

AMINO ACID AND VITAMIN COMPOSITION OF *SACCHAROMYCES FRAGILIS* GROWN IN WHEY

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SUMMARY

Saccharomyces fragilis grown in a whey medium was analyzed for amino acid and vitamin contents. Qualitatively, the amino acid composition of *S. fragilis* was similar to that reported for other yeasts. The quantity of the individual amino acids, however, was greater in the whey-grown *S. fragilis*. Lysine, in particular, was present at the level of 11.14 g/16 g. N, compared to the range of 3.8 to 6.9 g/16 g. N found in other microorganisms.

An extraction procedure applied to whole, dried *S. fragilis* cells removed 40% of the total weight and 28% of the N of the cell preparation. The amino acid composition of the residual protein fraction was the same as that of the whole cells, but the amounts of the component amino acids varied from 35% of the histidine to 100% of the serine and valine in the whole cell preparation.

Thiamine, pyridoxine, riboflavin, niacin, folic acid, pantothenic acid, p-aminobenzoic acid, choline, inositol, and biotin were present in *S. fragilis* in concentrations within the ranges reported for other microorganisms.

The role of dried yeast as a source of proteins, vitamins, and other growth factors in human and animal nutrition is well established. Generally, strains of *Saccharomyces* and *Torulopsis* yeasts have been used for food purposes. In addition to baker's yeast and debittered brewers' yeast, *Saccharomyces* yeasts have been grown in molasses and in unhopped grain media as primary yeast for food and animal feed. *Torula* yeast, although capable of good growth on molasses medium, has been used extensively in the conversion of carbohydrate-containing industrial wastes into protein- and vitamin-rich products (18, 19, 23, 27).

Whey produced in the manufacture of cheese has often been treated as a waste. However, in Germany during the protein shortage of World War II, attempts were made to grow *Torula utilis* (8) and *Saccharomyces lactis* (22) on whey. The propagation procedures involving these strains of yeast were not economically practical, and the use of whey as a medium for yeast growth has not been exploited commercially. Recently, a modified procedure for the growth of *Saccharomyces fragilis* on whey has been reported (29). In addition to utilizing the waste whey, thus reducing to some extent the increasingly important problem of water pollution, the growth of yeast gives a product that might be used for animal feed and human diet supplementation.

Analyses of the various strains of yeasts grown on different media indicate that, qualitatively, the amino acid composition of the proteins and the vitamin contents are the same. Quantitatively, the individual amino acid concentrations

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appear to vary only slightly within a narrow range, regardless of the strain of yeast or the medium in which the yeasts are grown (5, 10). The vitamins, on the other hand, do show a wide range of variability depending on the concentration of the particular vitamin, or precursor, in the medium, the synthetic ability of the yeast, and the growth conditions (21).

The amino acid and vitamin composition of *S. fragilis* have not been reported previously. The concentration of the individual amino acids and vitamins, however, should not vary considerably from the range of values found in the other yeasts. The data reported in this paper confirm the fact that the composition of *S. fragilis* grown in whey is similar to that of other yeast species used as a supplement in human and animal nutrition.

MATERIALS AND METHODS

Organism. The conditions for the growth of *S. fragilis* NRRL Y1109 have been described previously (29). The yeast, grown in Italian cheese whey in the pilot plant apparatus (28), was concentrated to a cream containing 15-18% yeast solids in a DeLaval separator. The yeast was washed twice with equal volumes of cold tap water and reconcentrated with the separator. The washed yeast was dried on a double-drum drier.

Nitrogen. Total nitrogen was determined by hydrolysis of the sample according to a modification of the Koch-McMeekin method (14), followed by micro-Kjeldahl distillation into boric acid.

Protein. The protein contents of the samples were calculated on the basis of $N \times 6.25$.

