $2^1/_2$ hours using 2.5*N* hydrochloric acid for wheat and rice. These studies were conducted on high carbohydrate-containing material and may have less applicability on foods such as meat. Cystine values obtained with short periods of hydrolysis may be in error due to peptide stimulation of the assay organisms (*1*, *4*).

This study was undertaken to determine the combinations of hydrolysis time and method which would result in maximum readings for methionine and cystine when assayed by microbiological means. Consideration was also given to a comparison of turbidimetric and acidimetric determinations of the growth of the test organism.

Experimental

Sample Preparation. Three eviscerated Hoosier White birds, each weighing approximately 1250 grams, were cooked in a reel-type oven at 325° F. (163° C.) for 1 minute per 10.5 grams of carcass weight. The birds were cooked in a shallow open pan, loosely covered with aluminum foil, and allowed to cool prior to taking samples. Breast and thigh muscles (minus skin) were combined and ground together three times. Portions for analysis were dried for 24 hours in a vacuum oven at 65° C. Fat was removed by Soxhlet extraction with petroleum ether for 15 hours. The moisture-free, fat-free samples were pulverized with a mortar and pestle, and 1 gram of this powder was used for each hydrolysis treatment. Thirty-milligram samples of the pulverized material were analyzed for nitrogen by the micro-Kjeldahl method.

Hydrolysis Procedure. The meat samples were hydrolyzed by the sealed-tube method or under reflux for 1, 2, 4, 8, 16, or 24 hours. For each combination of time and method, four samples (treated as two replicates, each containing two samples) were hydrolyzed and analyzed for methionine and cystine. The sealed-tube hydrolyses were done in borosilicate glass tubes 18 mm. in outside diameter and originally 16 inches long. Tubes became progressively shorter with each use and were discarded when the sample and acid filled more than one third of the tube. One gram of the pulverized moisture-free, fat-free sample was placed in the tube and 10 ml. of 3*N* hydrochloric acid were added. The sealed tubes, when cool, were shaken vigorously and placed in the autoclave in a horizontal position. The autoclave was then heated and maintained at 15 pounds pressure (approximately 120° C.) for the specified time. One-gram samples to be refluxed were placed in 300-ml. flat-bottomed round boiling flasks with 10 ml. of 6*N* hydrochloric acid and connected to condensers. Flasks were heated on a hot plate on high with a Powerstat setting of 70. Refluxing was timed from the fall of the first drop.

Hydrolyzates were rinsed from the hydrolysis container and brought to a pH of 4.0. One-half gram of activated charcoal was added, the solution filtered, and the residue washed until the volume of filtrate was almost 250 ml. The pH was then adjusted to 6.8, the volume made to 250 ml., and the solution frozen in 4-ounce polyethylene bottles until used for assay of amino acids.

Determinations. Methionine and cystine were determined both turbidimetrically and acidimetrically. From the standpoint of time, turbidimetric determinations are more desirable, as readings can be made more rapidly than titrating and the tubes need be held only 16 to 20 hours rather than 72. *Leuconostoc mesenteroides* P-60 ATCC No. 8042 was the test organism. The media used were Difco dehydrated Bacto-Methionine assay medium and Bacto-Cystine assay medium. Five milliliters of the rehydrated medium were placed in each 16 × 125 mm. screw-cap culture tube with additional test sample or standard and distilled water to make a volume of 10 ml. After inoculation, the tubes were incubated at 30° C.

After 16 to 20 hours of incubation, turbidimetric determinations were made in the test tube attachment of the Beckman DU spectrophotometer at a wave length of 660 microns as recommended by the Society of American Bacteriologists (*6*). Acidimetric titrations were made after 72 hours using 0.1*N* sodium hydroxide. The solutions were stirred with a magnetic stirrer while being brought to an end point pH of 7.0 as measured by a Beckman Model H pH meter. Standard amino acid and test material growth response curves were plotted on log-log paper as suggested by Block and Weiss (*3*), except that 11 × 16 inch, 1 × 2 cycles paper was used rather than $8^1/_2$ × 11 inch, 2 × 2 cycles paper.

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Results and Discussion

Acidimetric *vs.* Turbidimetric Determinations. In general, the methionine values determined turbidimetrically were somewhat lower than those determined acidimetrically. Turbidimetric readings at the higher levels of methionine were difficult, as the growth curve failed to maintain a straight line when plotted on log-log paper. This was particularly true with the standard curve responses (Figure 1). The correlation coefficient was computed for the methionine values of 34 samples analyzed turbidimetrically and acidimetrically, and found to be 0.218. This correlation was not statistically significant at the 0.05 level, and it indicates that turbidimetric determinations are of questionable value for quantitative determination of methionine. Turbidimetric determinations were even less applicable to the determination of cystine. All methionine and cystine values in the discussion of the hydrolysis experiment are made acidimetrically with 0.1*N* sodium hydroxide.

