The Tyrosine Content of Farm Feeds

CARL M. LYMAN, B. J. CAMP, and HELEN M. TILLER

Texas Agricultural Experiment Station, A&M College of Texas System, College Station, Tex.

A procedure for the stabilization of tyrosine during the acid hydrolysis of food materials containing carbohydrates is based on the principle of adding excess tryptophan which reacts with the carbohydrate, leaving the tyrosine free. Using this method for hydrolyzing the samples, the tyrosine content of 138 different kinds of farm feeds was determined by microbiological assay with *Leuconostoc mesenteroides* as the test organism.

The essential amino acid content of farm feeds used in poultry and swine rations has been reported by Lyman, Kuiken, and Hale (*11*).

Although tyrosine is not one of the essential amino acids, tables on the essential amino acid content of feedstuffs are not entirely complete without data on tyrosine, because the quantitative requirements of animals for phenylalanine are greater if tyrosine is not present in the diet. The sparing action of tyrosine has been shown for rats by Armstrong (*2*), for young pigs by Mertz, Henson, Beeson (*12*), and for chicks by Almquist and Grau (*1*).

One of the major difficulties in the determination of tyrosine in materials containing carbohydrates is that during acid hydrolysis tyrosine is destroyed, with the formation of humin substances (*7, 10*). For this reason, alkaline hydrolysis has been recommended by Block and Weiss (*3*). Although it seems to have been the preference of most investigators, Hodson and Krueger (*8*) found a 10% loss after barium hydroxide hydrolysis, and Bolling and Block (*4*), as a result of 38 experiments on the recovery of tyrosine added to lactoglobulin and six experiments with tyrosine alone, reported a loss of from 11 to 17% of the added tyrosine after 5-hour hydrolysis with 5*N* sodium hydroxide in an oil bath at 115° to 125° C. The experience of the present authors has been much the same as the above reports. The procedure used by Kuiken, Lyman, and Hale (*9*) for the stabilization of tryptophan during alkaline hydrolysis did not completely stabilize tyrosine.

The purpose of the present communication is to report a new method for the stabilization of tyrosine during the hydrolysis of feedstuffs containing carbohydrates, and to report the tyrosine content of feedstuffs and materials with potential value as farm feedstuffs.

Experimental

Hydrolysis of Samples. A 1-gram sample ground to pass a 60-mesh sieve and

Table I. Stabilization of Tyrosine during Acid Hydrolysis[a] in the Presence of Carbohydrate

Added before Hydrolysis			Tyrosine Recovery	
Starch, g.	Tryptophan, g.	Tyrosine, mg.	Mg.	%
			Mg.	%
0.0	0.0	10.0	9.1	91
0.5	0.0	10.0	8.3	83
0.0	1.0	10.0	10.1	101
0.5	1.0	10.0	9.8	98

[a] 100 ml. of 6*N* HCl, refluxed 24 hours.

Table II. Stabilization of Tyrosine during the Acid Hydrolysis of Feedstuffs[a]

Feedstuff	Tyrosine Found in Sample, % Without tryptophan	Tyrosine Found in Sample, % With tryptophan[b]
Oats	0.35	0.42
Wheat	0.48	0.54
Soybean	1.65	1.92
Corn	0.28	0.48
Tuna fish meal	2.39	2.34
Polished rice	0.21	0.46

[a] Grams of sample, 100 ml. of 6*N* HCl, refluxed 24 hours.

[b] Grams of tryptophan added prior to hydrolysis.

Table III. Tyrosine Content of Farm Feeds and Materials with Potential Values as Feed Ingredients

Product	No. of Samples	Average Crude Protein,[a] %	Average Tyrosine Content, % Sample	Average Tyrosine Content, % Crude protein
ALGAE				
Ascomeal	1	5.75	0.24	4.17
Cladophora rupestris	2	28.12	0.99	3.52
Laminaria cloustoni	1	10.91	0.27	2.47
Neptune's Bounty	1	6.01	0.14	2.33
Rhodymenia Palmata	1	20.69	0.61	2.96
ANIMAL BY-PRODUCTS				
Blood meal	1	80.14	2.21	2.76
Bone meal, steamed	1	15.77	0.49	3.11
Digesta bone	1	7.97	0.24	3.01
Digester tankage	2	60.81	1.63	2.68
Dried buttermilk	2	28.31	1.46	5.16
Dried skimmed milk	1	33.50	1.89	5.64
Dried whey solubles	1	28.09	0.80	2.85
Dried whole whey	2	12.31	0.35	2.84
Dried hydrolyzed whey	1	19.79	0.68	3.44
Dried sweet whole whey	1	12.69	0.34	2.68
Meat and bone scrap	2	50.02	1.22	2.43
Meat, bone, poultry scrap	1	51.93	1.25	2.41
FERMENTATION FEEDS				
Dried brewer's and distiller's grains				
Brewer's dried grains	1	20.21	1.22	6.03
Distiller's dried grains	1	29.50	1.17	3.97
Corn distiller's dried grains, Distillers' Feed Research Council, composite sample	1	25.77	1.38	5.36

