Determination of Tryptophan in Cereals[1]

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ABSTRACT

A method for the analysis of tryptophan in cereals is described which employs NaOH solution containing histidine to hydrolyze the sample in an O-ring joint tube under N_2. For cereal products an 8-hr. hydrolysis was found optimum. Interfering substances are removed by an ion-exchange column. Tryptophan is measured colorimetrically by *p*-dimethylaminobenzaldehyde. Results are in good agreement with mean values reported in the literature and are generally higher than those obtained by enzymatic hydrolysis.

Reliable information concerning the tryptophan content of cereals is essential, especially when cereals are a major source of protein. The loss of tryptophan by oxidation and by reaction with other amino acids has led to an extensive search for a method that is both reliable and convenient. Several spectrophotometric methods have been used in determining tryptophan in intact soluble proteins (1,2). Methods also have been developed for the estimation of tryptophan following its release by alkaline or enzymatic hydrolysis of proteins.

In the analysis of cereals, difficulties are encountered in applying these methods because of the insolubility of some of the proteins and the production of unwanted colors by reaction of the solvent or reagents with the nonprotein fraction of the food (3). Furthermore, the high content of carbohydrates and their degradation products produced during hydrolysis interfere with subsequent analysis for tryptophan. In this paper a method is described which is based on hydrolysis with a sodium hydroxide solution containing histidine in an O-ring joint tube under nitrogen, followed by an ion-exchange treatment to remove interfering substances and the subsequent determination of tryptophan with para-dimethylaminobenzaldehyde (*p*-DAB). This procedure has proved useful for the analysis of cereals. Data obtained by ion-exchange chromatography following alkaline hydrolysis and by colorimetric determination with *p*-DAB after enzymatic hydrolysis with pronase (4) are presented also.

EXPERIMENTAL

Materials

Foods. The foods used in this work were commercial samples of white-wheat flour (General Mills); whole-wheat flour (Pillsbury); converted long-grain rice (Uncle Ben); yellow corn meal, enriched and degerminated (Aunt Jemima); rolled oats (Quaker Oats); whole chick peas; nonfat dry milk (Carnation); and wheat starch (Paygel P, General Mills). *Opaque-2* corn was kindly supplied by Edwin T.

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Proteins. Lysozyme (Grade 1, egg white) was purchased from Sigma Chemical Company. Gluten and zein were purchased from General Biochemicals Corporation. Rice glutelin was prepared by the method of Tamura and Kenmochi (5).

Reagents. L-Tryptophan (Mann, chromatographically pure) was used as a standard. Pronase (Calbiochem, B grade) was used for enzymatic hydrolysis. The ion-exchange resin, Dowex 50W X 8 (ionic form H^+, 200 to 400 mesh), was washed and fines were removed prior to packing of columns (6).

Alkaline Hydrolysis. A sample weighing between 100 and 500 mg. and containing approximately 500γ of tryptophan was weighed into an 18-ml. polypropylene tube. Five milliliters of 10N NaOH and 5 ml. of water containing 50 mg. of histidine were added. The charged tube was immersed in boiling water, the sample was stirred vigorously, and the tube was placed in an O-ring joint tube (7) which then was immersed in boiling water and flushed with nitrogen. The sample in the tube was heated in an air oven at 120°C. for 8 hr.

Ten milliliters of 5N HCl was used to adjust the hydrolysate to pH 5. After centrifugation, the precipitate was washed with deionized water. The combined supernatant and washings were passed through a column (2 X 40-cm.) containing Dowex 50W X 8 (2 X 3-cm. resin bed). The resin was then washed with 100 ml. of water to remove chloride ions, carbohydrates, and other interfering materials. The tryptophan was then eluted with 40 ml. of 2N NH_4OH. The effluent collected was evaporated to dryness in a Buchler flash evaporator, and the residue was dissolved in water.

