and 1.6 mg. of toxaphene–i.e., 106%–was recovered. A 45-gram butterfat sample, to which no toxaphene was added, was carried through the same procedure and no measurable interferences were encountered.

Discussion

Figure 1 shows the absorption curve measured against isopropyl alcohol for thiourea–potassium hydroxide alone, and the absorption curves for toxaphene and Strobane corrected for the absorption of thiourea–potassium hydroxide. The peak absorption for toxaphene is in the vicinity of 310 mμ and for Strobane at about 320 mμ. The absorption of the thiourea reagent becomes appreciable in this region and contributes a considerable fraction to the total absorption; however, above 380 mμ the absorbance due to thiourea is less than 0.025. Errors due to this reagent blank can be eliminated either by correcting for the blank or by reading absorbance against the reagent itself. As the chances of introducing errors due to the much higher readings of the blank are greater at the lower wave length, in actual practice, readings were made at 400 mμ even though some sacrifice in sensitivity was entailed. The absorbance, corrected for the reagents, at three wave lengths for several concentrations of toxaphene and Strobane are plotted (Figure 2). Beer's law is followed at each wave length, but the sensitivity is somewhat lower at the higher wave lengths. As a matter of convenience 400 mμ was selected for preparing the working curves.

The constitutents of toxaphene do not all have the same absorptivity. For example, recrystallization of toxaphene from methanol yields approximately 15% of a white noncrystalline solid fraction whose absorptivity is about 40% less than that of the mixture itself. However the technical commercial samples seem to be uniform since four separate samples gave approximately the same working curves. The standard sample used in establishing the final curves was one of a series of standard insecticide samples sponsored by the Entomological Society of America and distributed through the Nutritional Biochemicals Corp., 21010 Miles Ave., Cleveland 28, Ohio.

To see what interference might be expected from other chlorinated insecticides, 20 mg. each of aldrin, dieldrin, methoxychlor, DDT, lindane, BHC, TDE, chlordan, and heptachlor were carried through the reaction. Interferences were encountered only from heptachlor and chlordan. The color produced by both these insecticides was considerably greener than that due to toxaphene or Strobane, but of the same magnitude. This interference may be minimized in practice by either the sulfuric acid treatment or proper choice of condition for carrying out the chromatographic separation.

In the cleanup procedures for determining toxaphene in butterfat, concentrated sulfuric acid, rather than mixtures of concentrated and fuming sulfuric acid, proved satisfactory. Toxaphene is stable in the presence of fuming sulfuric acid and, for other vegetable or animal extracts, treatment with this may be preferred.

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ENZYMATIC ANALYSIS

Quantitative Determination of L- Glutamic Acid by L- Glutamic Acid Decarboxylase (from *E. coli*)

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An accurate method adaptable for the routine determination of L-glutamic acid in foods and pharmaceuticals, raw materials used in manufacturing monosodium glutamate, and plant process streams was needed. A modification of the method of Umbreit and Gunsalus, using L-glutamic acid decarboxylase prepared from *Escherichia coli* ATCC strain 4157 gives values, which are accurate and reproducible within narrow limits, and is well adapted to routine analytical work.

THE ENZYMATIC DECARBOXYLATION OF AMINO ACIDS and particularly glutamic acid has been investigated. The preparation of bacterial enzymes specific for the decarboxylation of L(+) glutamic acid, L(+) lysine, L(−) histidine, L(+) arginine, L(−) tyrosine, and L(+) ornithine has been described (*4, 5, 7–9, 21*). The application of these preparations to the rapid analysis of amino acids in protein hydrolyzates has also been discussed. A suspension of *Clostridium perfringens* strain SR 12 (NCTC No. 6784) has been described (*7*) and has been claimed to be specific for decarboxylation of L-glutamic acid. However, Meister, Sober, and Tice (*14*) found that this suspension of *Clostridium perfringens* SR 12 yielded equivalent amounts of carbon dioxide from L-aspartic acid at about 10% of the rate for L-glutamic acid decarboxylation. Methods were described for the separate determination of aspartic and glutamic acids by decarboxylation. Krebs (*13*) found that this same suspension decarboxylates L-glutamic acid and hydrolyzes L-glutamine. Schales, Mims, and Schales (*17*) reported the preparation of clear solutions of an enzyme from higher plants which specifically decarboxylated L-glutamic acid. Schales and Schales (*18*) described the enzyme kinetics of L-glutamic acid decarboxylase from carrots, and the quantitative determination of L-glutamic acid by L-glutamic acid decarboxylase prepared from squash (*19*). Umbreit and Gunsalus (*24*) prepared enzyme suspensions of *Escherichia coli* ATCC strain 4157, which specifically decarboxylated L-glutamic acid, and hydrolyzed L-glutamine.

