Survey Hydrolysis Procedure For Lysine Analysis[1]

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ABSTRACT

The hydrolysis procedure for liberating amino acids from protein-containing materials has been simplified for a screening analysis for lysine. It is proposed for use in surveying for high-lysine varieties in large collections of agricultural commodities. The method has been tested on safflower, wheat, barley, and milo and compared with the standard analytical procedure used in this laboratory. The method affords high reproducibility for all commodities. The precision of the method for safflower is better than for grains. However, the results are quite satisfactory for picking up high or low values in any population. One person can readily handle 300 samples in a normal working week.

The problem of meeting lysine needs of populations consuming high levels of cereals and low levels of animal proteins ranks high in the consideration of nutritionists. The discovery of types of corn high in lysine (1) is providing a great stimulus to further research on improving the protein quality of other cereals and oilseed meals by genetic means. Such nutritionally improved products could have great significance in animal as well as in human nutrition. For plant-breeding studies very large numbers of lysine analyses are needed. Conventional analytical procedures are costly and time-consuming. This paper presents a simple hydrolysis method suitable for the determination of lysine in the thousands of samples that might be involved in a search for high-lysine types in an oilseed or cereal grain seed collection.

There are several methods for determining lysine in protein hydrolysates, including ion-exchange chromatography (2), microbiological assay (3), enzymatic decarboxylation (4,5), paper chromatography (6), reaction with fluorodinitrobenzene (7), and reaction with picric acid (8). All require the prior hydrolysis of the protein to its constituent amino acids. Suitable hydrolytic conditions for quantitative liberation of amino acids have been the subject of many studies (9,10,11). Amino acid analysis of crude materials such as foods and feeds requires special sample preparation (e.g., oil removal and uniform sampling) before hydrolysis if accurate results are desired for all amino acids. These steps are time-consuming and limit the number of samples that can be processed with a reasonable expenditure of manpower.

Generally, nutritional evaluation of a food or feed commodity is based on its amino acid composition as related to its nitrogen content, expressed as g. of amino acid per 16 g. of nitrogen. Thus, the need for quantitative recovery of all hydrolysate could be eliminated if the relative recoveries of lysine and nitrogen were the same. Previous work (9,12) on hydrolytic conditions has shown that some amino acids are either subject to degradation or are slowly liberated during hydrolysis. Lysine, however, is completely liberated by 24 hr. and is stable throughout the whole hydrolysis procedure. In the present work, experiments were undertaken to determine which of the usual precautions in the hydrolysis of proteins could be

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eliminated when only lysine and nitrogen values were needed, in the hope that a procedure could be developed that would allow hundreds of samples to be processed rapidly and economically with a minimum of labor, materials, and specialized laboratory equipment.

EXPERIMENTAL

Two methods of protein hydrolysis have been compared. The first is our "conventional" procedure involving all the precautions necessary for accurate amino acid determination. The second is the new simplified "survey" procedure proposed for lysine determination. The "conventional" procedure, called procedure A, is fully described in a previous paper (12). However, a brief review of the method is presented for comparative purposes.

Oil-free powdered meal, 40 mg., is hydrolyzed in a nitrogen or evacuated atmosphere with a large excess of 6*N* HCl. Hydrolysis is carried out for 24 hr. at 110 ± 1° C. The hydrolysate is quantitatively recovered, filtered through a Millipore® filter, and taken to dryness. All traces of residual HCl are removed by repeated water evaporations of the dried hydrolysate. It is then dissolved in an appropriate amount of pH 2.2 diluting buffer for analysis.

In the survey method, approximately 2-g. samples of whole seeds are measured by means of a calibrated scoop into flint glass screw-cap vials (10 dram, Owens Illinois No. 60957-L, No. 3), held in a suitable rack (e.g. Van Waters and Rogers No. 60935). To each sample, 20 ml. 6*N* HCl is added by means of an automatic pipet, and the vial caps (Owens Illinois No. 24-400, pulp and vinyl liner) are firmly closed with a pair of pliers. The samples are then heated in an oven at 110°± 2° C. for 24 hr. Very tight closure of the vials prevents losses of HCl during hydrolysis (e.g. less than 3%). Preliminary experiments showed that vapor losses up to 20% during hydrolysis did not affect the results. After cooling, the vial contents are filtered through 15 cm. Whatman No. 12 filter papers held in plastic powder funnels into 10-dram polyethylene vials. Neither the hydrolysis vials nor the filter papers need be washed, since lysine and nitrogen losses are proportionate. The filtered hydrolysates are then evaporated to dryness at 60°C. in a vacuum oven at full aspirator vacuum. The inner surfaces of the oven are coated with liquid Neoprene (Glidden No. N-200-1), with Teflon tape applied to surfaces subject to wear or abrasion. A piece of heavy polyethylene sheeting, placed over the entire inside portion of the door and gasket just prior to closing, further protects both from acid fumes. An infrared heat lamp controlled by a rheostat is used to warm the outside of the door to prevent acid vapor condensation on its otherwise unheated surface. The dried residues in the vials are dissolved in a few ml. of water and re-evaporated to remove traces of acid. (For testing purposes water was again added and evaporated.) After final drying the vial lids are replaced and the samples stored until assayed.

For comparing the abridged survey method with the conventional quantitative method, hydrolysates prepared by each were analyzed by the ion-exchange chromatographic method of Spackman, Stein, and Moore (2); a Phoenix automatic amino acid analyzer was used, modified with Beckman spherical resins and high pressure columns (12). Nitrogen concentrations of diluted hydrolysates were determined by micro-Kjeldahl analyses (13).

