Table II. Organic Matter Content of Typical Humid Region Soils

Soil Type[a]	Depth of Soil Sampled, Inches	Organic Matter, % Standard chromic acid[b]	Organic Matter, % Colorimetric quick test
Orlando f.s.	3	4.29	4.0
Scranton s.	6	3.68	3.5
Scranton s.	5	3.16	3.0
Ducker s.l.	6	4.06[c]	4.0
Gainesville l.f.s.	7	2.42	2.5
Leon s.	5	1.82	1.5
Plummer f.s.	8	1.53	2.0
Blanton f.s.	3	1.23	1.5
Rex f.s.	8	1.84	2.0
Blanton f.s.	3	1.76	1.5
Greenville l.f.s.	6	0.90	1.0
Gainesville l.f.s.	4	3.79	4.0
Arzell f.s.	3	0.27	0.0
Brookston c.	6	2.50[d]	2.5
Montgomery s.c.l.	6	3.15[d]	2.5
Bono c.l.	6	>4.0[d]	4.0
Rafferty s.c.l.	6	>4.0[d]	>4.0
Chalmers s.c.l.	6	4.0[d]	3.5

[a] F.S. (fine sand), s (sand), l.f.s. (loamy fine sand), c (clay), s.c.l. (salty clay loam), c.l. (clay loam).
[b] Recorded data from survey of Alachua County, Florida.
[c] Recorded soil samples, College of Agriculture, University of Florida.
[d] Selected group of northern humid region heavier textured soils.

ing solution. Two drops of the chlorine solution are added; the tube is immediately sealed over with the thumb and shaken vigorously for exactly 1 minute (oxidation time is critical). After shaking, the suspension is allowed to settle for exactly 5 minutes. The entire soil suspension is now filtered through a Whatman No. 1 filter paper, and 2 ml. of the clear filtrate is caught in a marked 16 × 150 mm. test tube. Immediately, 3 drops of the *o*-tolidine solution are added, the tube is swirled for mixing, and then a period of 15 minutes is allowed for the full color to develop.

The shade of deep orange to pale yellow color is compared directly with the liquid standards and the amount of organic matter is estimated to the nearest 0.5%.

Results

Results obtained with the colorimetric test for organic matter in some typical humid region soils are given in Table II. Comparisons are made with several soil types which have been tested for organic matter by the standard chromic acid procedure.

Literature Cited

(1) Am. Public Health Assoc., "Standard Methods for the Examination of Water and Sewage," 9th ed., pp. 92–5, Lancaster Press, Lancaster, Pa., 1946.
(2) Edson, S. N., and Thornton, G. D., *Quart. J. Fla. Acad. Sci.*, **17**, 83 (1954).
(3) Gammon, N., and others, Fla. Agr. Expt. Sta., Bull. **524** (1953).
(4) Peech, M., and others, U. S. Dept. Agr., Circ. **757** (1947).
(5) Purvis, E. R., and Blume, J. M., Va. Truck Agr. Expt. Sta., Bull. **106** (1941).

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GLUTAMIC ACID DETERMINATION

Paper Chromatography in Routine Determination of Glutamic Acid in Production

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The determination of glutamic acid in protein hydrolyzates by McFarren's quantitative chromatographic method has been applied successfully to the analysis of products obtained in glutamic acid production. The addition of 2-butanol to the phenol solvent improved the separation of glutamic acid from the other amino acids and led to the formation of round and compact spots.

IN THE PRODUCTION OF GLUTAMIC ACID from gluten it is important to have a reliable and convenient method for determining glutamic acid. Irrespective of the method used to separate this amino acid from the gluten hydrolyzate, it is essential to check the glutamic acid content of a wide variety of process liquors and salt cakes, some of which may contain a high concentration of sodium chloride.