The purpose of the work described herein was to develop an accurate method adaptable for routine determination of L-glutamic acid in foods, raw materials used in manufacturing monosodium glutamate, and plant proc-

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ess streams. A modification of the method of Umbreit and Gunsalus (*24*) offered the greatest promise and was selected for further study and development. The modified method has been successfully utilized to analyze a wide range of materials. Following the completion of this work, a report appeared by Najjar and Fisher (*15*), who prepared enzyme suspensions of *E. coli* ATCC No. 11246, which specifically decarboxylated L-glutamic acid.

Dried dead cells of *E. coli* suspended in a solution buffered at pH 5.0 are used as a reagent. This suspension contains enzymes, one of which quantitatively decarboxylates L-glutamic acid to γ-aminobutyric acid, and another of which hydrolyzes L-glutamine to L-glutamic acid followed by decarboxylation. The enzyme liberates 1 mole of carbon dioxide from 1 mole of L-glutamic acid. Pyridoxal phosphate functions as the coenzyme of L-glutamic acid decarboxylase. From 1.5 to 2.5 mg. of L-glutamic acid can be measured manometrically in the Warburg apparatus with a precision within 1%.

Experimental

Preparation of Enzyme. Reagent is prepared from acetone-dried cells of *E. coli* ATCC strain 4157 by Hac's modification (*11*) of the method of Umbreit and Gunsalus (*24*). In this report, the enzyme activity is expressed as $Q_{CO_2}^{28}$ which is equal to the microliters of carbon dioxide evolved per hour per milligram of dried cell preparation at 28° C., using 5 mg. of pure L-glutamic acid as the substrate. Each batch of enzyme should be tested for activity as soon as it is prepared.

In a small beaker, 60 mg. of dried enzyme preparation with 0.9 ml. of pyridoxal phosphate solution, enough 0.2*M* potassium acid phthalate to bring the suspension to pH 5.0, and sufficient distilled water to bring the total volume to 6.0 ml. are mixed. The pyridoxal phosphate solution is prepared by dissolving 4 mg. of pyridoxal phosphate in a minimum amount of approximately 2*N* hydrochloric acid, adding 50 ml. of 0.133*M* phosphate buffer, adjusting the solution to pH 7.0, and bringing the total volume to 100 ml. with distilled water. The solution is stored under refrigeration (0° to 5° C.) and used not longer than 1 month.

Measurement of Activity. One and one half milliliters of 0.133*M* phthalate buffer (pH 5.0), containing 1.33% of sodium chloride, 1.0 ml. of L-glutamic acid solution (5 mg.) at pH 5.0, and 0.4 ml. of distilled water are placed in the main compartment of the reaction flask. To the side bulb of the flask is added 0.1 ml. of enzyme suspension, containing 1 mg. of the dried cell preparation and 0.6 mg. of pyridoxal phosphate. The suspension is at pH 5.0. The reactants are allowed to equilibrate at 28° C. for 20 minutes with the manometers closed, and an initial manometer reading is taken, followed by mixing the reactants. Another reading is taken 2 minutes after mixing, followed by a 15-minute reading. The reaction, $Q_{CO_2}^{28}$ = μl. of CO_2, is evolved in 15 minutes, multiplied by 4, and divided by the milligrams of enzyme used. This gives the microliters of carbon dioxide evolved per hour per milligram of dried enzyme preparation. The enzyme activity should be determined with a precision within 10%.

Table I. Stability of Enzyme

Storage Conditions	Initial Activity $Q_{CO_2}^{28}$	Activity One Month Later: Room temp. $Q_{CO_2}^{28}$	Activity One Month Later: 6° C. $Q_{CO_2}^{28}$
Air	433	344	370
Vacuum	433	395	..
Atmosphere of nitrogen	433	341	..
Atmosphere of CO_2	433	317	..
Atmosphere of oxygen	433	282	..
Atmosphere of hydrogen	433	208	..
Atmosphere of SO_2	433	67	..