Fractionation. A yeast protein fraction was prepared by treating the dried yeast according to the procedure of Roberts *et al.* (20). Five-gram quantities of yeast were suspended successively, with continuous stirring, in 150 ml. of the following solutions: 5% trichloroacetic acid for 30 min. at 5° C.; 75% ethyl alcohol for 30 min. at 40-50°; ether-70% alcohol (1:1) for 15 min. at 40-50°; 5% trichloroacetic acid for 30 min. in a boiling water bath. The suspensions were centrifuged between each solution, and the supernatant liquids discarded. The precipitates obtained after the hot 5% trichloroacetic acid treatment were resuspended in acid-alcohol, filtered, and washed on the filter with 300 ml. acid-alcohol, followed by an ether wash. The precipitates were air-dried. Three grams of a fluffy white powder were obtained from 5 g. of yeast.

Hydrolysis. Amino acid analyses were carried out on dried yeast and yeast protein preparations hydrolyzed with 6 N HCl (0.25 ml. acid/mg dry weight of sample). The evacuated, sealed tubes containing the acid and sample were heated in an oil bath at 110° for 24 hr. The acid was removed, and the hydrolysate dried and stored, in vacuo over P₂O₅ and soda lime.

Amino acids. The amino acid analysis was done by resin column chromatography according to Moore *et al.* (15). The dried protein hydrolysates were dissolved in citric acid buffer, pH 2.3, and aliquots placed on the short column for the determination of basic amino acids. The remaining amino acids were determined on the long column.

Vitamins. The following vitamin analyses of the dried yeast powder were carried out by the Wisconsin Alumni Research Foundation: choline (12), inositol (4), thiamine (3), vitamin B₆ (4), riboflavin (3), niacin (3), folic acid (3), pantothenic acid (16), p-amino benzoic acid (PABA) (1), and biotin (31).

RESULTS

Amino acid composition. The amino acid composition of the whole cells of *S. fragilis* is shown in Column 1 of Table 1. Quantitatively, the amino acids of

TABLE 1
The amino acid composition of *Saccharomyces fragilis*, other microorganisms, and yeast protein fractions

(The data are reported as grams/16 grams N)

	1	2	3	4	5	6	7	8
Amino Acid	<i>S. fragilis</i> whole cells	<i>S. fragilis</i> protein	12 Brewers' yeast ^a (av.)	4 Pri- mary yeast ^a (av.)	Com- posite protein (6- yeast)	<i>Torula</i> <i>utilis</i> ^a	<i>Strepto-</i> <i>coccus</i> <i>fecalis</i> ^a	<i>Esche-</i> <i>richia</i> <i>coli</i> ^a
Lysine	11.14	10.15	6.9	6.3	10.4	8.7	7.2	3.8
Arginine	7.37	7.08	5.4	4.5	5.7	7.6	3.4	4.2
Histidine	3.98	1.97	2.5	2.7	3.0	2.8	1.3	1.2
Aspartic Acid	10.40	11.16	7.2	7.2	10.2	7.6
Threonine	5.57	6.46	4.6	4.9	7.5	5.4	3.3	3.1
Serine	5.21	6.96	4.2	4.2	7.3	1.9
Glutamic Acid	15.24	13.26	10.9	10.9	7.9	14.5	10.1
Proline	4.31	4.0	4.0	4.9	2.6
Glycine	4.24	4.63	4.0	3.9	4.5	0.4	4.6
Alanine	7.21	8.17	5.5	5.5	7.8	5.7
Valine	5.72	7.78	4.9	4.7	8.0	6.3	4.5	6.1
Methionine	1.57	1.24	1.3	1.4	2.0	1.8	1.9	2.0
Isoleucine	5.05	6.00	4.3	3.9	6.1	7.9	4.1	4.3
Leucine	9.60	6.0	5.3	8.0	7.5	4.7	8.3
Tyrosine	4.57	3.42	3.3	3.4	4.1	2.2
Phenylalanine	5.05	5.39	3.0	3.2	4.4	5.1	2.8	2.8
Nitrogen	7.97	9.59	7.8	8.3	8.9	7.6	13.2	12.0
Reference			(6)	(6)	(6)	(13)	(25)	(2)

^a These analyses are of whole cell preparations.