Sodium hydroxide attacked the glassware, forming from 0.1 to 0.3 g. of sodium silicate per sample. Neither borosilicate Pyrex glass, alkali-resistant glass, nor Vycor tubes were satisfactory under the necessary hydrolytic conditions. In contrast, the polypropylene tube inserted in the O-ring joint served as an economical and simple lining which prevented occlusion with silicate. Furthermore, the O-ring joint tubes eliminate the sealing of tubes and their troublesome opening, and they may be used repeatedly.

Enzymatic Hydrolysis. Hydrolysis was carried out with the enzyme, pronase, as suggested by Cain (4). A pulverized sample weighing 50 to 300 mg. and containing approximately 250γ of tryptophan was mixed in a 60-ml. polyethylene bottle with 10.0 ml. of a freshly prepared 0.02% solution of pronase in a 0.03M sodium phosphate buffer, the pH of which was adjusted to 7.4. One drop of chloroform was added as a preservative, and the bottles, tightly capped, were shaken at medium speed in a water bath for 24 hr. at 40 ±1°C. Blanks containing enzyme solution only were assayed in the same manner. The mixture was then centrifuged (30 min. at 3,000 r.p.m.), and the supernatant was decanted. Aliquots of 1 ml. were used for analysis.

Ion-Exchange Procedure for Separation of Tryptophan from Interfering Substances

A method was developed to separate tryptophan after alkaline or enzymatic hydrolysis from chloride ions, carbohydrates, and other materials before treatment with *p*-DAB. The hydrolysate was adjusted to pH 5 first with 5N HCl, then with dilute HCl or NaOH. It was then centrifuged for 30 min. at 3,000 r.p.m. and the precipitate was washed with 20 ml. of water three times.

The combined supernatant and washings were placed on the column containing Dowex 50W X 8 and the rate of flow was set at 1 ml. per min. The resin was then washed with approximately 150 ml. of redistilled water to remove the interfering materials. The tryptophan was then eluted with 40 ml. of 2N NH_4OH with the rate of flow 1 ml. per 3 min. The effluent was collected in a 250-ml. round-bottomed flask. It was evaporated to dryness *in vacuo* with a rotary evaporator and a 50°C. water bath. The residue after evaporation was dissolved in 10 ml. of water. Aliquots of 1 ml. were analyzed.

The ion-exchange resin was regenerated by passing 100 ml. of 1N HCl through the column. After being washed with 150 ml. of redistilled water, it was ready for further use.

Determination of Tryptophan. Two methods were used for determination of tryptophan; namely, reaction with *p*-DAB under certain conditions, and ion-exchange chromatography.

Procedure B of Spies and Chambers (8) was adopted. Eight milliliters of 23.5N H_2SO_4 and 1.0 ml. of 2N H_2SO_4 containing 30 mg. of *p*-DAB were mixed and cooled to room temperature. Addition of 1.0 ml. of a water solution of tryptophan or 1 ml. of sample made the solution equivalent to 19N H_2SO_4. After being shaken, this solution was kept in the dark at room temperature for 1 hr.; then 0.1 ml. of 0.045% $NaNO_2$ solution was added. The solution was shaken to mix thoroughly, and the color was allowed to develop for 45 min. at room temperature in the dark. Absorbance at 590 nm. was then read in a Beckman DU spectrophotometer.

Tryptophan analyses also were carried out on a Beckman Model 120B automatic amino acid analyzer equipped with a high sensitivity cuvette. The method for the chromatographic analysis was based on the procedure of Spackman (9) for the determination of basic amino acids on the 15.0 X 0.9-cm. column.

RESULTS AND DISCUSSION

Effect of Time of Hydrolysis with Alkali

Three samples with an estimated tryptophan content of approximately 500γ were hydrolyzed for each time interval of 2, 4, 8, 16, and 32 hr. These contained 69 mg. of gluten, 69 mg. of gluten plus 500 mg. of wheat starch, and 500 mg. of white-wheat flour. Thus the concentration of starch was less than 5% in 10 ml. of 5N NaOH. Hydrolysis was carried out in the O-ring joint tube under nitrogen at 120°C., and followed by tryptophan determination with *p*-DAB. Figure 1 shows the curves illustrating the effect of hydrolysis time on tryptophan content of these hydrolysates. All three curves reached maximal values after 8 hr. at 120°C. in a hyperbolic fashion and then declined. Addition of starch to gluten caused a slightly higher maximum yield and protected the tryptophan during subsequent hydrolysis so that the curve paralleled that observed when wheat flour was hydrolyzed up to 32 hr. The presence of starch in cereals therefore resulted in preservation rather than destruction of tryptophan. These observations are in good agreement with those reported by Dreze (10).

