# AVAILABLE LYSINE IN FOODSTUFFS

by 4509

NIPA SOMNAPAN

B. S. (Hons.), Chulalongkorn University, 1963

M. S., Mahidol University, 1965

Bangkok, THAILAND

---

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

FOOD SCIENCE

Department of Biochemistry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

Approved by:

A. B. Pansky

Major Professor

THIS BOOK CONTAINS NUMEROUS PAGES WITH THE ORIGINAL PRINTING BEING SKEWED DIFFERENTLY FROM THE TOP OF THE PAGE TO THE BOTTOM.

THIS IS AS RECEIVED FROM THE CUSTOMER.

TABLE OF CONTENTS

# I. INTRODUCTION

In many areas of the world people are undernourished, malnourished, or even starved. At the same time, in other parts of the world food is abundant to the point of substantial excess (1). Some excess food can be processed and stored for later use or shipped where needed with little or no deterioration.

One of the processing methods, heat, has both beneficial and deleterious effects on the nutritive value of proteins. Some beneficial effects are inactivation of the trypsin inhibitor, growth inhibitors, hemagglutinins, and other toxic factors present (2, 3). Adverse effects are decreased availability of certain essential amino acids by interactions among themselves or with other components of the food or feed, or alteration of the amino acid molecule (4). Loss of amino acids depends primarily on the severity of heat treatment; presence of binding agents such as glucose, gossypol, etc.; and moisture content of the food. Lysine and methionine are the indispensable amino acids apparently most subject to such changes (5). Lysine generally is the limiting amino acid in cereal grains, which represent the major source of protein for most of the poor populations.

Animal protein contains a relatively higher concentration of lysine, and when added to cereal diets, markedly improves the biological value of the diets (6). The most severe effect of processing animal protein appears to be a decrease in the nutritional availability of lysine, which reduces the value as protein supplement. It is, therefore, of practical importance that the processing of high-protein foods should be controlled so that damage to the lysine is minimized (7).

Severe heat processing of cereals, such as toasting, puffing, and gun explosion techniques, has been reported to cause drastic reduction in the

nutritive value of cereal proteins. Less severe heat treatment, such as cooking or baking, does not have that effect (8, 9).

Generally it is accepted that part of the lysine in foods can become "unavailable" by reactions of the free ε-amino group with reactive components of the food (non-enzymatic browning). In general, this reaction, referred to as the Maillard reaction, is defined as the reaction of the amino group of amino acids, peptides, or proteins with "glycosidic" hydroxyl group of sugars. This reaction is followed by other more complex changes which result eventually in the formation of brown pigments and polymers (10).

Reaction between aldoses and protein in the "dry" state were intensively investigated by Lea and co-workers (11-15). The literature on non-enzymatic browning is extensive and is not reviewed here, since the chemistry of the Maillard type of browning has been clearly explained on the reactions between aldoses and simple amines (16).

Overheating, particularly in the absence of water (dry heat or frying), can reduce the nutritive value of protein by destruction of essential amino acids such as lysine, which is heat labile, or by tying the amino acid up in new chemical linkages not susceptible to enzymatic digestion. Availability also may be decreased by high temperature (140-150°), which causes denaturation of the protein (17).

One important reason for the interest of the food industry in the phenomenon of browning is its relation to nutrition. The literature suggests three separate considerations in this regard (1):

1) lowered consumption of browned foods because of poor palatability, appearance, and physical properties;

2) loss of nutritional value due to vitamin destruction, lowered

digestibility of the protein, or loss of biological value due to essential amino acid destruction or unavailability;

3) production of toxic substances or metabolic inhibitors.

## II. REVIEW OF LITERATURE ON METHODS FOR DETERMINATION OF AVAILABLE LYSINE

### A. Chemical methods

Availability of lysine in all kinds of processed food has been studied by many investigators. Results of biological measurement of protein quality have been compared with values from chemical methods. The most common chemical method for measuring lysine availability is based on the Sanger reaction, used to study free-amino groups in purified proteins by reaction with 1-fluoro 2,4-dinitrobenzene (FDNB) to give stable dinitrophenyl (DNP) compounds (18). Lea and Hannan (13) utilized dinitrophenylation to study the reduction of ε-amino groups during processing of milk products and reported that the ε-amino groups of lysine side chains and the small quantity of free α-amino groups at the end of polypeptide chains are capable of reacting with FDNB. The α-amino groups are derived from at least five amino acids, one of which is lysine.

Schwartz and Lea (19) reinvestigated the nature and reactivity of the free amino groups of casein by the FDNB method (18) using paper chromatography on chlorinated rubber (20) for separation and identification of the 2,4-dinitrophenyl amino acids. Terminal α-amino groups of aspartyl, glutamyl, lysyl, phenylalanyl, and valyl residues were identified. The α-amino groups were found to react at approximately the same rate as the ε-amino group of the lysine side chain.

"Nutritionally available lysine" is based on the assumptions that the reagent FDNB reacts with the free ε-amino group of lysine in crude material used, that ε-DNP lysine is the only compound that remains to give an extinction after acid hydrolysis and ether extraction, and that only those lysine molecules reacting with FDNB are available to the animal. The high correlation with biological assays suggests that these assumptions are substantially true for the particular animal products tested (21).

Carpenter et al. (22) determined lysine in a series of fish, whale, and meat products by the FDNB procedure and found a close correlation with corresponding results of chick feeding tests, under conditions in which the lysine contribution of the test materials was the most important nutritional consideration. Baliga et al. (23) determined free lysine ε-amino groups in samples of cottonseed meal by an adaptation of Sanger's procedure based on the reaction with FDNB. A relationship was found between the content of lysine with free ε-amino groups and protein quality, as determined in rat protein repletion tests.

A modification of the original FDNB procedure recommended by Carpenter (7) apparently overcomes interference by free arginine to which the original method was subject. Carpenter reduced some of the error in the analyses by reacting the ε-DNP lysine with methoxycarbonyl chloride, forming ε-DNP-α-methoxycarbonyl lysine which becomes ether soluble, leaving DNP-arginine in solution (24). The difference in color intensity of the hydrolyzate before and after reaction with methoxycarbonyl chloride and extraction with ether was taken as a measure of available lysine. It was found that samples that had been heated and had shown decreased nutritional value in feeding tests also showed lower "available lysine" values in the chemical procedure.

Martinez and associates (25) reported the relative importance of gossypol and raffinose in binding and destroying lysine and in impairing the nutritive value of cottonseed meals, as determined by the rat repletion method. Their results were highly correlated with content of free ε-amino groups of lysine of the protein and poorly correlated with total lysine.

Carpenter and March (26) reported on the loss of available lysine by cooking. They determined available lysine by chemical analysis and by biological assays on chicks and found that the FDNB method had correctly indicated a severe reduction in the biologically available lysine in biscuits containing both vegetable and animal ingredients. Boyne et al. (27) reported on protein quality in feedingstuffs. Different types of protein concentrates were evaluated as supplements to cereal protein for chicks by the gross protein value (GPV) procedure and by the net protein utilization (NPU) procedure with rats, in which the test material is the sole source of protein in the diet. For fish, meat, and whale meals the "available lysine" procedure, based on the reactivity of ε-amino groups with FDNB, correlated best with the GPV results.