The chromatography of amino acids on buffered paper was undertaken by Haugaard and Kroner (*1*) and was later adapted by McFarren and Mills (*3*) to the quantitative estimation of amino acids in protein hydrolyzates. The latter authors employed papers buffered at pH 12 and a phenol-buffer solvent at the same pH, for the quantitative estimation of glutamic acid. The salt was removed from the hydrolyzates by extraction with a 95% solution of di-2-ethylhexylamine in chloroform.

The present paper describes a procedure for the estimation of glutamic acid on buffered paper (pH 12) using a solvent system made up of buffer (pH 12) saturated phenol containing a small amount of 2-butanol. The important features of this solvent system are a clean separation of glutamic acid from the other amino acids, even in the presence of a high concentration of sodium chloride, and the formation of round and compact spots of uniform size, which greatly reduces the error involved in estimating the concentration of amino acid by direct densitometry. The results obtained using the paper chromatography method have been compared with those obtained by Olcott's method (*4*).

Materials

Buffered Filter Papers. Whatman No. 1 (18 × 1/2 × 22 1/2 inches) filter

papers were cut into two strips ($9^1/_4 \times 22^1/_2$ inches) and dipped in the buffer solution (pH 12) recommended by McFarren (*2*). The papers were dried and stored in a clean, dry place.

Chromatographic Chambers. Chromatographic chambers manufactured by the Shandon Co., London, were employed. In order to ensure saturation of the atmosphere within the chamber, the buffer-rich layer obtained on equilibration of the solvent was placed in borosilicate glass dishes at the bottom of the chamber as well as in a trough supported at the center of the chamber.

Solvent. Phenol (Mallinckrodt Chemical Works), purified by vacuum distillation, was shaken in a separatory funnel with the buffer (pH 12) and the mixture was allowed to stand overnight. The two layers were then separated. The phenol-rich layer was used for the development of the chromatograms and the buffer-rich solvent was used to provide a suitable atmosphere within the chromatographic chamber. Five per cent (by volume) of 2-butanol, saturated with the buffer (pH 12), was added to the buffer-saturated phenol solvent. The developing liquid was checked periodically for the presence of droplets of the buffer, as the presence of excess water caused streaking of the spots. The droplets disappeared when the solvent was shaken with a little fresh phenol.

Standard Amino Acid Solutions. An 8m*M* stock solution of glutamic acid was prepared by dissolving glutamic acid (Nutritional Biochemicals Corp.) in 10% 2-propanol. Measured volumes of this stock solution were diluted with 10% 2-propanol to yield 1, 2, 3, and 4m*M* solutions, which were used in the preparation of the standards.

Preparation of Gluten Hydrolyzates. Three 250-mg. portions of the sample were sealed in glass tubes along with 5 ml. of 6*N* hydrochloric acid. The tubes were autoclaved for 12 hours at a pressure of 15 pounds per square inch. After the digestion period the tubes were cooled and opened. The hydrolyzates were dried in a vacuum desiccator containing calcium chloride and sodium hydroxide. One tube was used to determine the quantity of 0.5*N* sodium hydroxide required to raise the pH to 6.4. The dried hydrolyzate was dissolved in 2 ml. of 10% 2-propanol and small amounts of sodium hydroxide solution were added to the tube by means of a semimicroburet. The pH was checked by means of a pH meter. As this involved unavoidable losses of hydrolyzate, this hydrolyzate was not used for the glutamic acid determination. The quantity of sodium hydroxide solution used above was added to the remaining two tubes, followed by 2 ml. of 10% 2-propanol, which acted as preservative. Sufficient distilled water was then added to each tube to adjust the volume to 5.0 ml. The mixtures were stirred thoroughly by means of a magnetic stirrer and were centrifuged to remove insoluble humin. The clear solutions were transferred to clean test tubes and labeled "stock hydrolyzate solutions." Each milliliter of the stock solution represented 50 mg. of gluten. Two sets of solutions, representing concentrations of 10, 20, and 30 mg. of gluten per ml., were prepared by diluting portions of these stock solutions.

