# Determination of Tryptophan in Corn (*Zea mays*)

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A method is described for the determination of tryptophan in corn proteins which are solubilized by Pronase hydrolysis and the hydrolyzate is analyzed colorimetrically. The Pronase-hydrolysis-following-water-extraction procedure is an accurate determination of the tryptophan content of components containing 84 to 89% of the total nitrogen of corn. The Pronase-hydrolysis-without-prior-water-extraction procedure is a simpler, though possibly less accurate, method for the determination of the tryptophan content of components containing over 90% of the total nitrogen. An upper limit value is determined for the tryptophan content of the water extract of corn, which contains 3 to 8% of the total nitrogen and unidentified interferers.

More accurate and convenient methods for the determination of tryptophan in corn are needed for use in genetic and nutritional studies. Lyman *et al.* (1956) determined tryptophan in corn and other farm feeds by microbiological analysis of their alkaline hydrolyzates. In addition to possible losses of tryptophan during alkaline hydrolysis of complex mixtures, the microbiological method of analysis, while generally reliable, is not so convenient as colorimetric analysis. However, colorimetric determination of tryptophan in corn presents difficulties not found in similar analysis of purified proteins owing to the low tryptophan content (<0.1%) and the presence of unidentified components which interfere in the analysis. For example, tryptophan could not be determined colorimetrically in the hydrolyzate of corn made with sodium hydroxide containing tryptophan-protective agents (Spies, 1967) because of such interference.

This paper describes a method for the determination of tryptophan in corn by an adaptation of Procedure W (Spies, 1967) in which proteins are solubilized by hydrolysis with the enzyme, Pronase, and the hydrolyzate is analyzed colorimetrically under conditions designed to minimize interference. In the present study, samples of corn were extracted twice with water. The residue was then hydrolyzed with Pronase, and the residue from the Pronase hydrolysis was extracted with 75% ethanol. The tryptophan and nitrogen contents of the separate extracts were determined and compared with those obtained by Pronase hydrolysis of corn without preliminary water extraction.

## MATERIALS

The term corn refers to ether-defatted, whole ground corn (*Zea mays*).

Two pulverized (to pass a 60-mesh or finer sieve), high protein, corn samples (Log 1 and Log 2, 12-15-66) were supplied by W. P. Williams, Jr., Clemson University, Clemson, S. C. 29631. These samples, designated 1 and 2, contained 2.54 and 1.96% nitrogen, respectively.

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Tryptophan standard and reagents have been described previously (Spies, 1967).

Pronase was obtained from Calbiochem, Los Angeles, Calif.

## METHODS

**Nitrogen.** Nitrogen was determined by the Kjeldahl micromethod.

**Tryptophan Determination.** The standard curve for tryptophan was prepared by Procedure G (Spies and Chambers, 1948). The water extracts, Pronase solution, and Pronase hydrolyzates of corn were analyzed by Procedure L (Spies and Chambers, 1949) except that reaction I was for 6 hours. For development of color (reaction II), 0.1 ml. of 0.045% sodium nitrite reacting for 30 minutes was used for tryptophan, the Pronase solution, and the water extracts of corn. For reaction II with the Pronase hydrolyzates of corn, 0.1 ml. of 0.090% sodium nitrite was used. Procedure N (Spies and Chambers, 1949), modified by clarification of the solution after reaction I by centrifugation and filtration through fritted glass, was used in attempts to analyze the dried alcoholic extracts. Transmittance was read at 600 mμ. Blank solutions were similar to test solutions, except that *p*-dimethylaminobenzaldehyde (DAB) was omitted.

**Procedure X** (determination of tryptophan, in the Pronase hydrolyzate, and the water extracts of corn. For this study, nitrogen and solids in the extracts also were determined).