Rao et al. (28) determined available lysine in plant proteins. The protein was dinitrophenylated, hydrolyzed in acidic media, and the ε-DNP-lysine produced was eluted from an ion exchange column. The eluate was determined spectrophotometrically at 435 nm. Lyman and Thomas (29) determined available lysine in food materials which are high in carbohydrate content by treating the sample with FDNB and reducing the destructive action of carbohydrate by adding thiodiglycolic acid immediately prior to hydrolysis. They separated the DNP-lysine from extraneous colored materials by ion exchange chromatography and subsequently determined it spectrophotometrically at 363 nm. Rao and McLaughlan (30) determined available lysine by the method of Carpenter (7)

and methionine by bioassay techniques. There was a considerable decrease in the amounts of available lysine and methionine in samples autoclaved 20 and 40 minutes. Plasma lysine levels correlated well with the GPV and FDNB-available lysine. Results of Rao and McLaughlan showed that it was possible to use plasma amino acid levels as an index of the nutritional availability of certain amino acids in foodstuffs.

Bocter and Harper (5) measured available lysine in meat, casein, and egg albumin before and after heat treatment in the presence of glucose by the rat growth assay and by the FDNB method. The results showed a high correlation between the value obtained by the FDNB method and the growth assay procedure for untreated egg albumin, beef protein, and casein. A discrepancy occurred only with autoclaved protein preparations. The FDNB method, as recommended by Carpenter, would not take rate of digestion into account nor the possibility that after digestion of a severely processed "food," many of the lysine moieties reacting chemically as "available" might remain bound in digestible peptide residues, unavailable to the rat.

Blom et al. (17) developed a sensitive method for determination of available lysine based on chromatographic separation and polarographic detection of the eluents. The results were compared with those obtained with the FDNB method. Experimental evidence was obtained which showed that interferences in the determination of available lysine can be largely diminished by adding dinitrophenol before hydrolysis of the FDNB-treated sample. However, the FDNB method is laborious and time-consuming for the routine analysis of proteins.

Okuyama and Satake (31) reported that trinitrobenzenesulfonic acid (TNBS) reacted with only primary amino groups of amino acids and peptides in an aqueous solution at pH 8 and at room temperature without undesirable side reaction.

No reaction occurs with the imidazole nitrogen of histidine, the guanidinium group of arginine, or the hydroxyl group of tyrosine or threonine. The resulting TNP-peptides, as well as TNP-amino acids, had a high molar extinction coefficient at 340 nm which were of similar magnitude. Satake et al. (32) reported that amino acids, peptides, and primary amines could be assayed spectrophotometrically by the quantitative TNP-lation with TNBS. TNBS has been tentatively considered to be a useful reagent for elucidation of the conformational and the physiological significance of amino groups in protein molecules. However, TNP-amino acids and TNP-peptides are not so stable as the corresponding DNP-derivative. Thus, they are photo-susceptible and are decomposed by ammonia (32), as indicated in the following equations:

Photolysis

$$\text{TNP-NH-CHR-COOH} \xrightarrow{h\nu} \text{TNP-NH}_2 + \text{R-CHO} + \text{CO}_2$$

Ammonolysis

$$\text{TNP-NH-CHR-COOH} \xrightarrow{NH_3} \text{TNP-NH}_2 + \text{NH}_2\text{-CHR-COOH}$$

Kotaki and Satake (33) have revealed that the TNP-NH linkage of α-amino acid is much less stable than the DNP-NH linkage; thus TNP α-amino acids are cleaved into picric acid and the original amino acids by acid or alkali.

$$\text{TNP-NH-CHR-COOH} \longrightarrow \text{TNP-OH} + \text{NH}_2\text{-CHR-COOH}$$

acid : 6N HCl, 110°, 8 hr

alkali: N NaOH, room temperature, 3 hr

Such a susceptibility of TNP-NH linkage provides some advantages as well as some restrictions for use of the TNP method in protein chemistry. TNP ε-NH linkages were rather resistant to the conditions, and more than 60% were capable of remaining intact even after the prolonged heating for 24 hours in 6N HCl. Habeeb (34) used TNBS for determination of the free amino groups in proteins and to measure the free amino groups of bovine serum albumin after

reaction with formaldehyde. Mokrasch (35) modified the method of Satake et al (32) with an increase in sensitivity and selectivity. He observed that the absorption spectrum of the product of the reaction between TNBS and derived amines is not identical to the absorption spectrum of the corresponding purified TNP-derivative. Mixtures of amines, amino acids, and proteins can be analyzed quantitatively for their constituents by selective extraction of the TNP-derivatives. Amines and most amino acids may be analyzed by the absorbance of the TNP-derivatives at 420 nm. Aromatic amino acids, proteins, and TNP-derivatives extracted into organic solvents can be estimated by absorbance near 340 nm.

Kakade and Liener (36) in 1969 reported a method involving the use of TNBS to determine specifically the lysine content of protein foodstuffs. The specificity of the technique for the ε-amino groups of proteins resides in the fact that, subsequent to acid hydrolysis of the TNP-protein, the α-TNP-amino acids may be extracted with ether whereas ε-TNP-lysine remains in the aqueous phase (33). The amounts of available lysine were similar to those obtained with the more commonly used FDNB method. This newer method could be applied with considerable accuracy to the determination of lysine in a low-molecular-weight protein such as insulin with relatively few lysine residues, where the total number of N-terminal amino groups exceeds the number of free ε-amino groups. The method is particularly useful for the determination of lysine residues in peptides as well as proteins composed of several peptide chains or subunits. Only in the unique situation in which lysine was the N-terminal amino acid would difficulty be encountered since, in this instance, α,ε-bis-TNP-lysine or small peptides containing this derivative would be extracted into the ether phase and thus would escape detection. This method has the advantages of simplicity, ease of handling, and rapidity. The TNBS method

should prove to be a valuable analytical tool in the routine evaluation of the nutritive value of protein foodstuffs in which lysine is the limiting amino acid or becomes limiting as a result of processing.

## B. Biological methods

Kuiken (37) estimated the availability of lysine in a few proteins by measuring quantity of ingested lysine excreted in the feces and found that lysine was more sensitive to heat destruction than other essential amino acids. Approximately a 10% reduction in available lysine was observed when solvent-processed cottonseed meal was autoclaved at 15 lb pressure for 60 minutes. Using techniques developed by Schweigert and Guthneck (38), Guthneck et al. (39) used the protein-depleted adult male rat for studying the quantitative utilization of lysine. They showed that a greater amount of lysine was excreted in the feces of animals fed cereal foods and unheated soybean flakes than those fed other test products. There was better utilization of lysine from dried eggs and skim milk (91%) and from fresh meats (84%) than from cereal and legume (71%) and cooked or processed meat (70%).

Gupta et al. (40) determined the availability of lysine from proteins using the growth of weanling rats as the index of availability. Availability was based on weight gains after correcting for differences in food consumption. The lysine content of the proteins and of the feces was determined by the microbiological procedure with Leuconostoc mesenteroides as the test organism. The availability of lysine from beef was 76%, from gelatin 74%, and that in other purified proteins tested was 90 to 104%. Values for the availability of the lysine in three cereal proteins differed considerably. The value for rice being highest, with those for wheat and corn following in order. The lysine

of roller-dried skim milk was less available than that of a spray-dried preparation.

Heller et al. (41) examined the effect of heating upon the availability of lysine from meat. The rate of body weight gain in the male weanling rat was used as the criterion for evaluating the utilization of lysine as determined microbiologically from unheated and heat processed meat samples. The apparent amount of available lysine was not affected markedly by any of the heat treatments used. The percentage of available lysine in pork, beef, and lamb muscle ranged from 74 to 92 in either raw samples or after standard cooking procedures. Severe heat treatment (autoclaving for 16 hours) substantially reduced the available lysine in those products.

Carpenter et al. (42) reported on the use of weight gains of young chicks as a measure of response to dietary sources of lysine. They compared the amount of lysine from chick growth assay results with chemical analysis for available lysine by the FDNB method and for total lysine in acid hydrolyzates of the test materials. For the two samples of highest quality, the biological assay values were slightly higher than those by chemical analysis.