S. fragilis compare well with the average concentration of amino acids in 12 strains of brewers' yeast (Column 3), four strains of yeast grown expressly for feed or food purposes (Column 4), and *T. utilis* grown on wood sulfite waste liquor as a feed yeast (Column 6). For the most part, the concentration of the amino acids is somewhat higher in *S. fragilis*. It is interesting to note that the concentration of lysine is considerably greater in *S. fragilis* than in the other yeasts. This factor may be of importance in considering *S. fragilis* as a dietary supplement, particularly in view of the importance of lysine in nutrition.

The amino acid analysis of the whole yeast cell does not represent the composition of the true yeast protein. The protein contents of the whole cell is generally computed from the total nitrogen determination (N × 6.25). The total nitrogen, however, includes nucleic acids, free amino acids, and nonprotein nitrogen compounds such as choline and glutathione. The pure protein may

actually account for only 64-67% of the total nitrogen (9), and can be determined only after the removal of the extraneous nitrogenous matter. There is, however, no standard procedure for preparing purified yeast protein. Whole yeast cells have been treated in various ways with a number of solvents. The results of these treatments may be questionable with respect to the quantity of nonprotein nitrogen removed.

A study was made of the protein fraction of *S. fragilis*. Five-gram aliquots of the whole yeast were extracted as described in Methods. A 40% loss in solids resulted from this treatment. This protein fraction contained 9.59% of N, or 60% protein. The fraction, therefore, was not pure protein. If the nonprotein nitrogen components had been effectively removed, the remaining protein may be combined with carbohydrate or lipids, both of which are present in the yeast cell. Analysis of the yeast protein fraction for amino acid concentrations yielded the results shown in Column 2, Table 1. The differences in the amino acid concentration (based on 16 g. N) between the whole yeast cell (Column 1) and the yeast protein do not appear to be very great.

The quantity of the amino acids extracted by the treatment was calculated as shown in Table 2. Total amino acid concentrations were determined for the

TABLE 2
Total, combined, and extractable amino acid content of *Saccharomyces fragilis* *

Amino Acid	Total (μ g.)	Combined (μ g.)	Extractable % total
Lysine	266	183	31
Arginine	175	127	27
Histidine	96	34	65
Aspartic Acid	260	200	23
Threonine	138	116	16
Serine	130	126	3
Glutamic Acid	377	239	37
Glycine	105	83	21
Alanine	179	147	18
Valine	142	140	1
Methionine	39	22	43
Isoleucine	125	108	13
Tyrosine	114	62	46
Phenylalanine	125	97	22

* Total amino acids in 5 g. dried *S. fragilis* prior to extraction described in Methods. Combined amino acids are the residual amino acids in the 3 g. material remaining after treatment. Extractable amino acids include the free amino acid pool, polypeptides, and soluble proteins. They are determined as the difference between the total and combined amino acids.

5 g. of yeast prior to extraction. The yeast protein amino acids, or combined amino acids, are the residual amino acids in the 3 g. of material remaining after extraction. The term combined amino acids is used to include any amino acids that may be complexed with carbohydrates or lipids, as well as the pure protein material. The differences between the total and combined amino acid values are the extractable amino acids. This fraction should include the components of the free amino acid pool, polypeptides, and soluble proteins.

The range of concentration of the extractable amino acids of *S. fragilis*, grown in whey medium under the given conditions and extracted as described,

varies considerably, from essentially no serine or valine to 65% of the total histidine present in the yeast. Approximately 45% of the methionine and tyrosine, and about one-third of the lysine and glutamic acid were extracted. The other amino acids were soluble in the treatment solutions to the extent of 13 to 27% of their total concentrations.

Vitamin composition. The vitamin composition of *S. fragilis* is shown in Column 1, Table 3. The concentration of the vitamins in *S. fragilis* compares well with that reported for other yeasts (Table 3).