Table III (Continued)

1-gram of DL-tryptophan were placed in a 500-ml. long-necked round-bottomed flask with ground-glass connection. One hundred milliliters of redistilled 6*N* hydrochloric acid were added and the material was boiled under a reflux condenser for 24 hours. Overheating of the flask above the liquid level was avoided by using an electric heater with a refractory top plate with a small hole in the center. Caking of the material on the side of the flask may lead to low results.

Using a hot water bath to heat the flask, the volume of hydrochloric acid was reduced to approximately 5 ml. by vacuum distillation. About 75 ml. of distilled water and 10 or 12 glass beads were added, and then the flask was swirled to remove any solid material adhering to the flask.

The solution was transferred quantitatively to a 250-ml. beaker, and neutralized with sodium hydroxide (pH 6.8) using a Beckman pH meter. A pH meter was found more satisfactory than an indicator dye in adjusting the pH, because of the dark color of most hydrolyzates.

The solution was diluted to 200 ml. in a volumetric flask and filtered. Most of the added tryptophan precipitates and is removed by this filtration.

Microbiological Assay. *Leuconostoc mesenteroides* P60 (ATCC No. 8014) was used as the assay organism. The basal medium was the same as that used by Lyman, Kuiken, and Hale (*11*) for the determination of arginine, phenylalanine, and lysine with the exception that tyrosine was omitted. Standard curves were prepared with L-tyrosine over the range 0 to 25 γ with points at 2.5-γ intervals.

The general procedure for maintaining the culture and conducting the assay has been described (*11*).

Results and Discussion

Stabilization of Tyrosine during Acid Hydrolysis. It has been known for many years that both tryptophan and tyrosine react with carbohydrates when boiled in acid solution. As a result of the reaction, dark-colored humin materials containing nitrogen derived from these amino acids are formed. Observation of the color development as the reaction proceeds suggested that the reaction between carbohydrate and tryptophan is considerably faster than the reaction between carbohydrate and tyrosine. By increasing the concentration of tryptophan to many times that of tyrosine, the reaction is shifted toward tryptophan to the extent that for all practical purposes the tyrosine is left completely free. This is the basis for the stabilization of tyrosine used in this investigation.

Table I is representative of several tests on the use of tryptophan for the stabilization of pure tyrosine boiled for 24 hours in redistilled 6*N* hydrochloric acid with and without the addition of starch. In the presence of starch without tryptophan, 17% of the tyrosine was lost. When tryptophan was added in addition to the starch, the recovery was

Table III. Tyrosine Content of Farm Feeds and Materials with Potential Values as Feed Ingredients (*Continued*)