De Muelenaere et al. (43) determined the availability of lysine in corn and rice proteins by growth and fecal analysis. The influence of the type of carbohydrate, amino acid pattern of the diet, and caloric content of the diet on these methods were influenced by changes in the composition of the diet and the method of calculating availability. Groups fed the basal amino acid diet lost weight, as did those receiving the smaller supplements of lysine. Groups fed the standard diets containing quantities of lysine that approximated the optimal requirements grew satisfactorily; a group receiving 1% L-lysine HCl gained 38.6 g/week. Availability values estimated by the fecal analysis also

show a slight decrease with increasing levels of protein in the diet but less than was observed for values obtained with the growth method.

## III. PURPOSE

To evaluate the use of the chemical method of Kakade and Liener (36) for determining reduction of available lysine when proteins were processed by heat treatment alone or in mixtures with carbohydrate, minerals, or oil.

## IV. MATERIALS

### A. Samples and sources

Isolated soybean protein (α-protein). Nutritional Biochemical Corporation, Cleveland, Ohio.

Fish protein concentrate. Viobin Corporation, Monticello, Illinois.

Casein. Nutritional Biochemical Corporation, Cleveland, Ohio.

Dried milk (spray-dried nonfat milk powder). Bennett Creamery, Ottawa, Kansas.

Glucose.

Cornstarch.

Cottonseed oil (Wesson oil).

Minerals (salts of $CuSO_4.5H_2O$, $Fe(NH_4)_2(SO_4)_2.6H_2O$, $Fe_2(SO_4)_3.xH_2O$).

Chick-peas (Cicer arietinum). Supplied by Department of Grain Science and Industry, K.S.U., Manhattan, Kansas.

Commercial soybean meal, extracted soybean meal, and processed soybean meal. Supplied by Department of Grain Science and Industry, K.S.U., Manhattan, Kansas.

## B. Chemical reagents

Sodium bicarbonate. ACS reagent grade, 4% aqueous solution.

Conc. hydrochloric acid. ACS reagent grade.

2,4,6-trinitrobenzenesulfonic acid. 1% aqueous solution, Eastman Organic Chemicals, Eastman Kodak Company, Rochester, N.Y.

Diethyl ether, anhydrous. ACS reagent grade.

## C. Instruments and apparatus

Analytical balance.

Autoclave. Operating pressure 15 psi, Barnstead Still & Sterilizer Co.

Electrically heated oven. Set at 150°.

Water bath. Provided with temperature control and shaking mechanism.

Spectrophotometer. Beckman, Model DU, provided with silica cells.

Filter paper (Whatman no. 50). Size 5.5 cm.

Glassware. Test tube, pyrex, size 20x150 mm;
centrifuge tube with lip, size 50 ml;
centrifuge tube with a tight-fitting screw cap containing a teflon liner, size 50 ml;
glass funnel, size 4.0 cm;
pipette, graduated, 1 ml, 10 ml, 15 ml.

# V. PROCEDURE

## A. Method of analysis

The method of Kakade and Liener (36) was followed. Weigh 20 mg of finely ground sample into a 20x150 mm test tube. Add 1 ml of 4% $NaHCO_3$, pH 8.5, then shake sample 10 minutes in a water bath at 40°. Add 1 ml of freshly prepared 1% trinitrobenzenesulfonic acid. Continue shaking in dim light for 2 hours. Add 3 ml of conc. HCl and invert a glass vial (50-ml centrifuge tube with lip) cover over the top of each tube. Autoclave under pressure 15 psi for 1 hour. Allow the hydrolyzate to cool to room temperature and filter through a Whatman no. 50 filter paper into a 50-ml glass tube provided with a tight-fitting screw cap containing a teflon liner; rinse the hydrolyzate from the test tube twice with 2.5 ml distilled water. At this stage the filtrate measures 10 ml. Extract twice by shaking the hydrolyzate with 10 ml diethyl ether for 1 minute in the capped tube to remove TNP-N-terminal amino acid, peptides, and picric acid. Remove the ether (upper) phase by aspiration each time. Eliminate residual ether from the aqueous phase by placing the uncovered tube in hot water for at least 5 minutes. Dilute the aqueous solution 1:40 with distilled water and read absorbance at 346 nm using a Beckman Model DU spectrophotometer. Prepare a blank by the same procedure except that the conc. HCl is added to the sample before adding TNBS reagent.

Calculate available lysine in gram per 100 grams of sample using the molar absorptivity value of ε-TNP-lysine, $1.46 \times 10^4$ $M^{-1}$ $cm^{-1}$.

Example of calculation:

Molar absorptivity of ε-TNP-lysine = $1.46 \times 10^4$ $M^{-1}$ $cm^{-1}$.

$$C = \frac{A}{1.46 \times 10^4}$$

where C = concentration in mole/liter

A = absorbance by spectrophotometer at 346 nm

1 mole of lysine = 146.2 g

If A = 0.250, dilution = 1:40, and

protein concentration is 20 mg/10 ml aqueous solution, then

$$\text{available lysine}^* = 146.2 \times \frac{0.250 \times 40}{1.46 \times 10^4} \times \frac{10}{10^3} \times 10^2 \times \frac{10^3}{20}$$

$$= 5.00 \text{ g}$$

*Available lysine in g per 100 g of protein.

## B. Preliminary experiment to determine sample size

To mix the sample thoroughly with carbohydrate and decrease the sampling error, the amount of the sample was increased from 10 mg, as stated in the original method, to 20 mg. In the experiment to determine the proper weight of the sample, 10, 20, and 30 mg samples were used and amount of available lysine was determined. The data (Table I) showed that increasing the sample size to 30 mg caused greater variation in results on replicated samples than was found on samples of 10 mg or 20 mg. Since results were more consistent on 20 mg samples, that size sample was chosen as weight for use in this experiment.

TABLE I

Absorbances of ε-TNP-lysine at 346 nm, when different sizes of sample were used (each sample determined in triplicate)

| | absorbance at 346 nm | | | |
|---|---|---|---|---|
| | a | b | c | average |
| Isolated soybean protein | | | | |
| 10 mg | .125 | .132 | .127 | .128 |
| 20 mg | .228 | .230 | .222 | .226 |
| 30 mg | .352 | .367 | .345 | .354 |
| Fish protein concentrate | | | | |
| 10 mg | .138 | .141 | .135 | .138 |
| 20 mg | .275 | .278 | .300 | .284 |
| 30 mg | .420 | .480 | .448 | .434 |
| Casein | | | | |
| 10 mg | .177 | .170 | .190 | .179 |
| 20 mg | .342 | .340 | .336 | .339 |
| 30 mg | .508 | .532 | .510 | .516 |

## C. Samples used and sample preparation

For investigating the effect of heat on proteins and on the mixtures of proteins and carbohydrate, minerals, and oil, the following procedures were used to prepare the sample:

Protein-carbohydrate mixtures. Since the samples of protein or carbohydrate were not fine enough, each sample was ground finely in mortar. For studies of protein-carbohydrate mixtures, 20 mg of each protein and of carbohydrate was weighed and mixed together thoroughly in a test tube.

Protein-mineral mixtures. $CuSO_4.5H_2O$, $Fe(NH_4)_2(SO_4)_2.6H_2O$ and $Fe_2(SO_4)_3.xH_2O$ were prepared in aqueous solution at concentrations of 0.025 g/ml. A quantity of 0.04 ml of salt solution was pipetted onto 20 mg of finely ground protein in a test tube.