When lysozyme, estimated to contain 500γ of tryptophan, was hydrolyzed in the absence of starch, the values at 8, 12, 16, 20, and 30 hr. were 375, 430, 440, 430, and 210γ, indicating that maximal values were not reached until the interval

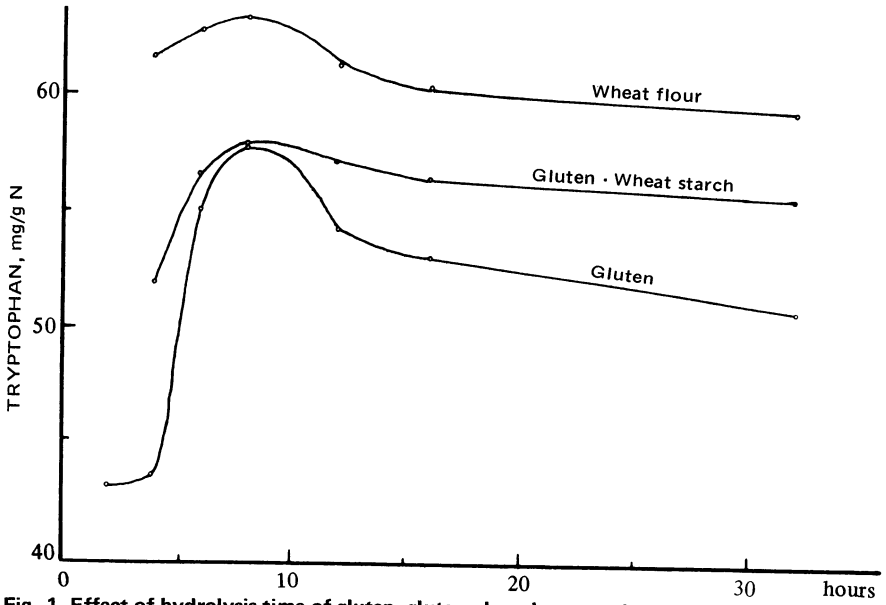

Fig. 1. Effect of hydrolysis time of gluten, gluten plus wheat starch, and white-wheat flour with 5N NaOH at 120°C.

between 12 and 20 hr. Therefore, it may be necessary to establish hydrolysis times for different materials if precise values are sought.

Recovery of Tryptophan

The protective action of histidine and starch on the recovery of tryptophan during alkaline hydrolysis was tested under several conditions based on the reaction with *p*-DAB. Results in Table I provide convincing evidence that the combination of histidine and starch can reduce or even prevent destruction of tryptophan in the free or bound state. However, recoveries were slightly lower (98.6 and 95.4%, respectively) when the same concentrations of starch and histidine were added to rice glutelin and zein. This observation may be related to the extremely low content of tryptophan in zein.

Ion-Exchange Procedure to Remove Interfering Substances

The ion-exchange procedure was developed to eliminate certain substances that interfere with the reaction between tryptophan and *p*-DAB. Destruction of cystine and cysteine during alkaline hydrolysis was confirmed by the absence of the characteristic peak following ion-exchange chromatography on an amino acid analyzer. Elimination of the decomposition products of sulfur-containing amino acids was complete since results obtained from the hydrolysates of solutions containing cystine, cysteine, methionine, and tryptophan were identical with solutions containing the same amount of tryptophan but no sulfur-containing amino acids. The removal of chloride ions and fructose was indicated by negative $AgNO_3$ and resorcinol tests, respectively.