DISCUSSION

S. fragilis can be grown in a whey medium to contain 50% protein. The strain of yeast and the medium on which it was grown have no influence on the amino acid composition of the protein and only some slight effect on the concentration of the various amino acids. The amino acids in *S. fragilis* do not differ from those found in other yeasts grown in various media, and the amounts of the amino acids fall, for the most part, within the range observed for other yeasts. *S. fragilis*, in common with most of the yeasts used for food purposes, is low in S-amino acids. *T. utilis* grown in sulfite liquor also was low in S-amino acids and had to be fortified with methionine to give values comparable to casein (11) in feeding tests.

Analysis of the whole yeast cells for amino acid composition can yield misleading information about the protein structure. The quantity of some amino acids may be reduced through reaction with carbohydrates during acid analysis. On the other hand, the apparent concentration of the amino acids in the yeast protein may be greater because the free amino acids, and those liberated from polypeptides by acid hydrolysis, are also included in the determination of amino acids. The extraneous nonprotein nitrogen and carbohydrate compounds should be removed from the yeast to avoid these errors. There is no standardized method for accomplishing this, and many procedures have been used (5). The method of Roberts *et al.* (20) has been used for the extraction of *Escherichia coli* and the materials removed by the various solvents were described in detail. Applying this procedure to *S. fragilis* removed 40% of the weight of the cell preparation and 28% of the nitrogen. The remaining material was not all protein; it may be protein combined with carbohydrate or lipids. The extractable amino acids are of interest in view of their possible importance in protein synthesis. For instance, serine and valine are present entirely in the protein, or combined fraction, whereas 65% of the histidine in the cells is present as extractable amino acid. The other amino acids are present in the extractable fraction in varying concentrations. The total nitrogen contents of the yeast will vary. *S. fragilis* growing in whey alone may have a nitrogen content of 4.9%, whereas in the media containing 1% $(\text{NH}_4)_2\text{SO}_4$, the growing cells may contain 8.2% N (30). Since the true protein fraction of the yeast will have the same amino acid composition and concentration under all conditions in which the yeast can remain healthy, only the amount of the amino acids in the extractable fraction can vary when the external N source is changed. The quantities of the

TABLE 3
Vitamin composition of *Saccharomyces fragilis* and other yeasts

Vitamin	<i>S. fragilis</i>	<i>Torula uttilis</i>	<i>Torula uttilis</i>	<i>Torula uttilis</i>	Brewers' yeast	Bakers' yeast	Whey
Thiamine ($\mu\text{g/g}$)	24.1	6-53	5.3	7-42	104-250	11-60	4-15
Pyridoxine ($\mu\text{g/g}$)	13.6	35	33.0	28-47	23-40	25-39	.5-15
Riboflavin ($\mu\text{g/g}$)	36.0	26-62	45.0	24-61	25-80	25-80	5-68
Niacin ($\mu\text{g/g}$)	280.0	210-535	417.3	375-690	300-627	293-482	3-22
Folic Acid ($\mu\text{g/g}$)	5.8	4-31	21.0	19-30	25-34	0-1
Pantothenic Acid ($\mu\text{g/g}$)	67.2	86-180	37.0	72-86	150-280	22-90
p-Aminobenzoic Acid ($\mu\text{g/g}$)	24.2	17-21	11-40	15-40	24-167
Biotin ($\mu\text{g/g}$)	2.0	1.1-1.9	2.3	1.1-1.7	1.1	0.8-2.4	.03-1.5
Choline (mg/g)	6.7	0.5-4
Inositol (mg/g)	3.0	3.5	2.8	0.5-6
References		(10)	(13)	(23)	(17)	(17)	(7)

amino acids in the extractable fraction may depend on the yeast strain, the composition of the medium, or the age of the yeast (24).

It is of interest to note that in the column chromatography of the yeast protein, or combined, amino acids, a small peak appeared in the 35th 2-ml. fraction, before any of the amino acids were eluted. The material in this peak gave a red color upon addition of the ninhydrin developing reagent. A similar peak has been isolated and identified as levulinic acid in acid hydrolysates of bean and potato fractions by Zacharius and Talley (32). The identity of the yeast protein material has not been established. This peak was not observed in the acid hydrolysates of whole dried yeast. The extraction treatment given to the yeast apparently made the observation of this material possible by (1) concentrating the material so the small quantity present could be detected, or (2) forming or exposing a precursor compound that yielded the unknown on acid hydrolysis.