Protein-oil mixtures. One drop of cottonseed oil was mixed with the 20 mg of finely ground sample of protein.

Processed proteins. 20 mg of finely ground processed protein was used.

The various samples and treatments used are summarized for convenience in Table 2.

## D. Statistical analysis

Correlation test (44) was used to determine the correlation coefficient (r) of protein efficiency ratio (PER) and of the average weight gain of rat[1] with available lysine of chick-peas used in diets in a rat growth test.

---

[1]Athia Bano's unpublished report, 1969.

TABLE 2

Sample and experimental conditions used

| | Processing time in hr | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | $\frac{1}{4}$ | $\frac{1}{2}$ | $\frac{3}{4}$ | 1 | $1\frac{1}{2}$ | 2 | 6 | 8 |
| Protein | | | | | | | | | |
| Casein | | | | | | | | | |
| dry heat[1] | +[3] | - | + | - | - | - | + | - | + |
| moist heat[2] | + | - | + | - | - | - | + | - | + |
| Fish protein concentrate | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| moist heat | + | - | + | - | - | - | + | - | + |
| Isolated soybean protein | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| moist heat | + | - | + | - | - | - | + | - | + |
| Protein + carbohydrate | | | | | | | | | |
| Casein + glucose | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| moist heat | + | - | + | - | - | - | + | - | + |
| Casein + starch | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| moist heat | + | - | + | - | - | - | + | - | + |
| Fish protein concentrate + glucose | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| moist heat | + | - | + | - | - | - | + | - | + |
| Fish protein concentrate + starch | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| moist heat | + | - | + | - | - | - | + | - | + |
| Isolated soybean protein + glucose | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| moist heat | + | - | + | - | - | - | + | - | + |
| Isolated soybean protein + starch | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| moist heat | + | - | + | - | - | - | + | - | + |
| Dried milk (casein + galactose) | | | | | | | | | |
| dry heat | + | - | + | - | - | - | + | - | + |
| Protein + minerals | | | | | | | | | |
| Isolated soybean protein + $CuSO_4.5H_2O$ | | | | | | | | | |
| dry heat | + | - | - | - | - | - | + | + | - |

TABLE 2 (continued)

| | Processing time in hr | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | $\frac{1}{4}$ | $\frac{1}{2}$ | $\frac{3}{4}$ | 1 | $1\frac{1}{2}$ | 2 | 6 | 8 |
| Isolated soybean protein + $Fe(NH_4)_2(SO_4)_2.6H_2O$ dry heat | + | - | - | - | - | - | + | + | - |
| Isolated soybean protein + $Fe_2(SO_4)_3.xH_2O$ dry heat | + | - | - | - | - | - | + | + | - |
| Protein + oil (Wesson) | | | | | | | | | |
| Isolated soybean protein + oil dry heat | + | - | + | - | + | + | + | - | - |
| Isolated soybean protein, ether-extracted after processing dry heat | - | - | - | - | - | - | + | - | - |
| Isolated soybean protein + oil, ether-extracted after processing dry heat | - | - | - | - | - | - | + | - | - |
| Foodstuffs | | | | | | | | | |
| Raw chick-pea | + | - | - | - | - | - | - | - | - |
| Chick-pea, heated in oven at 200° | - | - | + | - | - | - | - | - | - |
| Chick-pea, heated in oven at 250° | - | + | + | - | - | - | - | - | - |
| Chick-pea, heated in oven at 400° | - | + | + | - | - | - | - | - | - |
| Chick-pea, heated in oven at 450° | - | - | - | + | - | - | - | - | - |
| Chick-pea, steamed and dried | - | + | - | - | - | - | - | - | - |
| Commercial soybean meal | + | - | - | - | - | - | - | - | - |
| Nonfat soybean meal | + | - | - | - | - | - | - | - | - |
| Soybean meal | Final temp. meal from processing equipment was 250° F | | | | | | | | |
| Soybean meal | Final temp. meal from processing equipment was 300° F | | | | | | | | |

[1]Dry heat in an oven at 150°.
[2]Moist heat in an autoclave under pressure of 15 lb.
[3]Indicates trials conducted.

# VI. RESULTS

Influence of heat treatment on available lysine of casein, fish protein concentrate, and isolated soybean protein is presented in Table 3. There was little or no reduction of available lysine when the protein sample was heated in the oven or in the autoclave under a pressure of 15 lb for a half-hour. Dry heat at 150° reduced available lysine 10 to 20% in 2 hours and 43 to 48% in 8 hours. Moist heat under a pressure of 15 lb caused a lesser reduction in available lysine, only 3 to 10% in 2 hours, and 19 to 21% in 8 hours.

The available lysine in mixtures of protein and carbohydrate processed in a drying oven at 150° is shown in Table 4. Available lysine in samples processed in an autoclave is shown in Table 5.

TABLE 3

Effect of heat on available lysine of proteins
(g/100 g of sample)

| Sample | Processing time in hr | | | |
|---|---|---|---|---|
| | 0 | $\frac{1}{2}$ | 2 | 8 |
| Casein | | | | |
| dry heat[1] | 6.25 | 6.15 | 5.00 | 3.24 |
| moist heat[2] | 6.10 | 5.66 | 5.50 | 5.00 |
| Fish protein concentrate | | | | |
| dry heat | 5.60 | 5.50 | 4.92 | 3.14 |
| moist heat | 5.68 | 5.50 | 5.52 | 4.56 |
| Isolated soybean protein | | | | |
| dry heat | 4.30 | 4.30 | 3.66 | 2.46 |
| moist heat | 4.28 | 4.30 | 4.16 | 3.36 |

[1]Dry heat in an oven at 150°.
[2]Moist heat in an autoclave under pressure of 15 lb.

TABLE 4

Effect of dry heat at 150° on available lysine in mixtures of protein and carbohydrate (g/100 g of protein sample)

| Sample | Processing time in hr | | | |
|---|---|---|---|---|
| | 0 | ½ | 2 | 8 |
| Casein + glucose | 5.28 | 5.18 | 4.30 | 3.10 |
| Casein + starch | 5.90 | 6.15 | 4.83 | 3.28 |
| Fish protein conc. + glucose | 5.38 | 4.04 | 3.20 | 1.16 |
| Fish protein conc. + starch | 5.30 | 4.66 | 4.02 | 3.22 |
| Isolated soybean protein + glucose | 3.64 | 3.50 | 3.04 | 2.42 |
| Isolated soybean protein + starch | 4.18 | 4.02 | 3.30 | 2.48 |
| Dried milk[1] | 2.06 | 1.03 | 0.86 | 0.26 |

[1]Contains casein and lactose plus minerals and traces of other substances.

TABLE 5

Effect of moist heat at 120° (15 psi) on available lysine in mixtures of protein and carbohydrate (g/100 g of protein sample)

| Sample | Processing time in hr | | | |
|---|---|---|---|---|
| | 0 | ½ | 2 | 8 |
| Casein + glucose | 5.00 | 2.58 | 1.44 | 0.50 |
| Casein + starch | 5.58 | 5.48 | 5.36 | 4.46 |
| Fish protein conc. + glucose | 5.32 | 1.98 | 1.18 | 0.08 |
| Fish protein conc. + starch | 5.30 | 4.70 | 4.58 | 4.10 |
| Isolated soybean protein + glucose | 3.68 | 2.30 | 0.44 | 0.40 |
| Isolated soybean protein + starch | 3.88 | 3.96 | 3.16 | 3.12 |

Dry heat at 150° reduced available lysine in protein-glucose mixtures 16 to 40% in 2 hours and 33 to 78% in 8 hours. In protein-starch mixtures the available lysine was reduced 18 to 24% in 2 hours and 39 to 44% in 8 hours.