Procedure

The filter papers ($9^1/_4 \times 22^1/_2$ inches) were marked by a line drawn approximately 3 inches from one end of the 9-inch side. Seven dots were made along this line at intervals of 1 inch. The sheets were then placed on clean glass plates, one over the other in echelon, each sheet separated by a glass plate. A space of approximately 2 cm. was left between each successive glass plate and the line on which the spots were to be applied.

The standard amino acid solutions of 1, 2, 3, and 4m*M* concentrations were applied on alternate dots made on the filter paper, in 5-μl. quantities by means of a micropipet (Microchemical Specialties). The same volumes of each of the three concentrations of hydrolyzate were placed separately on the three remaining dots. A separate paper carrying the above standards was prepared to accommodate the duplicate hydrolyzate.

The papers were placed in the chromatographic chamber containing the buffer-rich layer, and were allowed to stand for approximately 2 hours. After this equilibration period, the stopper in the lid of the chamber was removed, and approximately 200 ml. of the solvent-rich layer was introduced into the trough. The papers were allowed to develop for 24 hours. The developed chromatograms were dried at room temperature and were then dipped in a water-saturated butanol solution containing 0.4% ninhydrin and 4% acetic acid. The papers were allowed to drain for a few minutes, and were then placed in an air oven at 60° C. for 15 minutes.

The rows of amino acid spots were cut out in strips, narrow enough to pass through the densitometer. The strips were pasted together and the maximum color density of each spot was measured with a photoelectric transmittance densitometer (Photovolt Co., New York, N. Y.). To compensate for the variation in the light absorption of the filter paper in the different sections of each strip, the densitometer was adjusted at zero at the beginning of each section.

Standard curves were prepared by plotting, on squared paper, the color density units against the molarity of the standard amino acid solutions. The amino acid concentration in the hydrolyzate was obtained by referring the color densities of the hydrolyzate spots to the standard curves.

Determination of Glutamic Acid by Olcott Method. The procedure followed in these determinations was essentially the same as that described by Olcott, except that the standard Sørensen titration method was used for determining amino nitrogen instead of the Van Slyke manometric method.

Results

The glutamic acid contents of the following products were determined by both methods.

A. Gluten manufactured by Canadian Breweries, Ltd.
B. Crude glutamic acid isolated from a gluten hydrolyzate by isoelectric precipitation (Series 1).
C. Crude glutamic acid isolated from a gluten hydrolyzate by isoelectric precipitation (Series 2).
D. Spray-dried mother liquor obtained after removal of precipitated glutamic acid (sodium chloride content approximately 40%).
E. Spray-dried casein-gluten hydrolyzate (Series 1). (Sodium chloride content approximately 35%.)
F. Spray-dried casein-gluten hydrolyzate (Series 2). (Sodium chloride content approximately 35%.)

The two results reported for each sample using the chromatographic method (Table I) were obtained from analyses of the duplicate hydrolyzate

Table I. Determination of Glutamic Acid

	Glutamic Acid, %		
	Paper Chromatographic Method		Olcott method
Sample	Series 1	Series 2	
A	27.4(0.67[a], 2.4)[b]	26.9(0.52[a], 1.9)[b]	24.8, 25.1
B	40.4(1.01, 2.5)	39.6(1.10, 2.7)	40.6, 41.0, 41.0
C	54.4(1.06, 1.9)	54.1(1.12, 2.1)	55.3, 55.1, 55.1
D	5.71(0.37, 6.5)	5.64(0.43, 7.6)	5.08, 5.18, 5.38
E	15.5(0.68, 4.4)	15.7(0.67, 4.3)	15.2
F	11.7(0.32, 2.7)	11.4(0.26, 2.3)	12.0

[a] Standard error.
[b] Coefficient of variation.

samples. Each result represents the average of eight actual determinations. Each result reported using Olcott's method was obtained from the difference between the average of three amino nitrogen determinations on the autoclaved and the average of three similar determinations on the unautoclaved samples.

