Quantitative Determination of Terminal Methionine, Leucine, and Lysine in Raw and Toasted Soybean Oil Meal

SIDNEY W. FOX, CAROL WARNER, and THOMAS L. HURST

Chemical Laboratory, Iowa State College, Ames, Iowa

Attempts were made to determine whether or not methionine or other residues are directly exposed in the course of "toasting" soybean oil meal. Newer methods of quantitative assessment of aminoid and carboxoid residues showed that no methionine residues are directly exposed as the result of toasting. *N*-Terminal lysine was found in both the raw and toasted materials. The terminality of lysine suggests that its susceptibility to destruction during overheating may be due to the fact that a large part of the lysine is in a relatively exposed position in the protein molecule, rather than protected by folding of the native molecule.

THE QUALITY OF A PROTEIN or proteinaceous food is not easily or simply reduced to a chemical basis. The proportions of essential amino acids are critical in such assessment (*21*), and the availability of such amino acids may substantially modify their nutritional utility (*19*). Little is known about the chemical nature of the linkages which may influence the availability of individual amino acids from protein.

The fact that nutritional availability of amino acids might be influenced by appropriate treatment of protein has long been recognized in the case of soybean protein. Osborne and Mendel reported in 1917 (*20*) that simple controlled heating improved the nutritional value of soybean protein. A large number of explanations has been offered for this phenomenon. In many of these explanations, some manner of increased availability of methionine has been suggested (*1, 3, 6, 7, 18, 19, 22, 23*).

The development of quantitative methods of analysis of aminoid and carboxoid terminal amino acid residues in mixtures of proteins (*4, 10–12*) permits a new approach to this problem. Significant amounts of methionine are not directly released by the usual heating. Studies were also performed with leucine and lysine. The data on lysine provide new information which may contribute to understanding of the behavior of this amino acid residue in food processing.

An additional problem investigated during this work was the effect of trichloroethylene extraction of soybean protein (*15*) upon these same amino acids.

Experimental Materials and Methods

Table I gives the source and treatment of the samples of soybean oil meal, which were made available through Joseph C. Picken, Jr., Veterinary Research Institute, Iowa State College. M2A and M3A meals were from the same original batch of beans. M4B and M14B were from different lots of beans.

Table I. Source and Mode of Preparation of Soybean Oil Meal Samples

Code No.	M2A	M3A	M4B	M14B
Oil extractant	TCE[a]	TCE	Hex[b]	Hex
Toasted	No	Yes	Yes	No
Plant	BP[c]	BP	SK[d]	SK

[a] Trichloroethylene.
[b] Hexane.
[c] Blooming Prairie, Minn. M3A reported as "toasted" for 31 minutes at 250° F.
[d] Spencer Kellogg, Des Moines, Iowa.

The aminoid and carboxoid treatments have been described (*4, 11*). The method depends essentially upon an assay of the hydrolyzate, followed by another assay after the *N*-terminal or *C*-terminal residues are converted to derivatives which are unavailable to the assay organism. Inasmuch as it was more convenient to dry the treated samples over steam than in a desiccator, controlled experiments were performed to study the effect of method of drying. Drying was carried to the point at which no more liquid was visible in the sample. Methods of assay are given in earlier publications (*4, 10–12*). Combinations of organism and medium used in this study were: leucine, aq; lysine, ms; and methionine, fp. The first symbol in each pair designates the organism—(a) *Lactobacillus arabinosus*, (f) *Streptococcus faecalis*, (m) *Leuconostoc mesenteroides*—and the second designates the medium employed (*12*).

Recovery experiments with lysine were performed in the same way as the others, with the designated amount of standard amino acid added prior to blocking treatment.