Autoclaving under a pressure of 15 lb reduced available lysine in protein-glucose mixtures drastically: 37 to 62%, 71 to 88%, and 89 to 98% in a half-hour, 2 hours, and 8 hours, respectively. In protein-starch mixtures the available lysine was reduced 4 to 18% in 2 hours and 19 to 23% in 8 hours.

Results of effects of various processing treatments on available lysine in feedingstuffs (chick-peas and soybean meal) are in Table 6. The available lysine in raw soybean meal was higher than in meal processed at 300° F, but not so high as in meal processed at 250° F. The reduction of available lysine in chick-peas when processed in 15 minutes at 250° or 400° was 21%. On processing for 30 minutes the available lysine in chick-peas was reduced 8% at 200°, 24% at 250°, and 29% at 400°.

Results of an investigation on the effect of dry heat on available lysine of processed protein-mineral mixtures are presented in Table 7. The quantity of available lysine in the mixtures of isolated soybean protein and minerals, as compared with that in the controls, showed that presence of minerals had essentially no effect on available lysine of the protein when the mixtures were heated for as much as 6 hours at 150°.

Effect of dry heat at 150° on available lysine in the mixtures of protein and oil was investigated. Tests were made to determine whether the oil or oxidation products of the oil reacted with lysine to decrease availability or whether the oil per se interfered in the reactions.

After processing the protein-oil mixture for 2 hours, the mixture was extracted twice with ether before determining the available lysine in the

resulting protein. Another sample was analyzed without extracting the oil. The results are in Table 8. Apparently lysine was reduced markedly when oil was present, but the amount of available lysine in the sample processed without oil (control) was almost the same as in the sample processed with oil but from which oil was extracted after processing. Since lysine was not tied up in a nonreversible form, such as in the browning reaction, it is possible that the oil in some way interfered in the analytical reactions.

The nutritive value of chick-peas was determined by comparing the amount of available lysine by the Kakade and Liener method (36) with the average weight gain and the protein efficiency ratio (PER) from a rat growth test[1] in which chick-peas were used as feedingstuffs. This comparison is presented in Table 9.

The correlation coefficient (44) between the average weight gain in rats and the available lysine was 0.9576 (n=6), whereas the correlation coefficient between the PER and the available lysine was 0.9611 (n=6).

---

[1]Athia Bano's unpublished report, Food and Feed Grains Institute, K.S.U., 1969.

TABLE 6

Available lysine of raw and processed soybeans and chick-peas (g/100 g of sample)

| Code | Treatment | g/100 g |
|---|---|---|
| 70-58 | Commercial soybean meal | 1.95 |
| 70-55 | Nonfat soybean meal | 2.24 |
| 70-59 | Soybean meal, processed at 250° F[1] | 1.91 |
| 70-60 | Soybean meal, processed at 300° F[1] | 1.49 |
| 70-61 | Raw soybean | 1.65 |
| MC-1 -70 | Raw chick-pea | 1.39 |
| MC-2A-70 | Chick-pea, heated oven at 400°, 30 min | 0.98 |
| MC-2B-70 | Chick-pea, heated oven at 400°, 15 min | 1.10 |
| MC-2C-70 | Chick-pea, heated oven at 250°, 30 min | 1.06 |
| MC-2D-70 | Chick-pea, heated oven at 250°, 15 min | 1.10 |
| MC-2E-70 | Chick-pea, heated oven at 450°, 45 min | 0.64 |
| MC-4 -70 | Chick-pea, steamed for 15 min and dried | 1.38 |
| MC-6 -70 | Chick-pea, heated in oven 200°, 30 min | 1.28 |

[1]Temperature at which meal left processing equipment. These temperatures given on Fahrenheit scale.

TABLE 7

Effect of dry heat at 150° on available lysine of isolated soybean protein-mineral mixtures (g/100 g protein)

| Sample | Processing time in hr | | |
|---|---|---|---|
| | 0 | 2 | 6 |
| Isolated soybean protein | 4.38 | 3.60 | 3.00 |
| Isolated soybean protein + $CuSO_4.5H_2O$ | 4.22 | 3.84 | 3.04 |
| Isolated soybean protein | 4.40 | 3.86 | 3.38 |
| Isolated soybean protein + $Fe(NH_4)_2(SO_4)_2.6H_2O$ | 4.02 | 3.88 | 3.20 |
| Isolated soybean protein | 4.40 | 3.88 | 3.20 |
| Isolated soybean protein + $Fe_2(SO_4)_3.xH_2O$ | 4.18 | 4.00 | 3.23 |

TABLE 8

Effect of dry heat at 150° on available lysine of isolated soybean protein-oil mixtures (g/100 g protein)

| Sample | Processing time in hr | | | | |
|---|---|---|---|---|---|
| | 0 | $\frac{1}{2}$ | 1 | $1\frac{1}{2}$ | 2 |
| Isolated soybean protein | 4.40 | 4.26 | 4.20 | 4.20 | 3.64 |
| Isolated soybean protein + oil | 4.37 | 4.10 | 2.64 | 1.42 | 0.62 |
| Isolated soybean protein, ether extracted after processing | - | - | - | - | 3.68 |
| Isolated soybean protein + oil, ether extracted after processing | - | - | - | - | 3.62 |

TABLE 9

Comparison of the average weight gain, the PER value in rat growth test[1] with available lysine (g/100 g of sample)

| | average weight gain[2] 0-4 weeks | average PER | available lysine g/100 g |
|---|---|---|---|
| Chick-pea, raw | 132.6 | 2.1 | 2.0 |
| Chick-pea, popped | 165.6 | 2.7 | 3.4 |
| Chick-pea, roasted 15 min | 140.0 | 2.3 | 2.0 |
| Chick-pea, roasted 30 min | 75.0 | 1.6 | 1.7 |
| Chick-pea, steamed 15 min | 142.8 | 2.4 | 1.9 |
| Chick-pea, steamed 30 min | 110.0 | 1.9 | 1.9 |

[1]Athia Bano's unpublished report, Food and Feed Grains Institute, K.S.U., 1969.

[2]Weight gain in g.

# VII. DISCUSSION

The lysine in the samples of protein such as casein, fish protein concentrate, and isolated soybean protein became less available, as determined by the method of Kakade and Liener (36), when proteins were subjected to dry heat treatment (150°) or moist heat (120° in an autoclave at 15 psi). After 2 hours dry heat reduced available lysine 10 to 20% and moist heat 3 to 10%. After 8 hours dry heat reduced available lysine 43 to 48% and moist heat 19 to 21%. When dry heat was applied to the mixtures of protein and glucose, available lysine was reduced 16 to 40% in 2 hours and 33 to 78% in 8 hours. After treatment with moist heat, available lysine in the mixtures of protein and glucose was reduced 37 to 62% in a half-hour, 71 to 88% in 2 hours, and almost 100% in 8 hours. Results at a half-hour were similar to those found by Rao and McLaughlan (30) who found decrease of available lysine was 65 to 68% in samples autoclaved 20 and 40 minutes.

In the absence of glucose, the moist heat treatment caused less reduction in available lysine of proteins than dry heat, but in the presence of glucose, the moist heat treatment caused greater reduction than dry heat.

Dry heat probably caused reduction of available lysine by both direct decomposition and the browning reaction (16, 17). The reduction of available lysine by moist heat was caused by the same reactions (16, 17), with, however, increased browning due to high humidity (13). The ε-amino groups of lysine are key reactants in the browning mechanism (1).