Storage of Enzyme. A dried enzyme preparation was subjected to the storage conditions indicated in Table I for 1 month to determine optimum conditions for the preservation of activity. According to the data obtained, either refrigeration or vacuum is helpful in minimizing loss of enzyme activity during storage. Therefore, if both vacuum and refrigeration were used, enzyme deterioration during storage might probably be reduced to a minimum amount, thus stabilizing the enzyme for long periods of time.

Aqueous enzyme suspension, containing 10 mg. of dried enzyme preparation per ml. and maintained at pH 5.0, was kept at −10° C. for several months with and without 0.5% sodium chloride. The suspension exhibited a slightly greater degree of stability in the presence of sodium chloride than in its absence. Time can be saved in control work by making a month's supply of the enzyme suspension with 0.5% sodium chloride, dividing it into aliquots (each one sufficient for one day's run), and storing the aliquots in a freezer until needed.

Using 1 mg. of enzyme per determination, 0.6 γ of pyridoxal phosphate was found to be an acceptable amount to add in order to realize the full potential activity of the enzyme. Also, 1 mg. of adenosine triphosphate plus 3 γ of pyridoxal hydrochloride (*22*) has accomplished similar results for L-glutamic acid decarboxylase.

Analytical Procedure for Pure L-Glutamic Acid. Reagents—Buffer. A 0.4*M* potassium acid phthalate solution is prepared and adjusted to pH 5.0 with sodium hydroxide solution.

A 0.2*M* potassium acid phthalate solution is prepared.

L-Glutamic acid decarboxylase enzyme suspension. One unit of L-glutamic acid decarboxylase is defined as that amount of dried enzyme preparation which will liberate 100 μl. of carbon dioxide from L-glutamic acid in 15 minutes, under the conditions used for measurement of enzyme activity. Ten units of the enzyme were sufficient to cause a reaction to go to completion within 20 to 30 minutes. When running a number of determinations, make up the enzyme suspension in the following order:

1. Add to 10 units of dried enzyme powder (International Minerals and Chemical Corp.), multiplied by the number of determinations to be run, 0.3 ml., per determination, of pyridoxal phosphate solution (prepared as described earlier).
2. Add enough 0.2*M* potassium acid phthalate solution to bring to pH 5.0.
3. Add 0.5 gram of NaCl per 100 ml. of final solution.
4. Add enough distilled water to bring the solution to a volume equal to 0.5 of the number of determinations.
5. After these materials are added, break up enzyme clots completely into a fine suspension, by using a mechanical stirrer or with a stirring rod.

Determination of L-Glutamic Acid. A 2-ml. aliquot of the sample to be analyzed, containing between 1.5 and 2.5 ml. of L-glutamic acid, is placed in the main compartment of the Warburg vessel and 0.5 ml. of 0.4*M* potassium acid phthalate buffer, pH 5.0, is added. To the side bulb is added 0.5 ml. of the enzyme suspension. The Warburg flask is installed in a water bath at 28° C. and shaken with the manometer stopcock open for 5 minutes. After closing the manometer stopcock, the reactants are equilibrated in the flask for 20 minutes (or until readings become constant) and then the enzyme suspension is mixed thoroughly with the solution in the main compartment of the flask. The manometer is read until carbon dioxide evolution is complete (20 to 30 minutes). The microliters of carbon dioxide produced are converted to milligrams of glutamic acid as follows:

$$\frac{\mu\text{l. carbon dioxide}}{224} \times 1.4713 = \text{mg. glutamic acid}$$

Umbreit, Burris, and Stauffer (*23*) describe in detail the calibration of the Warburg apparatus, and the calculation of the amount of gas evolved.

The α-value for carbon dioxide (milliliters of carbon dioxide dissolved in 1 ml. of liquid when the pressure of carbon dioxide above the solvent minus the vapor tension of the solvent is 760 mm. of mercury at temperature t) used in the calculations was 0.699 which is the solution of carbon dioxide in pure water at 28° C. The capacity of the Warburg flasks used was between 15 and 20 ml. Brodie's solution was used as the manometer fluid.