Results and Discussion

Table II indicates no significant difference in value regardless of how the hydrolyzates were dried. Lysine is a particularly sensitive amino acid (*14*) for such study, and inasmuch as no decrement is recorded for heat-drying as against drying in a desiccator, the lack of effect is certain for most other amino acids. This type of result has been observed in other similar comparisons made in this laboratory. Drying on a steam bath was less time-consuming than drying

Table II. Effect of Drying in Desiccator or over Steam after Hydrolysis and after Aminoid Treatment and Hydrolysis of Soybean Oil Meal M14B

Amino Acid Assayed	Hydrolyzate, %		PTC[a]-Treated Hydrolyzed SBOM, %	
	Dried over steam	Dried in desiccator	Dried over steam	Dried in desiccator
L-Leucine	3.2, 3.1	3.2	3.1, 3.1	3.0
L-Lysine	2.7, 2.7	2.8	1.6, 1.7	1.2
L-Methionine	0.61, 0.65	0.61	0.62, 0.65	0.68

[a] Phenyl isothiocyanate.

in a desiccator. Prolonged heating over steam—for instance—has, however, been found in this laboratory to be capable of causing more general destruction of amino acids (*9*).

Table III indicates that about 10% of added lysine carried through the same treatment as the soybean oil meal is recovered; if calculated on the basis of $\frac{c}{a+b}$, the recovery would be 16%. Recoveries of pure L-lysine similarly treated were virtually negligible (*13*). The meaning of an experiment involving recovery of an amino acid added to protein can be only approximate, but the results of these tests suggest that the value for terminal lysine requires little, if any, correction.

No other differences in Table IV appear to be significant.

Each left-hand figure in each couplet in Table IV was determined in a simultaneous assay with the other left-hand figures. The right-hand figures were all also from a single battery of assays.

In the application of aminoid treatments the possibility that the treatment is blocking free amino acids as well as *N*-terminal residues must be considered. This question is answered by the fact that no *C*-lysine is found. If lysine were free, the decrement after carboxoid treatment should be reflected in a decrement to correspond to the proportion present as free lysine. This concerted use of quantitative aminoid and carboxoid figures provides, in fact, some new utilities in the field of analysis of peptide-type compounds and mixtures found in biological entities.

These results with lysine indicate that a major proportion of the protein of soybean is structurally linear or φ-shaped rather than purely cyclic. It seems easier to understand the thermal susceptibility of lysine in heat processing of cereal and soybean (*7*, *14*) on the basis of structural terminality. A terminal lysine residue which is sufficiently exposed to react with phenyl isothiocyanate through its α-amino groups should be more susceptible to thermal decomposition than lysine residues protected by enclosure within a folded protein molecule. Likewise, decomposition of lysine residues by a mechanism involving reaction with carbohydrate would probably be facilitated by such full exposure of reactive amino groups (*5*).

Comparison of the results from hexane-extracted and from trichloroethylene-extracted meal reveal no significant difference. The trichloroethylene treatment does not decrease the assayable methionine content of the meal. One possible explanation for the long-term toxicity of trichloroethylene-extracted soybean oil meals (*15*) is conversion of methionine side chains to a toxic derivative, by analogy with the reaction of Agene (*2*). The hydrolyzed heated meal is evidently not toxic to the assay bacterium. Qualifications of this conclusion are based on the possibility that acid hydrolysis destroys the toxic molecule, or that toxicity could be introduced by a change too small to be measurable, and in the probability that mammalian toxicity introduced by this treatment may be without effect on *Streptococcus faecalis*.

The terminality of lysine in soybean meal has been established in this study and in other work. Five amino acids (lysine, leucine, methionine, phenylalanine, and valine) have so far been assayed in soybean oil meal. Only lysine has been found to be terminal. These results indicate that as far as percentage of *N*-terminal residue type is concerned, soybean protein is chemically highly akin to corn, rye, wheat, a river alga, and a reed from the same stream (*8*). Corn, rye, and wheat have been analyzed for 14 amino acids in the *N*-terminal position; only lysine has been found present in substantial proportion.

Table III. Recovery of Lysine Added to Soybean Oil Meal M14B and Carried through Aminoid Treatment

a L-Lysine in Sample, γ	b Added L-Lysine, γ	c Total L-Lysine by Assay, γ	$\frac{c-a}{b}$ Recovery of Added L-Lysine, %
2.4	30	5.6	11
5.6	60	10.0	7
8.0	90	17.7	11
		Average	10

The amino acid values in Table IV permit consideration of the problem originally brought under study, the effect of toasting upon methionine.