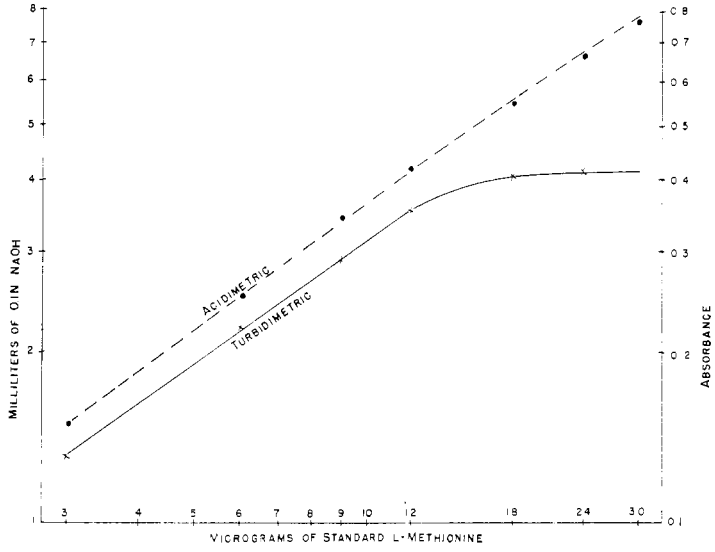

Figure 1. Typical growth curves for standard solutions of methionine

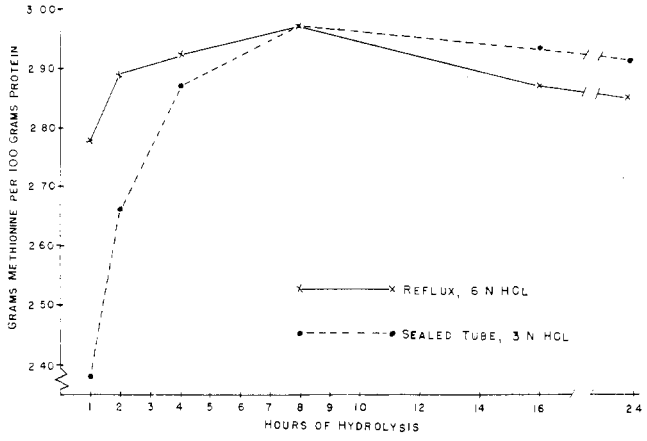

Figure 2. Effect of time and method of hydrolysis of methionine values of poultry meat

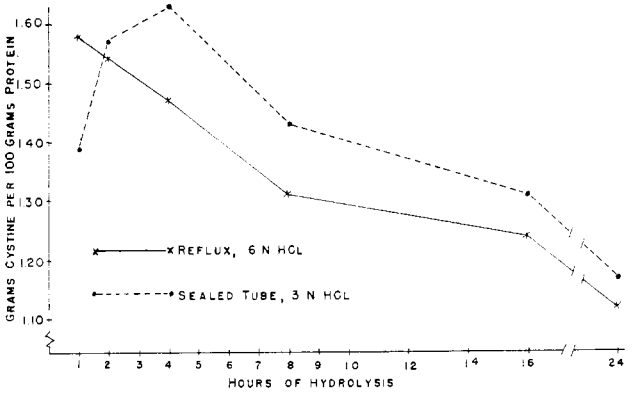

Figure 3. Effect of time and method of hydrolysis on cystine values of poultry meat

Optimum Hydrolysis Time and Method. The effects of time and method of hydrolysis are illustrated in Figures 2 and 3. It is readily apparent that sealed-tube hydrolysis with 3*N* hydrochloric acid requires a longer time than reflux with 6*N* hydrochloric acid to release the cystine from the protein; however, the sealed-tube method causes less destruction of the amino acids after the maximum has been reached. The time of reaching maximum values for the amino acids differs with each method and with each amino acid.

Statistical analysis by the analysis of variance method indicated that time and (time × method) interaction variations were highly significant for both cystine and methionine. Method differences were also significant in the methionine determinations. Replications were not significant at the 0.01 level for methionine or cystine. The standard deviation for subclass samples within replicates, as taken from the analysis of variance table, was 0.255 gram of methionine and 0.079 gram of cystine per 100 grams of protein.

Figures 2 and 3 show that the most suitable periods of sealed-tube hydrolysis are 4 hours for cystine and 8 hours for methionine. A sealed-tube hydrolysis for 6 hours might give near-maximum values for both methionine and cystine, with minimal destruction of either amino acid. The use of one 6-hour hydrolyzate would eliminate the need for a separate hydrolysis for each amino acid.

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Received for review July 31, 1959. Accepted January 20, 1960. Journal Paper 1466, Purdue Agricultural Experiment Station. From a thesis submitted to the Faculty of Purdue University by the senior author in partial fulfillment of the requirements for the degree of doctor of philosophy.