Product	No. of Samples	Average Crude Protein,[a] %	Average Tyrosine Content, %	
			Sample	Crude protein
FERMENTATION FEEDS (*Cont.*)				
Corn distiller's dried grains with solubles. Distillers' Feed Research Council, composite sample	1	27.82	1.19	4.28
Dried fermentation solubles				
Distiller's solubles	1	27.03	0.72	2.66
Corn distiller's solubles. Distillers' Feed Research Council, composite sample	1	27.72	1.05	3.79
Cane sirup butyl fermentations solubles	1	25.13	0.70	2.79
Dried corn fermentation solubles	1	32.13	1.29	4.01
Molasses ethyl fermentation solubles (Vacatone 40)	1	7.85	0.09	1.14
Malt sprouts	1	31.83	0.71	2.23
Brewer's dried yeast	1	43.37	1.48	3.41
Torula yeast	1	46.35	1.67	3.60
FISH BY-PRODUCTS				
Fish meals				
Anchovy meal	1	58.72	2.16	3.66
Angola fish meal	1	65.03	2.35	3.61
Fish meal	1	64.74	2.23	3.45
Flounder meal	1	56.48	1.57	2.78
Herring fish meal, Canadian	1	73.00	2.56	3.51
Herring full meal, Canadian	1	72.06	2.12	2.94
Herring full meal, Norwegian	1	66.59	2.26	3.39
Menhaden fish meal	3	61.21	1.97	3.21
Pilchard full meal	1	63.87	2.07	3.24
Redfish meal	2	58.50	1.90	3.25
Sardine meal	1	53.97	1.79	3.32
Sardine fish meal, Chilean	1	69.59	2.32	3.33
Sardine waste–fish meal	1	59.35	1.94	3.27
Tuna fish meal	2	60.80	2.07	3.40
White fish meal	1	69.06	2.33	3.37
Crab meal	1	36.87	1.40	3.80
Fish solubles				
Condensed fish solubles	2	69.12 (dry wt.)	0.61	0.88
Condensed menhaden fish solubles	1	64.66 (dry wt.)	0.87	1.35
Menhaden fish meal with solubles	1	61.87	2.23	3.60
Dry fish solubles on alfalfa meal carrier	1	39.26	0.61	1.55
Desiccated and defatted fish meal	1	80.30	2.65	3.30
Desiccated, defatted, and deodorized fish flour	1	79.60	2.79	3.51
Fish glandular and liver hydrolyzate	1	63.66 (dry wt.)	1.80	2.83
GRAINS				
Barley, Cordona	1	13.93	0.52	3.73
Barley, Goliad	1	13.97	0.41	2.86
Barley, av.		13.95	0.46	3.29
Buckwheat seed	4	11.52	0.38	3.21
Corn, Country Gentlemen	1	10.81	0.52	4.81
Corn, yellow	3	9.93	0.45	4.53
Feterita	1	11.50	0.66	5.74
Hegari, early	1	9.63	0.39	4.05
Kafir, sugary	1	8.28	0.54	6.52
Kafir, waxy	1	9.56	0.39	4.08
Kafir, combine	1	9.47	0.43	4.54
Manitoba, yellow	1	14.25	0.63	4.42
Milo	1	11.34	0.50	4.41
Oats, Alamo	1	12.15	0.48	3.95
Oats, Mustang	1	11.60	0.46	3.97
Oats, Ranger	1	10.45	0.42	4.02
Oats, av.		11.40	0.44	3.98
Redbine, 66	1	9.78	0.37	3.78
Rice, Century Patna (polished)	1	8.41	0.45	5.35
Rice, Rexoro (polished)	1	8.66	0.46	5.31
Rice (polished), av.	1	8.53	0.46	5.33
Rice, Century Patna (rough)	1	7.81	0.40	5.12
Rice, Rexoro (rough)	1	8.16	0.40	4.90
Rice (rough), av.		7.98	0.40	5.01
Rye, Balboa	1	10.47	0.41	3.92
Rye, Tetra Petkus	1	9.53	0.33	3.46
Rye, av.		10.00	0.37	3.70
Shallu	1	9.54	0.43	4.51
Shrock	1	11.59	0.51	4.40
Spelt, White Spring	1	14.00	0.46	3.29