Warburg apparatus sensitive to smaller changes in gas pressure may be obtained by use of a Warburg flask of 5-ml. capacity, manometer of smaller size bore, and by substituting 2,2,4-trimethylpentane (which has less density) for Brodie's solution. For each set of samples analyzed at any time, a blank determination must be made on a 0.5-ml. aliquot of the enzyme suspension, used together with the materials described in the preceding section, except that distilled water is used in place of the sample of L-glutamic acid.

Precision and Accuracy of Analytical Procedure for Pure L-Glutamic Acid. To test the variability of the method, repeated analyses of the same sample of L-glutamic acid were made by one individual. Nine determinations, using 1.5 mg. of L-glutamic acid per determination, gave recoveries of 97.5% of theory with a standard deviation of ±1.0%. Eight determinations, using 2.0 mg. of L-glutamic acid per determination, gave recoveries of 97.5% of the theory with a standard deviation of ±0.7%.

The usefulness of such an analytical method depends primarily on its complete measurement of L-glutamic acid and its specificity for this compound. As the L-glutamic acid used was believed to be of the highest purity obtainable, the analytical values were considered to be 2.5% below the true values. Gale (*6*) has shown that the carbon dioxide output from the decarboxylation of L-lysine, L-histidine, L-glutamic acid, L-arginine, L-ornithine, and L-tyrosine, by their respective decarboxylase preparations, represented 96 to 98% of theoretical. According to Hanke (*12*) the decarboxylation of L-lysine and L-tyrosine, by their respective decarboxylases, produced 97 to 98% of the theoretical yield of carbon dioxide because the decarboxylase preparations contain some L-amino acid oxidase which oxidizes the L-lysine or L-tyrosine to a slight extent before decarboxylation is complete.

A correction factor was not incorporated in this method because the average result of 10 determinations from day to day could vary as much as 2% (96 to 98), although the precision of the average result in any one day could be within ±0.3 standard deviation. Whether the low results obtained with pure L-glutamic acid (96 to 98%) are due to an erroneous α-value for carbon dioxide in the reacting solution, is open to question. A possible proof may lie in determining the α-value for carbon dioxide in the presence of the final reacting products.

Table II. Effect of Sodium Chloride Concentration on Accuracy of Determination of Pure L-Glutamic Acid

% NaCl per assay	Zero	1.0	1.67	2.0	3.0	4.0	5.0	6.5	13.0
% L-GA found	98.0	98.7	98.1	98.3	98.6	100.6	99.1	101.2	103.5
Standard deviation	±0.3	±0.4	±0.5	±0.7	±0.4	±0.7	±0.8	±0.3	±0.6
No. of determinations	8	10	10	10	10	19	10	10	10

The validity of the blank determination has also been questioned. The authors experience was that numerous determinations of pure L-glutamic acid gave similar results, although the blank for each determination may have been different. The same results were obtained for hydrochloric acid—hydrolyzed wheat gluten. Therefore, it is felt that the blank determination is advisable.

The effect of varying concentrations of a typical salt sodium chloride on the determination of pure L-glutamic acid was studied in order to find the salt concentration at which the L-glutamic acid values start to change significantly owing to the effect of salt upon the solubility of carbon dioxide. Pure L-glutamic acid, 2.0 mg., was decarboxylated per assay, in the presence of varying concentrations of sodium chloride (Table II).

With the materials encountered in this work, dilution of samples to approximately 0.1% of L-glutamic acid content, preparatory to analysis, invariably brought the inorganic salt concentration well below 4%, eliminating the need for correcting L-glutamic acid results.

Samples containing a small concentration of L-glutamic acid (0.1% or below) in the presence of high concentrations of the alkali salts, can be treated in the following manner, to separate the salts from L-glutamic acid (*3*):

The sample is adjusted to pH 0.5 with hydrochloric acid and anhydrous isopropyl alcohol is added until the solution contains an isopropyl alcohol concentration of 85%. The precipitated inorganic salts are filtered off and washed with 85% isopropyl alcohol. The filtrate and washings are collected and the isopropyl alcohol is evaporated by boiling, making certain that the pH of the solution remains below 0.5 during evaporation. After cooling, the solution is adjusted to pH 5.0 and made to volume.