Neither carboxoid methionine nor aminoid methionine is directly opened by the toasting process. Inasmuch as these treatments generally do not attack peptide linkages (*4*), the possibility of opening a linkage by these manipulations must be restricted to special non-peptide linkages such as that proposed by Johnson, Parsons, and Steenbock (*17*, *24*), if, indeed, any such linkage is directly split by heat. The results of these tests shed no light on the possibility that the toasting process opens in soybean protein other peptide linkages which permit closer approach of appropriate proteases to methionine linkages (*16*). This possibility is under further study.

An absence of direct effect on leucine linkages may also be noted from Table IV. Leucine was studied merely as an amino acid which might provide normal values.

Lysine was studied in part because of its known heat-susceptibility in cereal processing (*7*, *14*). Perhaps the most significant result from this study was the unexpected finding of aminoid terminality for lysine. A large proportion of the lysine was found in the terminal position in either raw or heated protein. This type of result is observed also in the data of Table II and in other experiments which reveal part of the structure of unpurified soybean protein and two of the extracted materials (*9*).

Table IV. Assays of Soybean Oil Meals before and after Aminoid and Carboxoid Blocking Treatments

Meal	Amino Acid	Hydrolyzate, %	Hydrolyzate of Aminoid-Treated SBOM, %	Hydrolyzate of Carboxoid-Treated SBOM, %
M2A	Leucine	0.75[a]	0.75	0.80
M14B	Leucine	0.74	0.75	0.76
M3A	Leucine	0.76	0.78	0.77
M4B	Leucine	0.75	0.72	0.75
M2A	Lysine	2.8, 2.2	1.7, 1.3	2.9, 2.3
M14B	Lysine	2.5, 2.5	1.4, 1.1	3.3, 2.6
M3A	Lysine	2.3, 2.6	1.0, 1.2	2.3, 2.6
M4B	Lysine	2.2, 2.3	1.0, 1.4	2.3, 3.3
M2A	Methionine	3.8	3.8	3.6
M14B	Methionine	3.4	3.4	3.4
M3A	Methionine	3.6	3.5	3.4
M4B	Methionine	3.6	3.4	3.7

[a] All figures = $\frac{\text{wt. of amino acid}}{\text{wt. of SBOM sample}} \times 100$.

Literature Cited

(1) Bell, J. M., Williams, H. H., Loosli, J. K., and Maynard, L. A., *J. Nutrition*, **40**, 551 (1950).

(2) Bentley, H. R., McDermott, E. E., and Whitehead, J. K., *Proc. Roy. Soc. (London)*, **B138,** 265 (1951).
(3) Clandinin, D. R., Cravens, W. W., Halpin, J. C., and Hart, E. B., *Poultry Sci.*, **25,** 509 (1946).
(4) De Fontaine, D., and Fox, S. W., *J. Am. Chem. Soc.*, **76,** 3701 (1954).
(5) Evans, R. J., and Butts, H A., *Food Research*, **16,** 415 (1951).
(6) Evans, R. J., and McGinnis, J., *J. Nutrition*, **31,** 449 (1946).
(7) *Ibid.*, **35,** 477 (1948).
(8) Fox, S. W., De Fontaine, D., and Homeyer, P. G., *Federation Proc.*, **14,** 213 (1955).
(9) Fox, S. W., De Fontaine, D., and Warner, C., unpublished experiments.
(10) Fox, S. W., Hurst, T. L., and Itschner, K. F., *J. Am. Chem. Soc.*, **73,** 3573 (1951).
(11) Fox, S. W., Hurst, T. L., Underwood, O., and Griffith, J., *Ibid.*, **77,** 3119 (1955).
(12) Fox, S. W., Hurst, T. L., and Warner, C., *Ibid.*, **76,** 1154 (1954).
(13) Fox, S. W., and Underwood, O., unpublished experiments.
(14) Fritz, J. C., Kramke, E. H., and Reed, C. A., *Poultry Sci.*, **26,** 657 (1947).
(15) Goss, W. H., *J. Am. Oil Chemists' Soc.*, **29,** 253 (1952).
(16) Ingram, G. R., Riesen, W. W., Cravens, W. W., and Elvehjem, C. A., *Poultry Sci.*, **28,** 898 (1949).
(17) Johnson, L. M., Parsons, H. T., and Steenbock, H., *J. Nutrition*, **18,** 423 (1939).
(18) McGinnis, J., and Evans, R. J., *Ibid.*, **34,** 725 (1947).
(19) Melnick, D., and Oser, B. L., *Food Technol.*, **3,** 57 (1949).
(20) Osborne, T. B., and Mendel, L., *J. Biol. Chem.*, **32,** 369 (1917).
(21) Rose, W. C., *Federation Proc.*, **8,** 546 (1949).
(22) Russell, W. C., Taylor, M. W., Mehrhof, T. G., and Hirsch, R. R., *J. Nutrition*, **32,** 313 (1946).
(23) Stevens, J. M., and McGinnis, J., *J. Biol. Chem.*, **171,** 431 (1947).
(24) Viswanatha, T., and De, S. S., *Indian J. Physiol.*, **5,** 51 (1951).