Table III. *(Continued)*

Product	No. of Samples	Average Crude Protein,[a] %	Average Tyrosine Content, % Sample	Average Tyrosine Content, % Crude protein
GRAINS (*Cont.*)				
Turghai, red	1	14.18	0.57	4.02
Wheat, Frisco	1	15.28	0.51	3.34
Wheat, Quanah	1	18.16	0.63	3.47
Wheat, Seabreeze	1	18.50	0.55	2.97
Wheat, Selkirk	1	17.00	0.54	3.18
Wheat, av.		17.23	0.56	3.24
GRAIN BY-PRODUCTS FROM MILLING AND PROCESSING				
Corn by-products				
Corn, coarse fiber	1	8.60	0.26	2.91
Corn, fine fiber	1	12.80	0.48	3.75
Corn oil cake meal (germ meal)	1	24.39	0.91	3.73
Corn glutelin	1	48.51	2.67	5.50
Corn gluten	1	62.65	3.72	5.94
Corn gluten feed	1	21.42	0.64	2.99
Corn gluten meal	1	43.55	1.97	4.52
Corn steep water	1	41.19 (dry wt.)	1.11	2.69
Hominy feed	1	9.97	0.43	4.31
Grain sorghum by-products				
Grain sorghum gluten meal	1	64.69	3.13	4.84
Grain sorghum oil cake meal (germ meal)	1	18.50	0.65	3.51
Milo, coarse fiber	1	10.03	0.39	3.88
Milo, fine fiber	1	23.22	1.14	4.91
Milo oil cake meal (germ meal)	1	21.94	0.84	3.83
Milo gluten	1	60.91	2.33	3.83
Milo gluten feed	1	26.38	0.90	3.41
Milo gluten meal	1	44.93	2.24	4.99
Milo heavy steepwater solids	1	37.66 (dry wt.)	0.93	2.47
Oatmeal feed	1	13.91	0.55	3.95
Oats, table	1	16.72	0.66	3.95
Rice by-products				
Rice bran	1	12.59	0.46	3.65
Rice bran, Century Patna	1	14.50	0.47	3.24
Rice bran, Rexoro	1	15.07	0.55	3.65
Rice bran, av.		14.05	0.49	3.48
Rice polishings	1	14.00	0.45	3.22
Wheat by-products				
Wheat bran	2	15.41	0.50	3.25
Wheat germ	1	27.45	0.81	2.95
Wheat germ meal	1	38.63	0.96	2.49
Wheat gray shorts	2	17.78	0.50	2.83
Wheat, Red Dog	1	17.39	0.57	3.28
Wheat, second clear	1	15.69	0.46	2.93
Wheat standard middlings	1	16.52	0.51	3.09
OILSEED RESIDUES				
Babassu meal	1	22.72	0.69	3.04η
Castor flour	1	65.03	2.07	3.18
Castor pomace	1	39.79	1.15	2.89
Copra meal	3	21.35	0.53	2.48
Cottonseed meal, solvent-extracted	2	47.60	1.50	3.15
Cottonseed flour (Proflo)	1	55.94	1.88	3.36
Linseed meal	1	45.25	1.04	2.30
Palm kernel meal, expeller	2	16.95	0.56	3.31
Peanut meal, solvent-extracted	2	46.19	1.69	3.66
Safflower meal	1	22.10	0.62	2.81
Sesame meal, expeller	3	45.44	1.55	3.41
Soybean meal, solvent extracted	2	45.75	1.83	4.00
Soybean protein (commercial)	1	80.32	3.12	3.88
Sunflower seed meal	1	41.26	1.05	2.54
Tung meal (commercial)	1	20.88	0.77	3.69
Tung meal (laboratory)	1	48.07	1.48	3.08
Walnut oil meal (av. protein)	1	13.03	0.44	3.38
Walnut oil meal (high protein)	1	19.57	0.62	3.17
PEAS AND BEANS				
Black-eyed peas	3	21.59	0.76	3.52
Chinese-red cow peas	1	22.54	0.81	3.59
MISCELLANEOUS				
Alfalfa leaf meal, dehydrated	2	19.27	0.84	4.35
Animal protein factor[b]	1	52.94	0.88	1.66
Antibiotic feed supplement[c]	1	46.13	1.54	3.34
Grape pulp, dehydrated	1	13.50	0.50	3.70

[a] Values not corrected to moisture-free basis. [b] Dried fermentation product prepared as source of vitamin B_{12}. Amino acid composition of products is due to carrier substance. Other animal protein factor preparations may be different in amino acid content according to process used. [c] Dried fermentation by-product from bacitracin production.

98%. Within the limits of error to be expected in a microbiological test, this represents complete recovery.

It is conceivable that the stabilization of tyrosine in a protein molecule might be quite a different matter from the stabilization of tyrosine added as the free amino acid. To test this possibility casein was hydrolyzed alone, with tryptophan, with starch, and with both tryptophan and starch. The values for the tyrosine content of the casein sample hydrolyzed without the addition of either tryptophan or starch were 4.66 and 4.69%. On the addition of tryptophan before hydrolysis, the value was raised to 4.88%. Although the slightly higher value appears to represent an improvement in the conditions of hydrolysis, the difference is within the experimental error of the microbiological test. When starch was added without tryptophan, the apparent tyrosine content was reduced to 4.19%. This destruction was prevented by the addition of tryptophan (4.70 and 4.71% tyrosine).

The sample of Labco casein used in this test contained 13.49% nitrogen without correction for moisture and ash. Based on the experimental value of 4.70% tyrosine in the sample, the tyrosine content calculated to 16% nitrogen is 4.47%. Literature values for the tyrosine content of casein vary considerably. Although a number of these are in good agreement with the above, the purpose of the experiment was to test tyrosine stabilization rather than to present a new value for the tyrosine count of casein. It appears that part of the discrepancy to be found in the literature concerning the tyrosine content of casein is due to the fact that whole casein is made up of several proteins of different amino acid composition (*5*, *6*).

Table II shows the difference in the values obtained for the tyrosine content of feedstuffs hydrolyzed with and without tryptophan. With a material like tuna fish meal which contains very little carbohydrate, adding tryptophan before hydrolysis made little or no difference. However, with material like polished rice, which is high in carbohydrate and low in protein, the tyrosine values were more than doubled.