To measure the recovery of tryptophan after the ion-exchange procedure, 100 ml. of 5N NaOH containing 25 mg. of a mixture of 18 amino acids commonly

TABLE I. RECOVERY OF TRYPTOPHAN HYDROLYZED[a] IN THE PRESENCE OF CERTAIN SUBSTANCES

Component Added to Hydrolytic Solution	Tryptophan			
	In gluten γ	Added γ	Found γ	Recovery %
None		500	403.0	80.6
Histidine		500	476.6	95.3
Starch		500	503.3	100.7
Histidine + starch		500	504.0	100.8
Gluten	182.3	300	470.6	97.6
Gluten + histidine	212.5	300	515.5	100.6
Gluten + starch	214.7	300	465.3	90.4
Gluten + histidine + starch	205.4	300	509.4	100.8

[a]Alkaline hydrolysis in O-ring joint tube under nitrogen.

TABLE II. TRYPTOPHAN CONTENT OF SELECTED SOURCES OF PROTEIN

Source	Nitrogen[a] mg./g.	Method of Hydrolysis and Determination			Literature Value[b] mg./g.N
		Alkaline		Enzymatic	
		p-DAB mg./g.N	Ion-exchange chromatography mg./g.N	p-DAB mg./g.N	
Lysozyme	169.8	451.0	548.0	387.0	456
Wheat gluten	135.7	56.0	53.4	54.2	61
Rice glutelin	130.8	86.2	...	73.1	85
Zein	149.2	4.1	...	1.0	4
White-wheat flour	18.2	79.5	82.5	63.1	70
Whole-wheat flour	27.2	59.9	79.1	45.2	72
Rice	10.6	91.0	102.0	84.2	64
Cornmeal	13.1	31.3	41.4	30.9	38
Oatmeal	26.6	68.5	81.2	53.3	75
Soy flour	87.1	86.4	108.0	77.6	86
Chickpea	29.1	81.7	134.0	64.4	51
Nonfat dry milk	54.0	90.2	89.0	83.9	90

[a]By macro-Kjeldahl.

[b]From Orr and Watt (11), except lysozyme (12) and glutelin (13).

present in cereals plus 500 mg. histidine, 500 mg. fructose, and 4.0 g. glucose was hydrolyzed, adjusted to pH 5, and diluted to a final volume of 200 ml. Ten milliliters of a 500γ tryptophan solution plus aliquots of the neutralized hydrolysate were passed through the columns. The recovery of tryptophan from 20 replicates on six different columns was 87.5 ±0.74%. Thus, a correction factor of 1.14 during the ion-exchange procedure was established. This factor was checked from time to time during the analysis of food samples, and the values were found to be reasonably constant. Adsorption and elution of 250γ of free tryptophan with a hydrolysate of white-wheat flour yielded a mean recovery of 100.5 ±0.8% of the free tryptophan adsorbed in five samples.

Tryptophan Content of Selected Sources of Protein

Tryptophan in certain purified proteins and protein sources which varied in the

proportions of carbohydrate and protein was determined: a) with *p*-DAB and by ion-exchange chromatography after alkaline hydrolysis followed by the ion-exchange procedure to remove interfering substances, and b) with *p*-DAB after enzymatic hydrolysis. These data are presented in Table II with values from the literature (11).

Since a relatively large amount of histidine was added to the samples, dilution became necessary to avoid overloading with histidine. For this reason ion-exchange chromatography for determination of tryptophan is less satisfactory following the present alkaline-hydrolysis procedure. This may account for the more elevated values found with those samples analyzed with ion-exchange chromatography than those with *p*-DAB on the same hydrolysate. On the other hand, values obtained with *p*-DAB were consistently higher after alkaline than after enzymatic hydrolysis under the conditions described, except for cornmeal. Tryptophan in *opaque-2* corn was considerably lower, however, than reported by Cain (4), who applied the method of Opienska-Blauth et al. (14) after hydrolysis with pronase. A yellow carotenoid pigment persisted in the analysis of corn samples. This discrepancy was probably responsible for the low values with *opaque-2* corn and with cornmeal in Table II. Further investigation is needed before a final conclusion can be drawn.

The data agreed reasonably well with mean values reported in the literature, indicating the validity of the proposed method.

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