LIBERATION OF LYSINE BY ACID AND ENZYMES FROM HEATED LYSINE-GLUCOSE MIXTURE

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Studies on the formation of humin in protein hydrolysates have demonstrated that heating causes a reaction between carbohydrates and certain amino acids (1–3). This reaction, often referred to as the Maillard reaction, has significance in connection with the development of brown color during the processing of foods. Further, this reaction appears to have nutritional importance, since recently it has been shown (4, 5) that heating reducing sugars in the presence of amino acids causes the latter to become nutritionally unavailable to chicks and microorganisms. Indeed, Patton et al. (6) have suggested that the decreased availability to chicks and microorganisms of lysine in heat-damaged soy bean oil meal (7, 8) is a result of this type of reaction.

Riesen et al. (8) and Clandinin (9) have shown that the liberation of lysine from heat-damaged protein materials by enzymatic hydrolysis is much lower than by acid hydrolysis. These workers suggest that values obtained by enzymatic hydrolysis give a more reliable index of the nutritive value of heat-treated protein materials than do those by acid hydrolysis.

The present study was undertaken to determine whether or not the heating of a lysine-glucose mixture in an autoclave for different periods of time affects the liberation of lysine by acid and enzymatic hydrolysis in a manner similar to that reported (8, 9).

Methods

Samples 1 to 9 (Table I), in quadruplicate, of 50 mg. of L-lysine monohydrochloride monohydrate (equivalent to 36.5 mg. of L-lysine) and Samples 10 to 18, in quadruplicate, of 50 mg. of L-lysine monohydrochloride monohydrate and 250 mg. of glucose, were placed in 125 ml. Erlenmeyer flasks and heated in an autoclave at 15 pounds steam pressure for 0, 4, 7½, 15, and 30 minutes and for 1, 2, 4, and 8 hours respectively. A second series of samples, identical to these except that 5 ml. of distilled water were added to each flask, was heated in an autoclave in the manner referred to above.
After heat treatment two samples of each quadruplicate in both series were subjected to acid hydrolysis and two to enzymatic hydrolysis. Acid hydrolysates were obtained by hydrolyzing the samples with 25 ml. of 2 N hydrochloric acid for 5 hours in the autoclave at 15 pounds steam pressure. Enzymatic hydrolysates were prepared by digesting the sample with 75 mg. of pancreatin (Nutritional Biochemicals Corporation, 3 X U. S. P. potency) and 25 mg. of crude intestinal hog mucosa (The Wilson Laboratories) in 50 ml. of 0.2 M disodium phosphate buffer solution for 72 hours at 37°. The amount of L-lysine liberated was determined microbiologically according to the procedure employed by Riesen et al. (8).

In Table I the values reported represent averages of two assays in duplicate.

### RESULTS AND DISCUSSION

The heating of L-lysine, dry or in the presence of water, produced no visible browning of the lysine or lysine-water solution. On the other hand, the heating of a mixture of L-lysine and glucose, dry or in the pres-
ence of water, resulted in visible browning of the mixture. The degree of browning was more pronounced in the dry series than in the water series. Further, the degree of browning increased as the time of heating increased in much the same way as was noted in the samples of soy bean oil meal of Clandinin et al. (7). Indeed, heating the lysine-glucose mixture without water not only caused various degrees of browning but also decreased the water solubility of the residues to such an extent that microbiological assay on the residues without acid or enzymatic hydrolysis was considered inadvisable.

The results of the microbiological assays, shown in Table I, indicate that heating L-lysine alone in an autoclave at 15 pounds steam pressure for 8 hours has no appreciable effect on its availability after acid or enzymatic hydrolysis. On the other hand, it is apparent from the data that heating L-lysine in the presence of glucose has a pronounced effect on its availability subsequent to acid or enzymatic hydrolysis. Further, the reduction in availability of lysine in the heated lysine-glucose samples is much more pronounced when they are heated dry than in the presence of water. It will also be noted from the values reported that the greatest decrease in the availability of lysine in the dry mixtures is effected during the 1st hour of heating. It is of interest to point out here the parallel between these results and the marked decrease in availability of lysine reported by Clandinin (9) as occurring in fish meal that was overheated for a short time during flame drying.

The data show that, in the samples without water, enzymatic hydrolysis released considerably less lysine from the heated lysine-glucose samples than did acid hydrolysis. This finding suggests the formation, on heating in the absence of water, of an enzymatic-resistant, acid-non-resistant linkage between lysine and glucose, such as the one postulated by Evans and Butts (10). If this is the case, the reaction between lysine and carbohydrate might consist first of the formation of an enzymatic-resistant but acid-non-resistant linkage, followed by the formation of an enzymatic- and acid-resistant linkage or disintegration of the lysine molecule.

**SUMMARY**

1. The liberation of lysine by acid and enzymatic hydrolysis from a lysine-glucose mixture, heated in an autoclave at 15 pounds steam pressure for varying periods of time, was greatly decreased by the heat treatment.

2. The decrease in liberation of lysine was greater when the mixture was heated dry than when heated in the presence of water.

3. The liberation of lysine by enzymatic hydrolysis from a heated lysine-glucose mixture was less than the liberation of lysine by acid hydroly-
sis, suggesting the formation on heating of an enzymatic-resistant linkage between glucose and lysine.

BIBLIOGRAPHY
