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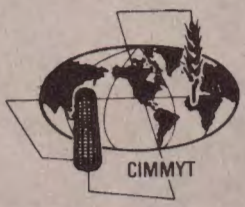

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A MODIFIED METHOD FOR RAPID TRYPTOPHAN ANALYSIS OF MAIZE

HORACIO HERNÁNDEZ H.[1] AND LYNN S. BATES[2]

Introduction

Due to their low lysine content, the cereal grains are generally considered of reduced nutritional quality in comparison to other protein sources. In maize, a second diet limiting amino acid is tryptophan. That increases in both of these amino acids are needed to improve the nutritional quality of maize has been known for many years. With the demonstrated improvement possible with opaque-2 maize, breeders are paying growing attention to improving nutritional quality through increasing the content of these two amino acids. However, the success of such research efforts depends ultimately upon the rapidity with which one can determine the lysine and/or the tryptophan content of individual plants or seeds in segregating populations.

From the observed relationship between lysine and tryptophan in the zein fraction and its relationship to the whole endosperm, it was believed a tryptophan value could serve as a single parameter for maize evaluation. This study demonstrates the feasibility of rapid tryptophan analyses and the magnitude of the correlation between tryptophan and lysine.

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Selection of a method

Microbiological assay, various chromatographic techniques, spectrophotometric analysis, and chemical estimations following alkaline or enzymatic hydrolysis have been commonly used for determining tryptophan in proteins. Unfortunately most of these methods require expensive equipment and highly trained personnel, or are time consuming due to the number of steps involved per determination.

Opienska-Blauth, et. al. (1) modified several earlier methods for use in biological materials. We selected their method for its simplicity and adapted it to routine maize evaluation. Several modifications were necessary.

A single step papain hydrolysis was utilized for protein solubilization. Charring of the samples was eliminated by diluting the sulfuric acid and substituting controlled heating for the heat of reaction lost. Reproducibility was found dependent upon particle size of the sample. The influence of pH, ionic strength of the acetate buffer, temperature, and hydrolysis time were studied. For routine work, 6 hr. hydrolysates were used since the differences between these and optimum 24 hr hydrolysates were very small. Anthocyanin pigments, irrespective of concentration, interferred with the characteristic violet-purple color of the reaction. This was due to an overlap of absorption spectra. Yellow carotenoid pigments did not affect the determination.

Methods and Materials

Reagents:

A) 270 mg of $FeCl_3.6H_2O$ was dissolved in 0.5 ml of distilled water and diluted to 1 liter with glacial acetic acid.

B) 30 N. sulfuric acid.

C) A volume to volume mixture of reagents A and B was prepared 1 to 2 hours prior to its use.

Papain solution. The enzyme (4 mg/ml) was dissolved in 0.1 N. sodium acetate buffer at pH 7.0. The enzyme solution was prepared daily.

Method:

Maize endosperm samples were prepared according to Mertz and Bressani (2) and Bates (3).

1. Eighty to one hundred and fifty mg of the defatted corn endosperm sample was weighed into a glass vial (approximately 22 x 75 mm) and 4 ml of papain solution added. The samples were capped, shaken, and placed in an oven at 65°C for 6 hr with 4 hourly shakings and two final hours of settling.

2. One ml of hydrolysate was pipetted into a colorimeter tube containing 4 ml of reagent C, the mixture was shaken vigorously and the color developed for 15 minutes at 65°C.

3. After cooling to room temperature, the absorbance of each sample was read at 545 mμ in a Bausch and Lomb Spectronic 20. The tryptophan concentration was calculated from standard curves (0 to 50 μg/ml) of DL-tryptophan treated similarly. (Fig. 1) Tryptophan was reported on a protein basis.

Quantification Studies:

The 6 hr enzymatic hydrolysis was compared with complete alkaline hydrolysis (2N barium hydroxide) in sealed tubes. Also the colorimetric method described above was used for these determinations following removal of the barium ion in the case of alkaline hydrolysis.

