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NUTRIENTS IN FERMENTATION PRODUCTS

Determination and Comparison of Amino Acid Composition of Yeast and Distiller's Solubles

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By monodimensional paper chromatography 15 amino acids were estimated in a strain of *Saccharomyces cerevisiae* and 13 amino acids in distiller's solubles. The materials and procedures used to procure the data are described.

PAPER CHROMATOGRAPHY occupies an important position in the separation and identification of small amounts of similar compounds in a complex mixture. The technique is well suited for the determination of amino acids in microgram quantities. Several methods for quantitative estimation of the individual amino acids have been proposed (*2, 5, 7*).

The separation and estimation of 15 amino acids in a strain of *Saccharomyces cerevisiae* and 13 in distiller's solubles are reported in this paper. The same procedure was used in the chromatographic examination of both the yeast and the solubles.

Materials and Reagents

Chromatographic Chambers. Two chambers were built from $^5/_8$-inch plywood, one to hold three trough and tray assemblies (Schaar and Co.) and the other to hold six. The chambers and lids were paraffined inside and the lids were held in place by wing nuts and bolts hinged to the sides of the box. Rubber tubing was glued around the top edge of the boxes, forming an effective gasket when the lids were tightened. Holes were drilled in the lid directly over each trough for the addition of solvent, after equilibration. These holes were stoppered with rubber stoppers. Atmospheric equilibration was accomplished by lining the walls of the chambers with filter paper, held in place by thumb tacks. The ends of the filter paper dipped into a photographic tray filled with solvent.

Solvent Systems. Solvent 1, 4 parts of phenol (Mallinckrodt, Gilt Label) to 1 part of water plus 20 mg. of 8-quinolinol per 500 ml. of solvent. A beaker containing 0.3% ammonia was placed in the chamber when this solvent was used.

Solvent 2, 2-butanol and 3% ammonia in the ratio of 3 to 1.

Solvent 3, 2-butanol, water, and formic acid in the ratio of 120 to 40 to 1.

Whatman No. 1, $18^1/_4 \times 22^1/_2$ inch chromatographic paper, and a Macbeth Ansco Model 12 color densitometer were also used.

Procedure and Results

Two liters of media from each of 24 pilot plant fermentations were centrifuged to provide the yeast samples used in the work described in this paper. The solids were washed twice and made up to a volume of 250 ml. with demineralized water. Five-milliliter samples of the resuspended yeast (containing about 32 grams of solids per liter) were hydrolyzed under total reflux for 18 to 20 hours with 10 ml. of 6*N* hydrochloric acid. Excess hydrochloric acid was removed by evaporation on a water bath, and the samples were placed in a vacuum desiccator. They were then taken up in exactly 1 ml. of isopropyl alcohol (the isopropyl alcohol inhibits bacterial action on the hydrolyzate). Further dilutions were made when necessary.

Samples of 2.5 grams of distiller's solubles from four different production dates were made up to 250 ml. with demineralized water. Five-milliliter aliquots of these samples were hydrolyzed under the same conditions as the yeast and taken up in 10% isopropyl alcohol. For the determination of tryptophan, the samples were also hydrolyzed with 5*N* barium hydroxide for 18 to 20 hours under total reflux. The barium was precipitated, after hydrolysis, by neutralization with sulfuric acid. The neu-