Application of Analytical Procedure to Crude Raw Materials

Protein Hydrolyzate. With this type of sample, normally an adjustment to pH 5.0 and a dilution to 1.5 to 2.5 mg. of L-glutamic acid per 2.0 ml. are sufficient. However, if hydrochloric acid is the hydrolytic agent and is present in excess, it is evaporated by boiling before proceeding with the adjustment of pH and volume. In this step, caution must be exercised to prevent conversion of glutamic acid to pyrrolidone carboxylic acid (*16*). If the solution is adjusted below pH 0.5 and kept at this pH during the heating of the L-glutamic acid solution, there is no danger of converting L-glutamic acid to pyrrolidone carboxylic acid.

Samples Containing an Organic Solvent. If the solvent is volatile, it is evaporated by boiling the solution at a pH below 0.5. If the solvent is not volatile, it is steam distilled in a sulfuric acid solution below pH 0.5.

Samples Containing Nitrate. Sulfuric acid instead of hydrochloric acid is used as the hydrolytic agent for samples containing nitrate, if such samples must be hydrolyzed prior to L-glutamic acid determination. Some amino acids are oxidized when hydrochloric acid is used as the hydrolytic agent in the presence of nitrate (*25*).

Samples Containing a Reducing Agent. Such samples are heated in acid medium with hydrogen peroxide until tests indicate the complete oxidation of the reducing agent. Care must be exercised that all excess hydrogen peroxide is destroyed by boiling in acid solution below pH 0.5, as the crude enzyme preparation contains catalase which acts on hydrogen peroxide to release oxygen.

Samples Containing Metal Ions. The metal ions, other than the alkali metal ions in the sample, are precipitated as carbonates and filtered off prior to L-glutamic acid determination if such metal ions are found to retard or inhibit decarboxylase activity at the concentrations at which these ions are present in solution. If the reaction is not completed in the normal time, lower concentration levels of the sample are tried to find whether the same results may be obtained. In addition, complete recovery of a known amount of added pure L-glutamic acid will give good evidence of the absence of inhibitors.

Samples Containing Small Percentages of L-Glutamic Acid. Aqueous suspensions of this type are adjusted to pH 5.0 and made to volume. A 2-ml. aliquot of this aqueous suspension is used for L-glutamic acid analysis. This method of handling water-insoluble sub-

stances containing small percentages of L-glutamic acid has been found to be the simplest and most reliable.

Food Samples Other Than Meat, Oil, and Fat. Forty grams of a representative part of the food sample is weighed and dispersed in a Waring Blendor with 100 ml. of distilled water for 10 minutes, or longer if necessary, to obtain a fine suspension. If the food sample is a mixture of solid and liquid, the entire sample is ground into a fine suspension before weighing a representative portion for analysis. For food samples containing more than 0.6% of L-glutamic acid, the final concentration for analysis should be more dilute than 40 grams of sample in 250 ml. of aqueous suspension.

The homogenate is diluted to approximately 200 ml. with distilled water, and heated at 90° C. for 10 minutes, to destroy any enzymes present. L-Glutamine, when present in fresh foods, interferes with the determination of L-glutamic acid because of the presence of L-glutaminase in the enzyme preparation. By chemically converting the salt of L-glutamine to the corresponding salt of L-pyrrolidone carboxylic acid (*26*), this inference may be eliminated as follows:

To the fresh food homogenate is added 80 ml. of 1.0*M* phosphate buffer at pH 6.5, and it is made to 200 ml. with distilled water, and refluxed for 2 hours. In this way, only pre-existing glutamate is measured, as the rate of transformation of glutamate to the corresponding salt of pyrrolidone carboxylic acid at pH 6.5 is slow enough that the 2-hour heating time does not alter the glutamate significantly (*27*). The suspension is cooled to room temperature and adjusted to pH 5.0 with hydrochloric acid. Finally, a few drops of benzyl alcohol are added to disperse the foam and the volume is adjusted to 250 ml.

A representative part (2 ml.) of this suspension is used for analysis as soon as possible after its preparation. To facilitate the transfer of this preparation, a 2-ml. measuring pipet with 0.25 inch of its tip cut off is used.

Meat Samples. A representative part of the sample, 40 grams, is weighed and dispersed in a Waring Blendor with 100 ml. of distilled water, until a fine suspension is obtained. The residue is filtered and washed with hot water. The filtrate and washings are collected together, adjusted to pH 5.0, and finally made up to a 250-ml. volume.