It was observed that the reduction of available lysine in the processed fish protein concentrate-glucose mixture was greater than in the casein-glucose mixture or the isolated soybean protein-glucose mixture. There is no apparent explanation for those differences unless, perhaps, the high

concentration of minerals in fish protein concentrate catalyzed the reaction.

When protein-starch mixtures were treated by either dry heat or moist heat, the reduction of available lysine was in the same range as in the sample of pure protein only. The reduction of available lysine was caused by glucose and not by starch, possibly because of the smaller number of reducing groups in starch than in glucose.

The browning reaction which occurred when dried milk was processed by dry heat at 150° reduced available lysine 50%, 58%, and 87% in 1/2, 2, and 8 hours, respectively. Neither casein nor lactose brown readily when heated alone but they do so when heated together (45-48).

Conkerton et al. (49) reported that autoclaving cottonseed meal for 2 hours changed the type of protein material and reduced the lysine 40%. Evans and Butts (50), who studied the effect of heat on soybean oil meal, reported that 40% of the lysine was destroyed by 4 hours of autoclaving. Kuiken (37) determined available lysine by a rat feeding method and reported a 10% reduction of available lysine when solvent-processed cottonseed meal was autoclaved 15 psi for 1 hour. Netke and Scott (51) determined available lysine in soybean oil meal by chick growth assay and found that autoclaving soybean oil meal reduced available lysine 26% in 2 hours and 40% in 3 hours.

In Table 6 are the results from the present studies when various samples of raw and processed soybeans and chick-peas were analyzed for the amount of available lysine. In short-time (15 minutes) processing of chick-peas at 250° or 400° the available lysine was reduced 21%. When chick-peas were processed for 30 minutes at different temperatures, the available lysine was reduced 8% at 200°, 24% at 250°, and 29% at 400°. Increasing the processing time at 400° from 30 minutes to 45 minutes did not cause appreciable further decrease in

available lysine. The maximum reduction of available lysine occurred when chick-peas were heated in the oven at 450° for 45 minutes. When chick-peas were steamed 15 minutes and dried, the available lysine was almost the same as in raw chick-peas. Increasing the temperature of processed soybean meal from 250° F to 300° F reduced available lysine 22%. It was noted that there were no differences in the available lysine in commercial soybean meal and processed soybean meal at 250° F.

Although conditions were different so that results cannot be compared directly, reductions of available lysine by autoclaving protein-glucose mixtures in the present study tended to be greater than found by others in natural products in which smaller amounts of reducing carbohydrates were present.

Hodge and Rist (52) suggested that non-enzymatic browning in sugar-amine systems might occur through the following reactions: (1) reducing sugar and amino compounds condense to form N-glycosides; (2) the N-glycosides rearrange spontaneously to form desoxyaminoketoses; (3) the desoxyaminoketoses are dehydrated spontaneously to nitrogenous reductones; (4) the labile desoxyaminoketoses undergo Strecker degradation, forming aldehydes and $CO_2$; and (5) the nitrogenous reductones react slowly alone and rapidly with amino acids to produce brown pigments.

Lea et al. (14) studied the interaction between casein and glucose when heated in the solid state. They found that the most rapid and, in the earliest stages, virtually the only reaction occurring was a combination of glucose molecules with free amino groups of the protein, which consist mainly of the ε-amino groups of lysine.

It is known that some minerals might interfere in certain analytical procedures (53). When $CuSO_4.5H_2O$, $Fe(NH_4)_2(SO_4)_2.6H_2O$, and $Fe_2(SO_4)_3.xH_2O$

were mixed with protein and heated at 150° for 2 hours and 6 hours, the amount of available lysine was comparable to that of the control at 0 hour (Table 7). Thus, presence of minerals at levels used had no effect on the determination of available lysine by the Kakade and Liener method (36).

When the samples were mixed with oil and heated at 150°, the apparent available lysine was reduced up to 83% in 2 hours. It has been suggested that polyunsaturated fatty acids in oil might be changed to aldehydes and acids by oxidation (54). The oxidation products of oils, particularly carbonylic compounds, react with the ε-amino groups of the lysine side chains in the protein molecule to reduce the amount of available lysine (55, 56). As part of the present study, a test was made which indicated that components of oil used, or oxidation products of those oils, did not react with ε-amino groups to decrease available lysine. After heating the protein-oil mixture and protein without oil for 2 hours, they were extracted twice with ether. Values for available lysine which were obtained on the extracted, processed protein with oil were the same as in the sample processed without oil. Since lysine apparently was not tied up in a nonreversible form, such as in the browning reaction, it is possible that oil in some way interfered directly in analytical reactions of this method.

Kakade and Evans (57) used Carpenter's method (7) for comparison with enzymatic methods for the available lysine determination. It was pointed out that the observed improvement in the nutritive value of navy beans when heated at 121° for 5 minutes was detected only by the enzymatic determination of available lysine and not by the chemical method. In this case heat had a beneficial rather than an adverse effect, possibly by breaking bonds rather than forming enzymatic resistant bonds.

Heat treatment of raw chick-peas by popping improved the nutritive value. Average weight gain and PER values obtained in studies with rats fed chick-peas receiving different treatments[1] were found to be linearly correlated with available lysine by the Kakade and Liener method (36) which was determined in the present study. The correlation coefficient (r) between average weight gain and available lysine was 0.9576, whereas the correlation coefficient for PER and available lysine was 0.9611. A correlation between PER and available lysine also was found by McLaughlan et al. (58). A high correlation was found between the available lysine by the FDNB method and growth assay procedure for untreated egg albumin, beef protein, and casein (5). The discrepancy occurred only with the autoclaved protein preparations. Heller et al. (41) reported a marked decrease in the utilization of the lysine by an animal growth assay when the meat was autoclaved at 120° and 15 psi for 16 hours, but there was little or no loss of lysine as determined microbiologically.

Results of studies reviewed indicate that amino acid availability, particularly of lysine, is an important factor in determining the nutritive value of proteins. Methods to determine availability that do not require animal feeding tests have been studied for some time. Kakade and Liener (36) suggested that their method is applicable for low-molecular-weight protein and that it might be valuable as a rapid chemical method for determining the available lysine of protein foodstuffs that have high carbohydrate contents, since the color caused by presence of carbohydrate did not interfere as in the Carpenter method (7). Results of the present investigation, together with additional information from the literature reviewed, indicated that determination of available lysine by the method of Kakade and Liener should be useful

---

[1]Athia Bano's unpublished report, 1969.

for the rapid evaluation of the nutritive value of foodstuffs in which lysine is the limiting amino acid. High carbohydrate contents or presence of minerals did not appear to interfere in the use of the method, but it apparently cannot be used for samples of protein foodstuffs processed with oil.

## VIII. SUMMARY

The method of Kakade and Liener, based on the determination of ε-amino groups, was used to investigate content of available lysine in proteins processed by heating at 150° in an oven or autoclaved at 120° (15 psi). The studies also included the effect of carbohydrate, oil, and minerals on the reduction of available lysine during the processing. Proteins used in this investigation were casein, fish protein concentrate, and isolated soybean protein. In addition, feedingstuffs that were used in some animal studies were analyzed for available lysine.

Dry heat at 150° reduced available lysine in proteins 10 to 20% in 2 hours and 40 to 48% in 8 hours and, in protein-glucose mixtures, 16 to 40% in 2 hours and 33 to 78% in 8 hours. Moist heat at 120° (15 psi) reduced available lysine in proteins 3 to 10% in 2 hours and 19 to 21% in 8 hours and, in protein-glucose mixtures, 37 to 62% in a half-hour, 71 to 88% in 2 hours, and almost 100% in 8 hours. In the absence of glucose, the moist heat (15 psi) caused less reduction of available lysine in protein than dry heat at 150°, but in the presence of glucose the moist heat treatment caused greater reduction of available lysine than dry heat.