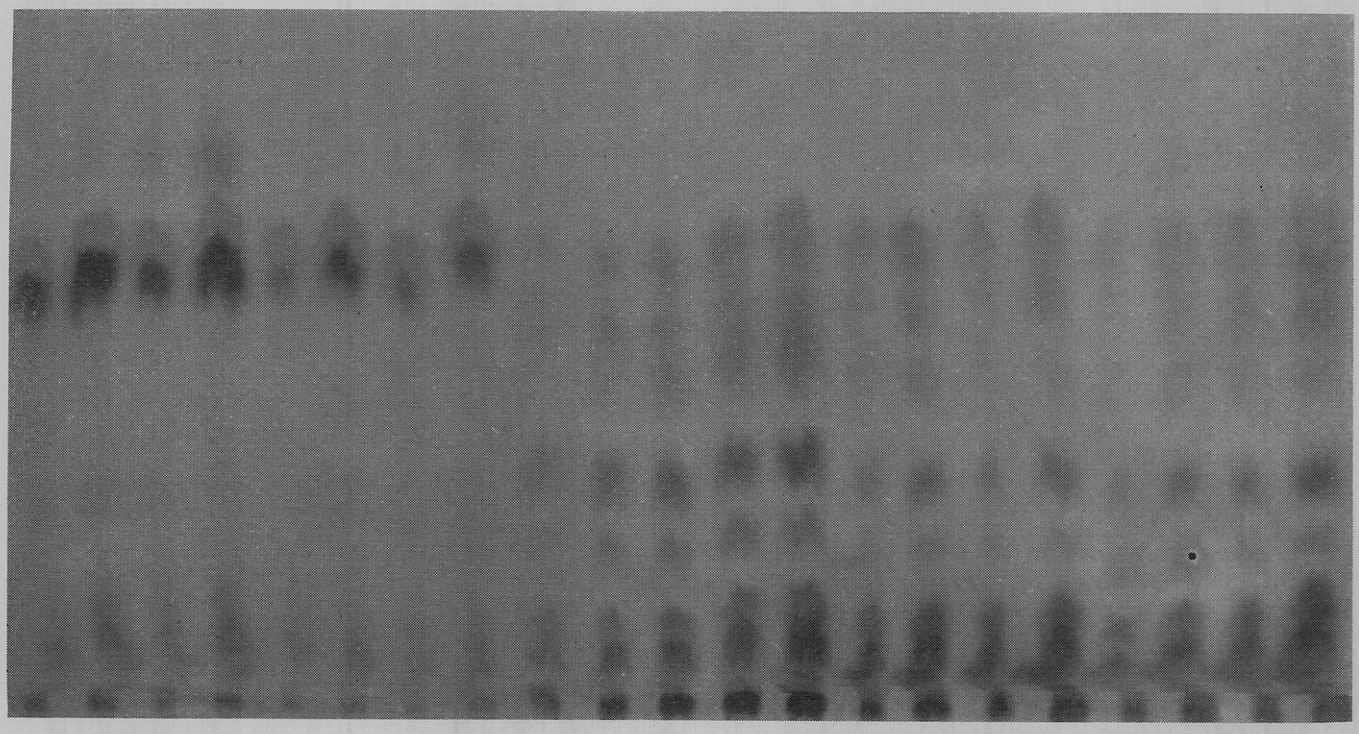

Figure 1. Typical chromatograph

Solvent 3

tralized samples were filtered, partially evaporated, decanted, and then evaporated. They were taken up in water, filtered and re-evaporated. The residues were taken up in exactly 1 ml. of 10% isopropyl alcohol.

Standard solutions of chromatographically pure amino acids were accurately made up in 10% isopropyl alcohol to give approximately 500 γ of α-amino nitrogen per milliliter. Aliquots of these stock solutions were pipetted into a 25-ml. volumetric flask to give a working standard solution with a concentration of 0.10 γ of α-amino nitrogen of each amino acid per 2.5 μl. of solution.

A 25-μl. pipet, graduated in 5-μl. divisions, was used to apply the samples to the chromatographic paper. The spots were applied $1^1/_2$ inches from the $22^1/_2$-inch edge and 1 inch apart. The standard amino acid solution was applied in the middle of the sheet in increments of 0.1, 0.2, 0.3, 0.4, and 0.6 γ. No more than 5 μl. was applied at a time, and the spot was thoroughly dried with a hair dryer between applications. The samples were applied at two concentrations, 2.5 and 5 or 5 and 10 μl., depending upon the dilution. Triplicates or quadruplicates were run on each assay. Sheets prepared with the individual amino acids replacing the unknowns indicated the relative positions of the amino acids in the resolved chromatograms.

The prepared papers were placed in the chromatographic chamber, but no solvent was placed in the troughs. The photographic tray was filled, the lid clamped on, and the system allowed to equilibrate. Two hours are enough for equilibration with phenol, but 4 to 6 are required for the butanol systems. A beaker containing 50 ml. of 0.3% ammonium hydroxide was used in the phenol chamber as suggested by Consden, Gordon, and Martin (*4*). After equilibration, monodimensional descending chromatograms were run at room temperature. The development periods were 18 to 20 hours with solvent 1, 60 to 65 hours with solvent 2, and 40 to 45 hours with solvent 3. The chromatograms were dried in a darkened hood. Those developed with phenol were dipped in ether and redried.

Resolutions. Solvent 1 resolves amino acids as the following spots, reading from the point of application: (1) aspartic acid, (2) glutamic acid, (3) cystine-serine, (4) glycine, (5) threonine, and (6) tyrosine. The rest of the acids are bunched together as one elongated spot.

Solvent 2 gives the following sequence: (1) glutamic acid–aspartic acid–cystine, (2) lysine, (3) arginine, (4) glycine-serine, (5) histidine-threonine, (6) alanine, (7) tyrosine, (8) valine-methionine, (9) isoleucine, and (10) leucine-phenylalanine.

Solvent 3 gives the following resolution: (1) lysine, (2) arginine-histidine, (3) glycine–aspartic acid–serine, (4) threonine, (5) alanine–glutamic acid, (6) valine-tyrosine, (7) methionine, (8) isoleucine-phenylalanine, and (9) leucine.

The resolved chromatograms were developed with a freshly prepared 0.2% ninhydrin in acetone containing 1% acetic acid, as described by Toennies and Kolb (*8*). The acetic acid was omitted in developing the color of the solvent 3 chromatograms. An interval of 18 to 20 hours in a darkened hood was allowed for color development. A typical chromatogram is shown in Figure 1.

The maximum density method, as outlined by Block (*2*), was used to estimate the amino acids. The maximum density of the colored spots was determined with the densitometer using a green filter with a maximum transmittance at 546 mμ. Standard curves of density *vs.* concentration were plotted on each run for each amino acid, yielding curves of the type shown in Figure 2. The unknown values of the hydrolyzates were read from the curves.