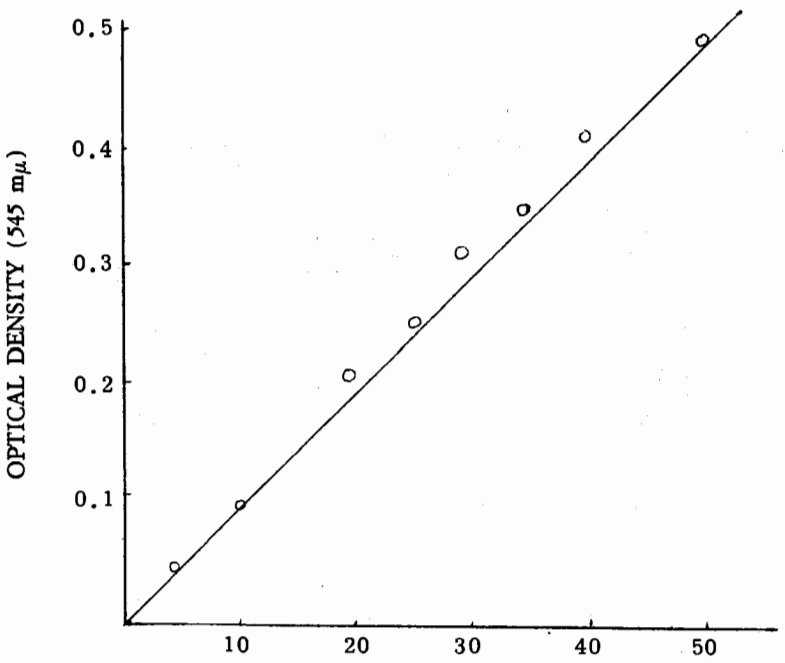

FIGURE 1. Standard curve of trvptophan..

Although papain cannot completely hydrolyze all the protein, the high correlation between values of percent tryptophan in protein as determined by alkaline hydrolysis versus enzymatic hydrolysis ($r = 0.98$) and the slope of the regression line (1.06) indicates that essentially all the tryptophan has been made available for the reaction as shown in Table 1.

Lysine Correlation:

Using a Beckman 120C amino acid analyzer, 55 samples were analyzed for lysine. The same samples were evaluated for tryptophan by the modified method described herein, Table 2. A highly significant correlation was obtained, $r = 0.85$ with the regression equation, $y = 0.3601 + 4.0745\ x$ where $y =$ per cent of lysine in protein and $x =$ per cent of tryptophan in protein.

TABLE 1. Enzymatic hydrolysis vs alkaline hydrolysis.*

Sample Number	6 hours Enzyme Hydrolysis % Tryptophan in Protein	24 hours Alkaline Hydrolysis % Tryptophan in Protein
0067	0.36	0.40
0105	0.31	0.42
0224	0.40	0.48
0264	0.38	0.41
0279	0.86	0.85
0618	0.57	0.64
2034	0.47	0.56
2035	0.92	0.98
2036	0.91	1.02
2037	0.40	0.35
2038	0.33	0.29
Tuxpeño (o_2) F_2	0.75	0.88

* $r = 0.98$.

TABLE 2. Ratio between lysine and tryptophan in corn endosperm protein.