It has been found that one operator, carrying out the survey procedure, can hydrolyze 100 samples per day the first 3 days of a week. He can filter and

evaporate the HCl the day each batch is removed from the 110° oven. Utilizing the weekend for final drying, he can easily prepare 300 samples each week for analysis.

RESULTS

A number of experiments were carried out to establish the validity of several specific short-cuts taken and of the over-all method. In a preliminary experiment, whole thin-hulled, brown-striped safflower seeds (19% hull, 51% oil) and ground, defatted seeds from the same batch were hydrolyzed and analyzed by the conventional high-precision procedure. Analyses showed no difference in the lysine-nitrogen ratios. Hence, high levels of oil provided no interference. Further, the results showed that the seeds did not have to be ground prior to hydrolysis. Since commercial types of safflower have a much higher hull content and the hulls are extremely tough and dense, it was thought that further experiments were needed to demonstrate the feasibility of hydrolyzing whole seeds. The results, in Table I, show the comparison of analyses of whole commercial safflower seeds (33% hull, 40% oil) hydrolyzed by the survey method with analysis of ground defatted seed from the same batch hydrolyzed by the conventional hydrolysis procedure.

TABLE I. EFFECT OF HYDROLYSIS METHODS ON APPARENT LYSINE CONTENT OF SAFFLOWER[a]

Sample and Hydrolysis Method	Weight g.	Lysine μM/ml.	N mg./ml.	Ratio No.[b] μM lysine/mg. N	Lysine g./16 g. N
Defatted meal: procedure A	0.040	0.194	0.158	1.23	2.87
	0.040	.206	.165	1.25	2.92
				Mean 2.90	Std. Dev. 0.0289
Whole seeds: survey	2.0	.356	.279	1.28	2.98
	2.0	.388	.292	1.33	3.11
	2.0	.287	.235	1.22	2.86
	2.0	0.236	0.188	1.26	2.94
				Mean 2.97	Std. Dev. 0.1044

[a] Commercial thick-hulled variety, 15.4% protein.
[b] An easily calculated value for reporting survey results. Once a typical value has been established, further calculations to desired units would be necessary for only those samples deviating from the normal.

Although the actual levels of lysine and nitrogen varied markedly among the survey hydrolyzed procedure samples, the ratios were the same for all, so that the values for g. lysine/16 g. nitrogen checked quite well. This shows that the hydrolysis was complete in spite of the intact thick hulls and oil content. Also, varying solution losses subsequent to hydrolysis are unimportant since the amounts of lysine and nitrogen lost were proportionate. Hence, it was established that there is no need for quantitative manipulations prior to the actual determinations of lysine and nitrogen on the final aliquot.

Interest in surveying for high-lysine varieties extends to grain commodities as well as oilseeds. Therefore, the survey hydrolysis method was tried on whole-grain samples of wheat, barley, red milo, and white milo. At the same time a portion of each of these samples was finely ground and hydrolyzed by the conventional procedure. Table II shows the results obtained. Individual sample results are presented to show the reproducibility of lysine and nitrogen ratios by each method.

For grain commodities, as opposed to oilseeds (i.e. safflower), the survey method of hydrolysis gives lysine values 9 to 11% higher than those of the conventional method. We attribute the higher lysine-to-nitrogen ratios obtained in the survey procedure to increased losses of humin nitrogen caused by the grain's high carbohydrate content relative to the amount of hydrolyzing agent. This would presumably be the case with other high-carbohydrate commodities as well. However, since the survey method of hydrolysis gives reproducible results, the slightly elevated g. lysine/16 g. N values would not affect the value of the method for selecting high individual samples from a breeding study or a world collection. The precise lysine figures would be determined on samples of interest by the conventional procedure.

In conclusion, the survey hydrolysis procedure affords the following time-saving

TABLE II. EFFECT OF HYDROLYSIS METHODS ON APPARENT LYSINE CONTENT OF GRAINS

	Procedure A g. lysine/16 g. N	Survey Procedure g. lysine/16 g. N
Wheat (15.1% protein)	2.68 2.50 2.44	2.75 2.66 2.83 2.82
Mean Std. Dev.	2.54 0.1249	2.77 0.0785
Barley (11.6% protein)	3.39 3.29	3.65 3.75 3.68 3.72
Mean Std. Dev.	3.34 0.0707	3.70 0.0440
Red milo (10.4% protein)	2.11 2.07	2.32 2.30 2.34
Mean Std. Dev.	2.09 0.0283	2.32 0.0200
White milo (10.3% protein)	1.99 1.95	2.13 2.18 2.16 2.20
Mean Std. Dev.	1.97 0.0283	2.17 0.0298

advantages over the conventional procedure for use in screening studies: 1) elimination of sample grinding, 2) elimination of fat-extraction steps, 3) samples need not be weighed, 4) no deoxygenation of hydrolysis mixture necessary, 5) quantitative recoveries are not required, 6) simultaneous oven evaporations of 100 or more samples.

Thus, the stated purpose of the method has been accomplished, namely, the ability to hydrolyze thousands of samples not practical by conventional means, owing to unreasonable labor and equipment demands. Systematic errors introduced by the modifications do not affect its use for screening purposes.

The described procedure and equipment has now been utilized in a survey of the world collection of safflower seeds involving over 2,000 samples. Future papers will describe the survey and the results obtained.

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