A compilation of the results is given in Table I. As the distiller's solubles were derived from the fermentation of corn, rye, and barley malt, the amino acid values on these products are included for comparison (*1*). Average values are given in Table II for the per cent total protein present in yeast, corn, rye, malt, and the solubles used in these experiments. Protein in the original

material was determined by the Kjeldahl method.

Discussion

When a solvent is allowed to run over a strip of filter paper, the paper tends to dehydrate the solvent to some extent.

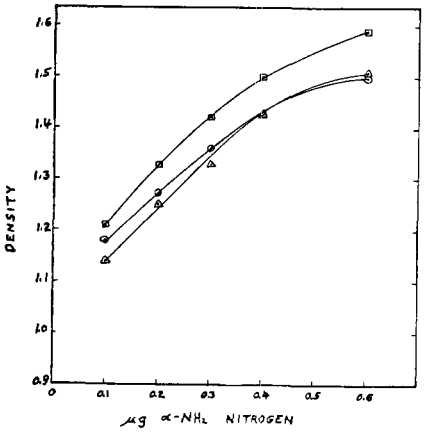

Figure 2. Standard curves for leucine-phenylalanine

Solvent 2

⊙ Run D
△ Run E
⊡ Run F

In a saturated atmosphere this dehydration is made up by the condensation of some water vapor on the paper and the composition of the solvent remains uniform. Therefore, it is essential to keep the atmosphere saturated with all the constituents of the solvent. In the use of butanol, for example, the rate of development was erratic if the equilibration time was shortened. The volume of liquid in the bottom is immaterial as long as there is an excess; in the 28 × 28 × 28 inch cabinet 1000 ml. were adequate.

Table II. Protein Content as Determined by Kjeldahl Method

Yeast	51.8
Solubles	27.2
Corn	8.6
Rye	11.7
Malt	11.6

Some of the work reported in this paper is part of a study of the utilization of leucine by *Saccharomyces cerevisiae*. The chromatograms to the left of the standard spots in Figure 1 are of centrifugates and the dark spots are leucine that was added to the indicated fermentations, 27–464 and 27–465. The chromatograms to the right are indicative of the composition of yeast cells.

Three solvents are necessary for the monodimensional determination of the amino acid composition of a protein hydrolyzate such as yeast, because no one solvent is capable of giving more than five to seven distinct readable spots.

Precautions must be taken to minimize the effect of extraneous variables—e.g., no more than 5 μl. of liquid should be applied to the filter paper at one time. As considerable fluctuation of uncontrolled variables occurs even under ideal circumstances, particularly in the fermentation process, the data are best analyzed using a statistical approach. The data on yeast were derived from a minimum of 11 fermenters (threonine) to a maximum of 18 fermenters (leucine-phenylalanine and isoleucine-phenylalanine spots). Individual chromatograms used to get the information tabulated in column 1 of Table I ranged from 28 (threonine) to 129 (alanine).

As only four different samples were used in the estimates of solubles, fewer determinations were required for the same degree of confidence in the chromatographic results. The data on solubles presented in column 2 of Table I are based on a minimum of four spots on each of five sheets or a total of 20 values for each spot. The position of each hydrolyzate on each sheet was in accordance with a table of random numbers, to avoid any distortion in the data due to sheet position.

The 15 amino acids listed in Table I account for 56.2% of the protein in the yeast. This compares favorably with the 50.9% reported by Block and Bolling (*3*) on 12 amino acids.

The presence of only a trace of tryptophan in the solubles is attributed to the method of production. Effluent from the still is screened and the thin liquid is evaporated to a sirup, which is dried into thin sheets of solubles on steam-heated drums. The pH of the still effluent is about 4. Tryptophan is probably destroyed under these conditions (*6*).

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Table I. Comparative Amino Acid Compositions as Per Cent of Protein

Amino Acid	Yeast	Solubles	Corn[a]	Rye[a]	Malt[a]
Alanine	2.9	9.8	...	...	...
Arginine	3.3	6.8	4.3	3.3	3.3
Aspartic acid	5.4	3.2	...	...	...
Glutamic acid	7.1	1.1	22.3	19.9	16.7
Glycine	1.1	...	...	...	...
Histidine	1.5	...	3.2	2.2	2.0
Isoleucine	3.3	6.2	6.4	3.9	4.7
Leucine	4.8	6.0	14.9	5.5	6.0
Lysine	6.7	7.6	3.2	3.3	4.0
Methionine	2.2	3.4	2.1	0.6	1.3
Phenylalanine	3.8	5.6	5.3	3.9	4.0
Serine	4.6	...	8.5	3.9	3.3
Threonine	3.0	7.8	3.2	2.2	2.7
Tryptophan	...	Trace	0.5	0.6	1.3
Tyrosine	2.5	4.8	2.1	1.1	1.3
Valine	4.0	1.7	5.3	3.9	4.7
Total	56.2	64.0	81.3	54.3	55.3

[a] As reported microbiologically by Baumgarten, Mather, and Stone (calculated to per cent of total protein) (*1*).