Discussion

The addition of 2-butanol to the buffered solvent recommended by McFarren (2) improves the separations of glutamic acid from the other amino acids, and leads to the formation of round, compact spots. Following the procedure described in this paper, glutamic acid was separated in the presence of relatively high concentrations of sodium chloride. The same solvent system has been used successfully for the determination of aspartic acid, serine, glycine, threonine, and alanine, as well as glutamic acid.

A heating period of 15 minutes at 60° C. is recommended for the rapid development of the ninhydrin color. Higher temperatures give spots that tend to fade quickly and are unsatisfactory for quantitative work, unless the color intensities are measured immediately following the heating period. Spots which do not fade appreciably in a week may be obtained by simply allowing the chromatograms to hang in the dark for 24 hours, instead of heating them at elevated temperatures.

The divergence between duplicate determinations made on a given hydrolyzate may be as high as 20%. In order to check this point, eight separate analyses were made on each of six hydrolyzates (series 1) and the standard errors were computed. The coefficient of variation was approximately 2.0% for sample C (54% glutamic acid), but rose to 6.5% for sample D (5.7% glutamic acid). In a similar analysis on a second series of hydrolyzates, similar values were secured. It may be concluded that eight replicate determinations on a single hydrolyzate afforded a reasonably reliable estimate of the glutamic acid content of the products examined.

Literature Cited

(1) Haugaard, Gotfred, and Kroner, T. D., *J. Am. Chem. Soc.*, **70,** 2135 (1948).
(2) McFarren, E. F., *Anal. Chem.*, **23,** 168 (1951).
(3) McFarren, E. F., and Mills, J. A., *Ibid.*, **24,** 650 (1952).
(4) Olcott, H. S., *J. Biol. Chem.*, **153,** 71 (1944).

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MOISTURE IN GRAIN

Hygroscopic Equilibria of Rough Rice at Elevated Temperatures

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The hygroscopic equilibria of rough rice have been determined at 80° to 111° F. for moisture contents of 11 to 22%, dry basis. The data have been correlated by an Othmer plot in which temperature, moisture content, and vapor pressure data are presented graphically for use in storage, packaging, processing, and drying. The plot permitted calculation of the relative isosteric heat of adsorption of water by rough rice of varying moisture content. Hygroscopic data were examined by the Harkins-Jura equation, which indicated that adsorption of water occurs as a condensed film over 14 to 22% moisture content. Application of the Brunauer-Emmett-Teller equation to available data on rough rice indicated that adsorption of moisture as a unilayer occurs over a range from dryness to approximately 7% moisture content. The amount of water, which constitutes a unilayer of adsorbed water on rough rice, is approximately 7 grams per 100 grams of dry rough rice and is identical calculated by Harkins-Jura or Brunauer-Emmett-Teller equations. Adsorption of water on rough rice occurs in three stages. The first, 0 to 7% moisture content, represents a unilayer of water molecules; the second is characterized by addition of an equal number of molecules to the already adsorbed unilayer; and the third may be considered a multilayer addition of water from approximately 14% moisture to saturation.

THE MOISTURE CONTENT of a material in hygroscopic equilibrium depends upon the relative humidity of the surrounding atmosphere but varies widely with different materials. The amount of moisture contained in a hygroscopic material in equilibrium with the surrounding atmosphere is properly defined as the hygroscopic moisture; industrially this hygroscopic moisture is frequently termed "moisture regain" and is expressed in parts of water per 100 parts of dry material by weight. Inasmuch as the moisture contents of hygroscopic materials seriously affect the storage and keeping qualities of such materials, equilibrium moisture data are directly applicable to the solution of storage, processing, and drying problems.

Rice, like other grains, is hygroscopic in nature—that is, it gains or loses moisture when the vapor pressure of water in the space surrounding the grain is greater or less than the vapor pressure exerted by the moisture within the grain. This equilibrium moisture content has previously been determined for rice and other grain by exposing samples of the grain to an atmosphere of constant temperature and relative humidity main

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