**First Water Extract.** To 500 mg. of corn in a tared, 15 × 125 mm., screw-capped, test tube were added 10 ml. of distilled water and one drop of toluene preservative. The corn was suspended by shaking. The tube was placed horizontally on a Thomas clinical rotator and shaken at 40 r.p.m. at 32° to 35° C. for 22 to 24 hours. The suspension then was centrifuged in the capped tube for 30 minutes at about 4000 G. The clear supernatant solution was decanted and weighed. One-milliliter aliquots of this extract were used for tryptophan and nitrogen determinations. The tube, with sample and residual water, was weighed to make the calculation in footnote *e*, Table I.

**Second Water Extract.** Ten milliliters of water was added to the tube containing the residue from the first extraction which was shaken for 30 minutes and centrifuged

Table I. Tryptophan, Nitrogen, and Solids in Corn Extracts

| | pH[a] | | Tryptophan Extracted[b] | | Nitrogen and Solid Extracted | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Nitrogen[c] | | Solid | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| First water extract[d] | 6.05 | 6.19 | 0.0075 ±.0001 | 0.0125 ±.0006 | 3.4 | 8.4 | ... | ... |
| Second water extract[e] | 6.24 | 6.56 | 0.0033 ±.0007 | 0.0025 ±.0002 | 0.07 | 0.3 | ... | ... |
| Pronase hydrolyzate following water extractions | 5.88 | 5.99 | 0.0490 ±.0016 | 0.0492 ±.0022 | 88.7 | 84.4 | 24[f] | 24[f] |
| Ethanol (75%) following water and Pronase extractions | | | | | 1.6 | 0.6 | 1[g] | 1[g] |
| Pronase hydrolyzate without prior water extraction | 5.67 | 5.63 | 0.0488 ±.0009 | 0.0521 ±.0006 | 91.2 | 90.3 | 22[h] | 22[h] |
| Ethanol following Pronase | | | | | 5.3 | 2.9 | 2[g] | 2[g] |

[a] pH of final extract; pH of starting Pronase solution, 7.24.
[b] Grams of tryptophan in extract per 100 grams of corn. Average of triplicate determinations. Precision expressed as average deviation of the mean.
[c] Per cent of total nitrogen of corn sample contained in the extract.
[d] Values based on volume of water added to sample.
[e] Values based on volume of water added, but corrected by subtracting amount remaining from first extraction.
[f] Per cent of total solid removed by two water extractions and the Pronase hydrolysis.
[g] Per cent of the total solids removed by 75% ethanol extraction.
[h] Per cent of the total solids removed by Pronase hydrolysis.

as above. The clear supernatant solution was decanted and weighed. One-milliliter aliquots of this extract were used for tryptophan and nitrogen determinations. The tube with sample and residual water was weighed to make a calculation in the next step.

**Pronase Hydrolysis Following Water Extraction.** A measured volume of aqueous Pronase solution was added to the tube so that the final total volume was 5.0 ml., inclusive of the residual water from the second water extraction. The concentration of the added Pronase was adjusted so that the final concentration of Pronase was 2.0 mg. per ml. One drop of toluene was added, the tube was shaken at 32° to 35° C. for 48 hours, the suspension was centrifuged as above, and the clear supernatant solution was decanted. One-milliliter aliquots were used for tryptophan and nitrogen determinations. The tryptophan and nitrogen contents of the Pronase solution were determined and subtracted, respectively, from the totals found in the Pronase hydrolyzates.

**Solid Extracted by Water and Pronase.** The residue remaining after the Pronase hydrolysis was washed twice with 10-ml. portions of water. These extracts were discarded, and the residual solid was dried in a vacuum over calcium chloride and weighed.

**Ethanol Extraction.** To the residue from the foregoing treatments was added 5.0 ml. of 75% ethanol. The suspension was shaken as described above for 24 hours. After centrifuging, the supernatant solution was decanted. A 2-ml. aliquot of this solution was evaporated to dryness in a 25-ml. Erlenmeyer flask for attempted tryptophan determination. Nitrogen was determined on the remaining solution.