Tyrosine Content of Feedstuffs. The tyrosine content of various classes of materials used in feeds for poultry and swine is given in Table III. The table also includes materials which are not now used as feedstuffs, but appear to have potential value for such use.

Different general classes of feedstuffs are characteristically high or low in certain amino acids. For example, feedstuffs of animal origin are in general higher in lysine than products of vegetable origin. Corn and the grain sorghums are high in leucine and the majority of the oilseed meals are high in arginine. Examination of the tyrosine

values reported here fails to reveal any particular class of materials which is either very high or very low in tyrosine.

No literature values could be found for the tyrosine content of many of the farm feeds reported in this communication. Gunness, Dwyer, and Stokes (*7*) determined tyrosine in some food materials by microbiological assay after basic hydrolysis. The values reported by these investigators are somewhat lower for blood meal, milk, rye, brewer's dried yeast, soybean meal, and alfalfa leaf meal than those given in the present communication. In the case of wheat, corn, and barley, the values obtained in the present investigation are either in satisfactory agreement or they are slightly lower for some varieties than those reported by Gunness, Dwyer, and Stokes (*7*).

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CORN NUTRIENT LOSSES

Chemical Changes in Corn during Preparation of Tortillas

RICARDO BRESSANI, RAMIRO PAZ Y PAZ, and NEVIN S. SCRIMSHAW

Institute of Nutrition of Central America and Panama, Guatemala, Guatemala, C. A.

Corn consumed in the form of flat cakes (tortillas) is the principal food of lower income families in Central America. Important changes in nutritive value result from heating the corn in lime water to soften it for tortilla preparation. The changes in samples of white and yellow corn used by two families in a Guatemalan highland Indian village were determined. For the white corn, the combined physical and chemical loss from corn to masa, the dough from which the tortilla cakes are made, averaged 60% of the thiamine, 52% of the riboflavin, and 32% of the niacin, as well as 10% of the nitrogen, 44% of the ether-extractable portion, and 46% of the crude fiber. The yellow corn lost 65% of the thiamine, 32% of the riboflavin, 31% of the niacin, and 21% of the carotene originally present, as well as 10% of the nitrogen, 33% of the ether-extractable portion, and 32% of the crude fiber.

In spite of the great importance of tortillas as the daily staple for many people in Mexico and Central America, relatively few studies have been made of their chemical composition and nutritive value. Tortilla preparation is not standard among all countries where it is a basic food, and additional studies are required to determine the manner in which the different methods influence nutritive value.

Tortilla preparation in Mexico, as described by Illescas (*14*), involves the addition of one part of whole corn to two parts of approximately 1% lime solution. The mixture is heated to 80° C. for 20 to 45 minutes and then allowed to stand overnight. The following day the cooking liquor is decanted, and the corn, now referred to as "nixtamal," is washed two or three times with water without removing the episperm or the germ. The cooked corn is then ground to a fine dough called "masa." About 50 grams of dough are patted flat and cooked on both sides on a hot iron plate.

Pérez y Pérez (*22*) has presented data on the mineral and protein content, and Cravioto and coworkers (*2*) have studied the chemical composition of tortillas made in Mexico. They have reported relatively small losses in thiamine, niacin, and riboflavin, and a 40% loss in the carotene content of yellow corn. The phosphorus and iron contents increased 15 and 37%, respectively, and because of the treatment with lime water, the calcium increased 2010%.

Massieu and coworkers (*19*) and Cravioto and associates (*3*) showed that tortillas were deficient in lysine and tryptophan, and that during preparation considerable change occurred in the original histidine, threonine, arginine, and tryptophan content of the corn. Although Tapia and coworkers (*25*) reported that the Mexican preparation of tortillas impairs the biological value of corn proteins, Cravioto and associates (*4*) and Laguna and Carpenter (*17*) have shown that rats fed tortillas gained weight faster on a diet deficient in niacin and tryptophan than rats fed untreated corn. This has been confirmed by Squibb and coworkers (*24*).

Jaffé (*16*) described the method used for making tortillas (arepas) in Venezuela and showed large losses of fat, thiamine, riboflavin, and niacin. Both the germ and episperm are separated by mechanical maceration, leaving only the endosperm. The Venezuelan method thus gives a product nutritionally inferior to that prepared in Mexico and Central America.

The present work was undertaken to study the effects of the lime treatment on the chemical composition of corn in the making of tortillas in Guatemala. The data obtained in this study are of practical value to dietitians, nutritionists, and institutions attempting to study the prob-