Received for review February 17, 1955. Accepted April 21, 1955. Journal Paper J-2706, Iowa Agricultural Experiment Station, Ames, Iowa. Project 863, supported by Grants G-3025 and G-3025(C) from the National Institutes of Health, Public Health Service.

COTTONSEED MEAL IN POULTRY FEED

Gossypol-Cephalin Compound from Fresh Eggs of Hens Fed Cottonseed Meal

CHARLES L. WORONICK and C. R. GRAU

Department of Poultry Husbandry, University of California, Davis, Calif.

The cephalin fraction of egg yolks from hens fed gossypol is yellow in color, and possesses an absorption spectrum with maxima at 380 and 400 mμ. Gossypol was identified in the cephalin fraction after oxalic acid hydrolysis. The reaction of gossypol with ethanolamine and with ethylamine resulted in products having absorption spectra that were almost identical with the absorption spectrum of gossypol egg cephalin. It is concluded that the aldehyde groups of gossypol condense with the primary amino groups of phosphatidylethanolamine to form a Schiff base which is present in the egg.

THE METABOLISM OF GOSSYPOL in the animal body has not yet been elucidated, but there is abundant evidence that the feeding of this toxic yellow pigment to laying hens results in the producduction of egg yolks which may discolor during storage (*7, 9, 10*). Swensen Fieger, and Upp (*11*) concluded that gossypol is absorbed unchanged from the intestinal tract of the hen, and is deposited in the egg in the free form. Evidence presented in support of this conclusion was rather indirect, as free gossypol has never been isolated from egg yolk.

Egg yolks from hens fed gossypol contain a distinctive yellow component which is insoluble in acetone, but is soluble in hexane-acetone 3 to 1 by volume (*5*). Spectrophotometric estimation of the amount of this component in the eggs reveals a direct relationship with the amount of gossypol fed. In this paper, evidence is presented to show that the yellow component is a compound of gossypol with cephalin.

Experimental

Preparation of Cephalin. Cephalin was prepared from normal eggs and from eggs of hens fed cottonseed meal as a source of gossypol (hereinafter called "gossypol eggs"). Fresh egg yolks were exhaustively extracted with acetone (*5*), followed by three extractions with 3 to 1 hexane-acetone. The hexane-acetone extracts were combined in two separate flasks, and evaporated under reduced pressure. Cephalin was prepared from the fatty residues by using the following procedure, which is a modification of that developed by Folch (*4*). The solvent volumes are expressed on a per egg basis.

The fatty residue was extracted twice with 5-ml. portions of 95% ethyl alcohol, and once with 2 ml. of petroleum ether (boiling range 30° to 60° C.). The petroleum ether extract was evaporated under reduced pressure, and the resulting residue was dissolved in 1 ml. of diethyl ether. This mixture was allowed to remain at −10° C. overnight, and the appreciable white precipitate which formed was discarded. The supernatant fluid was diluted with 1 ml. of diethyl ether, and the cephalin was precipitated with 10 ml. of 95% ethyl alcohol, which was added slowly during stirring. After remaining at room temperature for an hour, the sample was centrifuged at about 2000 r.p.m. for 5 minutes, and the supernatant fluid was discarded. The yield was approximately 43 mg. per egg. The cephalin was stored at −10° C. in solution with diethyl ether, or as a precipitate under acetone. It was more stable in acetone. The normal egg cephalin was almost white in color, while the gossypol egg cephalin was yellow. Attempts to fractionate the cephalin by the method of Folch (*4*) were not successful.

Hydrolysis of Cephalin. Cephalin which had been stored in diethyl ether