**Solid Extracted by Ethanol.** The residue from the ethanol extraction was washed twice with 10-ml. volumes of 75% ethanol, dried in a vacuum over calcium chloride, and weighed.

**Pronase Hydrolysis without Prior Water Extraction.** To 500 mg. of corn was added 5.0 ml. of aqueous Pronase, 2.0 mg. per ml., plus one drop of toluene. The suspension was shaken for 48 hours at 32° to 35° C. then centrifuged, and the clear supernatant solution decanted. One-milliliter aliquots were used for tryptophan and nitrogen analyses. The residue was dried and extracted with ethanol as described above.

## RESULTS

Table I shows the tryptophan contents and the proportions of the total nitrogen and total solids in successive water extracts and in the Pronase hydrolyzates obtained both with and without prior water extraction. The proportions of total nitrogen and total solids extracted by 75% ethanol from the residues following Pronase hydrolysis and the pH values of each solution are also shown in Table I.

## DISCUSSION

The most significant tryptophan-containing fraction of corn is that solubilized by Pronase hydrolysis. Thus, 89 to 84 and 91 to 90% of the total nitrogen of the two samples were contained in the Pronase hydrolyzates obtained with and without prior water extractions, respectively. Only 3 to 8% of the total nitrogen was contained in the combined water extracts and 0.6 and 1.6% in the ethanol extracts following the successive water extractions and Pronase hydrolyses of these samples.

The tryptophan values (0.0490 and 0.0492%) for the Pronase hydrolyzates following water extraction are regarded as accurate because the preliminary water extraction

removed the water-soluble components of corn that interfere with the colorimetric determination of tryptophan. The tryptophan values (Table I) for the water extracts of corn are regarded as only fixing an upper limit on the tryptophan content of these fractions because of the unknown effect of interfering components in analysis of the water extracts, and because tryptophan is determined on the intact protein rather than on the hydrolyzate (Spies, 1967).

The tryptophan values (0.0488 and 0.0521%) obtained on the Pronase hydrolyzates without prior water extraction agree rather closely with those obtained on the Pronase hydrolyzates following water extraction. This agreement may be fortuitous, however, because the tryptophan content of Pronase hydrolyzates without prior water extraction should equal the sum of the tryptophan contents of the water extracts plus that of the subsequent Pronase hydrolyzate. The difference is attributed to the uncertainty of the actual tryptophan content of the water extracts and the unknown effect of interference by water soluble components in the analysis of the Pronase hydrolyzate without prior water extraction. Accordingly, if for convenience, the tryptophan values obtained on the Pronase hydrolyzate without prior water extraction are used in comparative studies, the foregoing considerations should be borne in mind.

Tryptophan could not be determined on the ethanolic extracts of corn meal because of an interfering brown coloration formed in the test. However, this fraction contains a negligible proportion of the total nitrogen and total solids of corn.

Lyman *et al.* (1956) reported 0.089 and 0.087% tryptophan, determined microbiologically, in two samples of corn which contained 11.1 and 8.1% crude protein, respectively. It is not possible to compare critically the accuracy of the microbiological and Pronase methods by comparing values obtained in these two works because different samples were used.

The pH of maximum activity of Pronase is between 7.5 and 8.5, according to Nomoto and Narahashi (1959). Nevertheless, Pronase dissolved in water, where the pH dropped from 7.2 to between 5.6 and 6.0 during the hydrolysis of the corn, was slightly more effective than when Pronase dissolved in 0.1$M$ phosphate buffered at pH 7.5 was used.

Forty-eight hours at 32° to 35° C. was the optimum time of digestion with Pronase. Treatment with one lot of Pronase was sufficient.

The basic method for the determination of tryptophan in corn described in this paper may be applicable to some other grains or grain products and possibly other defatted naturally occurring substances. However, this would have to be determined by further study which the author does not plan to make.


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