Variety or Cross	% Protein	% Lysine in Protein	% Tryptophan in Protein
Maizón x o_2 o_2 Seg. F_2	9.25	4.1	0.91
,, ,, ,, Normal F_2	10.69	2.5	0.47
,, ,, ,, o_2 Seg. F_2	8.25	4.4	0.75
,, ,, ,, Normal F_2	9.69	3.0	0.39
Puebla Group I x o_2 o_2 Seg. F_2	10.25	4.5	0.72
,, ,, ,, ,, ,, Normal F_2	12.00	1.9	0.33
,, ,, ,, ,, ,, o_2 Seg. F_2	11.50	3.0	0.77
,, ,, ,, ,, ,, Normal F_2	12.88	2.0	0.36
V-520 C x o_2 o_2 Seg. F_2	9.88	3.9	0.77
,, ,, ,, Normal F_2	10.63	1.4	0.36
V-520 C x o_1 o_1 Seg. F_2	10.44	2.0	0.46
,, ,, ,, Normal F_2	10.44	2.0	0.34
Tuxpeño, Tep. 66B 5089-1#	11.19	1.4	0.31
,, ,, ,, ,, -2#	11.25	1.5	0.31
,, ,, ,, ,, -3#	8.25	1.8	0.38
,, ,, ,, ,, -4#	8.94	1.6	0.36
,, ,, ,, ,, -5#	11.88	1.7	0.29
Celaya, Sint. I 180-180-0	9.56	1.7	0.36
,, ,, ,, 80-80-0	8.88	2.0	0.35
Puebla Sint. 180-180-0	8.56	2.0	0.49
,, ,, 80-80-0	7.94	2.2	0.50
Guatemala # 23480	10.19	1.7	0.46
Sto. Domingo # 11136	7.38	2.5	0.41
Celaya, Gto. 65 44S-1	11.87	1.5	0.27
,, ,, ,, -2	12.13	1.8	0.30
,, ,, 79 45S-1	10.86	1.9	0.34
,, ,, ,, -2	11.52	1.8	0.30
,, ,, ,, -3	8.69	1.9	0.39
,, ,, 85 46S-1	11.31	1.5	0.37
,, ,, ,, -2	11.06	1.6	0.20
,, ,, 86 47S-1	12.31	1.6	0.35

TABLE 2. (Cont.). Ratio between lysine and tryptophan in corn endosperm protein.

Variety or Cross	% Protein	% Lysine in Protein	% Tryptophan in Protein
Cónico Nort., Gto. 24 49S-1	14.25	1.6	0.37
,, ,, ,, ,, -2	12.19	1.6	0.34
,, ,, ,, ,, -3	11.69	1.7	0.30
,, ,, ,, 34 50S-1	9.25	2.0	0.41
,, ,, ,, ,, -3	12.25	1.4	0.30
,, ,, ,, ,, -4	10.63	1.6	0.45
,, ,, ,, 95 52S-1	11.19	1.2	0.28
Maíz Ancho K-5	8.44	2.5	0.37
,, ,, K-18	7.40	1.7	0.45
,, ,, K-20	7.06	1.9	0.50
,, ,, K-50	7.22	2.4	0.53
,, ,, K-51	8.69	2.1	0.44
,, ,, K-53	7.34	2.2	0.45
,, ,, K-55	6.97	2.9	0.46
,, ,, K-76	8.00	2.6	0.40
,, ,, K-81	6.99	2.8	0.53
,, ,, K-87	8.23	2.5	0.45
,, ,, K-96	7.07	2.6	0.51
,, ,, K-105	6.94	2.0	0.53
Chalqueño Waxy Ch-67	9.69	1.7	0.47
Bolita x o_2 5044	9.66	3.7	0.92
Pepitilla x o_2 5055	9.50	3.6	0.91
Tuxpeño V-520 C Cot. 65-B	8.12	1.5	0.40
Tuxpeño Grano duro SRV-65-A	10.53	1.3	0.33

Discussion

This modified method has been used extensively for over one year in screening CIMMYT's maize materials. Its outstanding features are the number of determinations —100 single samples or 50 duplicate determinations— per 8 hr working day. Only common, inexpensive equipment is necessary; no special training is needed except skill in handling simple, chemical glassware and reagents and in reading a colorimeter. The procedure is also adaptable to isolated experiment stations with electrical power.

Abstract

A rapid method for determining the tryptophan content of maize endosperm is described. The method is simple, accurate and reproducible. A highly significant correlation with lysine was established. The method has been used to evaluate large numbers of maize synthetics and segregating populations.

REFERENCES

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