Fat or Oil Samples. In a Waring Blendor with 100 ml. of benzene, a 40-gram sample is dispersed. The free L-glutamic acid or L-monosodium glutamate is extracted with two portions of 50 ml. of distilled water in a separatory funnel. The aqueous fractions are combined and then placed on a steam bath for 20 minutes in order to evaporate the benzene contained in the aqueous fractions. To the cooled aqueous fractions, 0.5 gram of cetyltrimethylammonium bromide (Cetavlon), is added. The solution is adjusted to pH 5.0, and made up to a final volume of 100 ml. This prepared solution is ready for L-glutamic acid determination. The Cetavlon keeps any oil or fat present dispersed in the aqueous medium. This preparation method includes food containing a high percentage of fat, such as bacon.

Table III. Test of Precision of Method

	HCl Hydrolyzed CSF Diluted, Mg./Detn.	NaOH Hydrolyzed CSF Diluted, Mg./Detn.	HCl Hydrolyzed Wheat Gluten Diluted, Mg./Detn.
Average	1.824	1.740	1.964
Standard deviation	±0.018	±0.029	±0.022
No. of determinations	10	10	10

Precision and Accuracy of Analytical Procedure for Crude Raw Materials. Determinations were made on samples of hydrochloric acid–hydrolyzed concentrated Steffens filtrate (CSF), sodium hydroxide–hydrolyzed concentrated Steffens filtrate, and hydrochloric acid–hydrolyzed wheat gluten. Table III shows that the reproducibility of the method is excellent and that an experienced operator can reasonably expect to reproduce a given value within an error of 1%.

A collaborative study of the method was conducted at six laboratories involving three companies, which included International Minerals and Chemical Corp., General Mills, Inc., and A. E. Staley Manufacturing Co. One large sample of hydrochloric acid–hydrolyzed wheat gluten was prepared and diluted to contain approximately 5 grams of L-glutamic acid per 100 ml. Aliquots were sent to each of six laboratories, and two operators from each laboratory made duplicate determinations on the sample over a 5-day period. The average determinations are listed in Table IV. The agreement between laboratories and different analysts was of a high order and the general reliability of the method is indicated as being very good.

Analyses were made in this laboratory for six proteins, the average L-glutamic acid contents of which had been reliably established (by other analytical procedures and reported in the literature) and the values obtained in comparison with known and generally accepted values were as shown in Table V.

Table V. L-Glutamic Acid Content of Various Proteins

Sample	% L-Glutamic Acid on Protein Basis: Literature	% L-Glutamic Acid on Protein Basis: L-GA decarboxylase (from E. coli) method
Wheat gluten (tech.)	35–36 (*1*)	35.2
Corn gluten (tech.)	24.5 (*2*)	21.2
Zein	23.5 (*15*)	23.4
Gliadin	46 (*1*)	45.8
Casein	19.7–21.5 (*20*)	21.1
Gelatin	10.2–10.8 (*20*)	10.8

Agreement between the two sets of data is considered to be good.

Tests for L-Glutamic Acid Decarboxylase Inhibitors and Specificity. The effect of common inhibitors on the action of the various decarboxylases has been summarized by Gale (*9*). L-Glutamic acid decarboxylase is sensitive to silver ion, mercuric mercury ion, potassium permanganate, potassium cyanide, and hydroxylamine at the 0.01*M* concentration level, and partially sensitive at this concentration to cupric copper ion, hydrazine, semicarbazide, and sodium azide. Although quantitative data are lacking concerning the effect of organic solvents and metal ions (other than the alkali metal ions) on L-glutamic decarboxylase, they are known to inhibit the enzyme at much higher concentrations than those listed for the common inhibitors.

Two tests are available to determine whether any material to be analyzed contains substances having an inhibitory effect on the activity of the enzyme. A variety of samples were analyzed at different dilutions on the presumption that (other conditions being the same) the more concentrated samples would show

Table IV. Average Determination per Laboratory and per Operator

Laboratory	Over-all Average G. L-GA per 100 Ml.	Operator A G. L-GA per 100 ml.	Operator B G. L-GA per 100 ml.
I	5.420	5.439	5.400
II	5.298	5.285	5.310
III	5.399	5.366	5.431
IV	5.457	5.460	5.453
V	5.430	5.423	5.436
VI	5.409	5.370	5.447

Table VI. Analyses of Wheat Gluten and CSF Hydrolyzates at Different Dilution Levels