The reduction of available lysine in processed fish protein concentrate-glucose mixture by treatment with both dry and moist heat was greater than in

the casein-glucose mixture or the isolated soybean protein-glucose mixture. In the absence of glucose, processing by dry and moist heat caused no greater reduction of available lysine in fish protein concentrate than in casein or isolated soybean protein.

The reduction of available lysine in protein-starch mixtures was in the same range as in the samples of proteins alone when they were processed by dry heat and moist heat for the same time and at the same temperature.

In chick-peas processed by heating for 30 minutes, the available lysine was reduced 8% at 200° to 29% at 400°. Increasing the time to 45 minutes did not cause appreciable further decrease in available lysine, unless temperature was increased to 450°. There was little reduction of available lysine when chick-peas were processed at 250° or 400° for only 15 minutes. Increasing the temperature for processing soybean meal from 250° F to 300° F reduced available lysine 22%.

Popping chick-peas improved the nutritive value. In chick-peas processed in different ways there was a linear correlation between both weight gain and PER in the rat growth test and available lysine by the Kakade and Liener method.

This chemical method appears to be useful for the rapid evaluation of the nutritive value of foodstuffs in which lysine is the limiting amino acid. High carbohydrate contents or presence of minerals apparently do not interfere in the use of the method, but it probably should not be used for samples of processed protein foodstuffs containing oil.

ACKNOWLEDGMENT

The author wishes to express her gratitude and sincere thanks to her major professor, Dr. D. B. Parrish, for his advice, encouragement, and guidance throughout her graduate studies at Kansas State University.

She also acknowledges the helpfulness of other members of the advisory committee: Drs. M. M. MacMasters, H. L. Mitchell, and W. S. Ruliffson.

Special thanks are due to Dr. C. W. Deyoe, Dr. Athia Bano, and Mr. Mir Shamsuddin in generously providing feedingstuffs.

The author is indebted to her parents, the Fulbright Foundation, and the Agency for International Development in providing the invaluable opportunity of study and research at Kansas State University.

## LITERATURE CITED


1. Patton, S. 1955 Browning and associated changes in milk and its products: a review. J. Dairy Sci., 38: 457.

2. Borchers, S., C. W. Ackerson and R. M. Sanstedt 1947 Trypsin inhibitor. III. Heat destruction of the trypsin inhibitor of soybeans. Biochem., 12: 367.

3. Liener, I. E., and E. G. Hill 1953 The effect of heat treatment on the nutritive value and hemagglutinating activity of soybean oil meal. J. Nutr., 49: 609.

4. Donoso, G., O. A. M. Lewis, D. S. Miller and P. R. Payne 1962 Effect of heat treatment on the nutritive value of protein: chemical and balance studies. J. Sci. Food Agr., 13: 192.

5. Bocter, A. M., and A. E. Harper 1968 Measurement of available lysine in heated and unheated foodstuffs by chemical and biological methods. J. Nutr., 94: 289.

6. Burton, B. T. 1965 The Heinz Handbook of Nutrition, 2nd ed. McGraw-Hill Book Company, Inc., New York, p. 51.

7. Carpenter, K. J. 1960 The estimation of available lysine in animal protein foods. Biochem. J., 77: 604.

8. Liener, I. E. 1958 Processed Plant Protein Food. Academic Press, Inc., New York, p. 79.

9. Liener, I. E. 1960 Nutritional Evolution of Food Processing. John Wiley & Sons, Inc., New York, p. 231.

10. Ellis, G. P. 1959 The Maillard reaction. Advan. Carbohydrate Chem., 14: 63.

11. Hannan, R. S., and C. H. Lea 1952 Studies of the reaction between proteins and reducing sugars in the "dry" state. VI. The reactivity of the terminal amino groups of lysine in model systems. Biochim. Biophys. Acta, 9: 293.

12. Lea, C. H., and R. S. Hannan 1949 Studies of the reaction between protein and reducing sugars in the "dry" state. I. The effect of activity of water, of pH and of temperature on the primary reaction between casein and glucose. Biochim. Biophys. Acta, 3: 313.

13. Lea, C. H., and R. S. Hannan 1950 Studies of the reaction between protein and reducing sugars in the "dry" state. II. Further observations on the formation of the casein-glucose complex. Biochim. Biophys. Acta, 4: 518.

14. Lea, C. H., R. S. Hannan and D. N. Rhodes 1951 Studies of the reaction between proteins and reducing sugars in the "dry" state. IV. Decomposition of the amino-sugar complex and the reaction of acetylated casein with glucose. Biochim. Biophys. Acta, 7: 366.

15. Lea, C. H., and D. N. Rhodes 1952 Studies of the reaction between protein and reducing sugars in the "dry" state. V. The reaction of D-galactose, 2-deoxy-D-galactose, D-glucosamine and N-acetyl-D-glucosamine with casein. Biochim. Biophys. Acta, 9: 56.

16. Renold, T. M. 1963 Chemistry of non-enzymatic browning. I. The reaction between aldoses and amine. Advan. in Food Res., 12: 1.

17. Blom, L., P. Hendricks and J. Caris 1967 Determination of available lysine in foods. Anal. Biochem., 21: 382.

18. Sanger, F. 1945 The free amino groups of insulin. Biochem. J., 39: 507.

19. Schwartz, H. M., and C. H. Lea 1952 The reaction between proteins and reducing sugars in the "dry" state. Relative reactivity of the α- and ε-amino groups of insulin. Biochem. J., 50: 713.

20. Patridge, S. M., and T. Swain 1950 A reversed-phase partition chromatogram using chlorinated rubber. Nature, 166: 272.

21. Carpenter, K. J., and G. M. Ellinger 1955 The estimation of "available lysine" in protein concentrates. Biochem. J., 61: XI.

22. Carpenter, K. J., G. M. Ellinger, M. I. Munro and E. J. Rolfe 1957 Fish products as protein supplements to cereals. Brit. J. Nutr., 11: 162.

23. Baliga, B. P., M. E. Bayliss and C. M. Lyman 1959 Determination of free lysine ε-amino groups in cottonseed meals and preliminary studies on relation to protein quality. Arch. Biochem. Biophys., 84: 1.

24. Baily, K. 1957 Invertebrate Tropomyosin. Biochim. Biophys. Acta, 24: 612.

25. Martinez, W. H., V. L. Frampton and C. A. Cabell 1961 Effects of gossypol and raffinose on lysine content and nutritive quality of proteins in meals from glandless cottonseed. J. Agr. Food Chem., 9: 64.

26. Carpenter, K. J., and B. E. March 1961 The availability of lysine in ground nut biscuits used in treatment of Kwashiokor 2. Brit. J. Nutr., 15: 403.

27. Boyne, A. W., K. J. Carpenter and A. A. Woodham 1961 Progress report on an assessment of laboratory procedures suggested as indicators of protein quality in feedingstuffs. J. Sci. Food Agr., 12: 832.

28. Rao, S. R., F. L. Carter and V. L. Frampton 1963 Determination of available lysine in oil seed meal proteins. Anal. Chem., 35: 1927.

29. Lyman, C. M., and M. C. Thomas 1965 Determination of available lysine in food material. J. Assoc. Offic. Agr. Chem., 48: 858.

30. Rao, M. N., and J. M. McLaughlan 1967 Vitamins and other nutrients. J. Assoc. Offic. Agr. Chem., 50: 704.

31. Okuyama, T., and K. Satake 1960 On the preparation and properties of 2,4,6-trinitrophenyl-amino acids and peptides. J. Biochem., 47: 454.

32. Satake, K., T. Okuyama, M. Ohashi and T. Shinoda 1960 The spectrophotometric determination of amine, amino acid and peptide with 2,4,6-trinitrobenzene 1-sulfonic acid. J. Biochem., 47: 654.

33. Kotaki, A., and K. Satake 1964 Acid and alkaline degradation of the TNP-amino acids and -peptides. J. Biochem., 56: 299.

34. Habeeb, A. F. S. A. 1966 Determination of free amino groups in proteins by trinitrobenzenesulfonic acid. Anal. Biochem., 14: 328.

35. Mokrasch, L. C. 1967 Use of 2,4,6-trinitrobenzenesulfonic acid for the coestimation of amines, amino acids, and proteins in mixtures. Anal. Biochem., 18: 64.

36. Kakade, M. L., and I. E. Liener 1969 Determination of available lysine in proteins. J. Anal. Biochem., 27: 273.

37. Kuiken, K. A. 1952 Availability of the essential amino acids in cottonseed meal. J. Nutr., 46: 13.

38. Schweigert, B. S., and B. T. Guthneck 1953 Utilization of amino acids from foods by the rat. I. Methods of testing for lysine. J. Nutr., 49: 277.

39. Guthneck, B. T., B. A. Bennett and B. S. Schweigert 1953 Utilization of amino acids from food by the rats. II. Lysine. J. Nutr., 49: 289.

40. Gupta, J. D., A. M. Dakroury, A. E. Harper and C. A. Elvehjem 1958 Biological availability of lysine. J. Nutr., 64: 259.

41. Heller, B. S., M. R. Chutkow, C. H. Lushbough, A. J. Siedler and B. S. Schweigert 1961 Utilization of amino acids from foods by the rat. V. Effect of heat treatment on lysine in meat. J. Nutr., 73: 113.

42. Carpenter, K. J., B. E. March, C. K. Milner and R. C. Campbell 1963 A growth assay with chicks for the lysine content of protein concentrates. Brit. J. Nutr., 17: 309.

43. De Muelenaere, H. J. H., M-L. Chen and A. E. Harper 1967 Assessment of factors influencing estimation of lysine availability in cereal products. J. Agr. Food Chem., 15: 310.

44. Fryer, H. C. 1968 Concepts and Methods of Experimental Statistics, 2nd ed. Allyn and Bacon, Inc., Boston, p. 226.

45. Kass, J. P., and L. S. Palmer 1940 Browning of autoclaved milk, chemical factors involved. Ind. Eng. Chem., 32: 1360.

46. Harland, H. A., R. Jenness and S. T. Coulter 1947 Changes produced in milk on heating. J. Dairy Sci., 30: 526.

47. Patton, S., and D. V. Josephson 1949 Observations on the application of the nitroprusside test to heated milk. J. Dairy Sci., 32: 398.

48. Patton, S. 1952 Studies of heated milk. IV. Observations on browning. J. Dairy Sci., 35: 1053.

49. Conkerton, E. J., W. H. Martinez, G. E. Mann and V. L. Frampton 1957 Changes induced by autoclaving a solvent-extracted cottonseed meal. J. Agr. Food Chem., 5: 460.

50. Evans, R. J., and H. A. Butts 1948 Studies on the heat inactivation of lysine in soybean oil meal. J. Biol. Chem., 175: 15.

51. Netke, S. P., and H. M. Scott 1970 Estimates on the availability of amino acids in soybean oil meal as determined by chick growth assay: Methodology as applied to lysine. J. Nutr., 100: 281.

52. Hodge, J. E., and C. E. Rist 1953 The Amadori rearrangement under new conditions and its significance for non-enzymatic browning reactions. J. Amer. Chem. Soc., 75: 316.

53. Brummel, M., C. M. Gerbeck and R. Montgomery 1969 Effects of metals on analytical procedures for amino acids and carbohydrates. Anal. Biochem., 31: 331.

54. Meyer, L. H. 1968 Food Chemistry: Fats and other lipids. Reinhold Publishing Corporation, New York, p. 36.

55. Lea, C. H., L. J. Parr and K. J. Carpenter 1958 Chemical and nutritional changes in stored herring meal. Brit. J. Nutr., 12: 297.

56. Lea, C. H., L. J. Parr and K. J. Carpenter 1960 Chemical and nutritional changes in stored herring meal 2. Brit. J. Nutr., 14: 91.

57. Kakade, M. L., and R. J. Evans 1966 Chemical and enzymatic determinations of available lysine in raw and heated navy beans (Phaseolus vulgaris). Can. J. Biochem., 44: 648.

58. McLaughlan, J. M., C. C. Rogers, D. C. Chapman and J. A. Campbell 1959 Evaluation of protein in foods. IV. A simplified chemical score. Can. J. Biochem., 37: 1293.

# AVAILABLE LYSINE IN FOODSTUFFS

by

NIPA SOMNAPAN

B. S. (Hons.), Chulalongkorn University, 1963

M. S., Mahidol University, 1965

Bangkok, THAILAND

---

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

FOOD SCIENCE

Department of Biochemistry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1970

The trinitrobenzenesulfonic acid method, proposed by Kakade and Liener, was used to determine the reduction of available lysine when the proteins (casein, fish protein concentrate, and isolated soybean protein) were processed by heat treatment alone or in the mixtures with carbohydrate, mineral, or oil. Dry heat in the oven at 150° and moist heat in the autoclave at 120° (15 psi) were used in this processing. Some feedingstuffs were analyzed to compare with the nutritive values obtained in a rat growth test.

Determination of available lysine is based on the assumptions that the reagent trinitrobenzenesulfonic acid reacts with the free ε-amino group of lysine in crude material, that ε-TNP-lysine is the only compound that remains in the aqueous phase after acid hydrolysis and ether extraction, and that only lysine molecules reacting with the reagent are available to the animal.

Dry heat at 150° reduced available lysine in protein up to 20% in 2 hours and 48% in 8 hours and, in protein-glucose mixtures, up to 40% in 2 hours and 78% in 8 hours. Moist heat at 120° (15 psi) reduced available lysine in proteins up to 10% in 2 hours and 21% in 8 hours and, in protein-glucose mixtures, 88% in 2 hours and almost 100% in 8 hours. In the absence of glucose, the moist heat (15 psi) caused less reduction of available lysine in protein than dry heat at 150°, but in the presence of glucose the moist heat treatment caused greater reduction of available lysine than dry heat. Dry heat probably caused reduction of available lysine by both direct decomposition and the browning; further reduction was found when moist heat was used, probably because of increased browning due to high humidity.

Reduction of available lysine in processed fish protein concentrate-glucose mixture by treatment with both dry heat and moist heat was greater than in the other two protein-glucose mixtures. Reduction of available lysine

in protein-starch mixtures was in the same range as in the samples of protein alone when they were processed by dry heat and moist heat for the same time and at the same temperature. In chick-peas processed by heating for 30 minutes the available lysine was reduced to 29% at 400°; increasing the time to 45 minutes did not cause appreciable further decrease in available lysine, unless temperature was increased to 450°. Increasing temperature of processing soybean meal from 250° F to 300° F reduced available lysine 22%.

In chick-peas processed by different methods it was found that popping improved the nutritive value. A linear correlation was found between both weight gain and protein efficiency ratio (PER) in the rat growth test and available lysine by Kakade and Liener method.

This chemical method appears to be useful for the rapid evaluation of the nutritive value of foodstuffs in which lysine is the limiting amino acid. High carbohydrate contents or presence of minerals apparently do not interfere in the use of the method, but it probably should not be used for samples of processed protein foodstuffs containing oil.