Approximate Mg. L-Glutamic Acid per Assay	Amount L-Glutamic Acid, Grams/100 Ml. Sample			
	Pure L-glutamic acid	HCl-hydrolyzed wheat gluten	Alkaline-hydrolyzed CSF	HCl-hydrolyzed CSF
0.5	0.192	9.62	2.21	2.58
1.0	0.187	9.78	2.28	2.52
2.0	0.191	9.64	2.29	2.50
2.5	0.192	9.64	2.27	2.44

Table VII. Recovery of Known Quantities of Pure L-Glutamic Acid Added to CSF and Protein Hydrolyzates

Hydrolyzate Sample	Pure L-Glutamic Acid Added, Mg.	L-Glutamic Acid in Sample, Mg.	Total L-Glutamic Acid Present,[a] Mg.	L-Glutamic Acid Found, Mg.	% Deviation[b]
Mason City, CSF, alkaline	0.978	1.004	1.982	1.984	+0.1
Mason City, CSF, acid	0.978	1.014	1.992	1.971	−1.0
San Jose CSF, acid	0.978	0.917	1.895	1.911	+0.8
San Jose CSF, alkaline	0.978	0.887	1.865	1.820	−2.4
Corn gluten end liquor	0.983	0.757	1.740	1.784	+2.6
Zein No. 1	0.983	1.090	2.073	2.089	+0.8
Zein No. 2	0.983	1.091	2.074	2.075	+0.1
Wheat gluten plant	0.983	1.003	1.986	1.950	−1.8
Wheat gluten, reflux	0.963	0.800	1.763	1.767	+0.2
Corn gluten	0.963	0.694	1.657	1.659	+0.1
Gliadin	0.963	0.832	1.795	1.814	+1.0

[a] Pure L-glutamic acid added + L-glutamic acid in sample.
[b] Deviation of L-glutamic acid found from total.

lower values if inhibitors were present. Applying this method of testing to the hydrolysates of several different proteins and to the hydrolyzates of two different samples of concentrated Steffens filtrate (including hydrochloric acid and sodium hydroxide hydrolyzates), no evidence of the presence of inhibitory factors was found. The data from a typical experiment in this series are shown in Table VI.

Another test for inhibition can be made by determining the recovery of pure L-glutamic acid added in known quantity to crude materials. When known amounts of pure L-glutamic acid were added to various samples of concentrated Steffens filtrate hydrolyzates and to hydrolyzates of several different proteins, the method accounted for the added quantities in all cases within a small range of error, as shown in Table VII. This indicates the absence of inhibitory substances in the particular samples tested.

The specificity of the enzyme is a matter of considerable importance in assaying crude raw materials. The glutamic, lysine, histidine, arginine, and ornithine decarboxylases are present in *E. coli* strains in varying concentrations and without any consistent pattern of distribution (*10*). *E. coli* strain 4157 yields a cell preparation, which after treatment with acetone contains L-glutamic acid, L-glutaminase, and catalase. As hydrogen peroxide and L-glutamine are not found in protein hydrolyzates, the enzyme becomes a specific agent for the measurement of L-glutamic acid in such material. In addition, the presence of hydrogen peroxide and L-glutamine in other types of samples containing L-glutamic acid presents no problem when analysis for L-glutamic acid is desired, as both interferences may be eliminated when the samples are prepared for analysis as described above. The specificity of this enzyme has been tested at pH 5.0, and is not implied for any other pH value.

Conclusions

With three different sources of L-glutamic acid decarboxylase available (*Cl. perfringens*, *E. coli*, and higher plants), *E. coli* has been selected as the most desirable for preparation of the enzyme because a commercial source of this enzyme is available. Moreover, the enzyme produced from *E. coli* has the advantages over that from higher plants of yielding lower blanks, reacting in an atmosphere of air rather than nitrogen, and completing the reaction, without the necessity of releasing any bound carbon dioxide, prior to its measurement. The enzyme from *E. coli* strain 4157 has been adapted to the analytical determination of L-glutamic acid by a procedure which is conveniently applicable to raw materials. It is readily applicable to control of commercial processes for production of L-glutamic acid, as well as to its quantitative estimation in foods and pharmaceuticals.

The L-glutamic acid values obtained are sufficiently accurate and dependable as judged by all available criteria. The method has been rendered specific for L-glutamic acid regardless of the nature and amount of impurities associated with the types of glutamic acid–bearing materials described.

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