Although the vitamin composition of the yeasts appears to be constant, the concentration of the vitamins will vary depending on the yeast strain, composition of the medium, or cultural conditions. Brewers' yeast contains more thiamine than bakers' yeast, as normally produced, but enriched bakers' yeast with considerably more thiamine has been produced commercially (17). Since it is known that yeast absorbs thiamine from the medium, this enrichment can be accomplished by the addition of thiamine to the growing yeast, or to the spent medium of nonproliferating yeast (26). Whey contains very little thiamine (Table 3); therefore, the contents of vitamin B₁ in *S. fragilis* could be expected to be low. Although the vitamin value is comparable to other yeasts, *S. fragilis* could be enriched by the addition of thiamine to the medium.

A further cause of reduced vitamin concentration is the yeast yield. Increased yeast yields result in less vitamin per unit cell (21). It is possible, therefore, that under the conditions in which the *S. fragilis* was grown on whey for maximum yields, greater vitamin contents could not be achieved.

REFERENCES

- (1) AGARWALA, S. C., AND PETERSON, W. H. An Improved Method for the Determination of p-Aminobenzoic Acid by *Neurospora Crassa*. Arch. Biochem. Biophys., 27: 304. 1950.
- (2) ANDERSON, R. F., AND JACKSON, R. W. Essential Amino Acids in Microbial Proteins. Appl. Microbiol., 6: 369. 1958.
- (3) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Methods of Analysis. 8th ed., 819, 823, 830. 1955.
- (4) ATKIN, L., SCHULTZ, A. S., WILLIAMS, W. L., AND FREY, C. N. Yeast Microbiological Methods for Determination of Vitamins. Pyridoxine. Ind. Eng. Chem., Anal. Ed., 15: 141. 1943.
- (5) BLOCK, R. J., AND BOLLING, D. The Amino Acids Yielded by Various Yeasts After Hydrolysis of the Fat-free Material—A Comparative Investigation, Arch. Biochem. Biophys., 7: 313. 1945.
- (6) BLOCK, R. J., AND WEISS, K. W. Amino Acid Handbook. C. C. Thomas, Springfield, Ill. 1956.
- (7) Chemical Composition of Milk, in "Standard Values in Nutrition and Metabolism." E. C. Albritton, ed. Wright Air Development Center Tech. Rept. 52-301, p. 111. 1953.

- (8) DEMMLER, G. Yeast Culture on Whey by the Waldhof Process. *Die Milchwissen*, 4: 11. 1950.
- (9) DIRR, K., AND DECKER, P. Quoted by H. E. Carter in "Yeasts in Feeding—A Symposium," p. 5. 1948.
- (10) EDDY, A. A. Aspects of the Chemical Composition of Yeast, in "The Chemistry and Biology of Yeasts." A. H. Cook, ed. Academic Press, N. Y. 1958.
- (11) HARRIS, E. E., HAJNY, G., AND JOHNSON, M. C. Protein Evaluation of Yeast Grown on Wood Hydrolysate. *Ind. Eng. Chem.*, 43: 1593. 1951.
- (12) HOROWITZ, N. H., AND BEADLE, G. W. A Microbiological Method for the Determination of Choline by Use of a Mutant of *Neurospora*. *J. Biol. Chem.*, 150: 325. 1943.
- (13) INSKEEP, G. C., WILEY, A. J., HOLDERBY, J. M., AND HUGHES, L. P. Food Yeast from Sulfitic Liqueur. *Ind. Eng. Chem.*, 43: 1702. 1951.
- (14) MILLER, G. L., AND MILLER, E. E. Determination of Nitrogen in Biological Materials. *Anal. Chem.*, 20: 481. 1948.
- (15) MOORE, S., SPACKMAN, D. H., AND STEIN, W. H. An Improved System for the Chromatography of Amino Acids on Sulfonated Polystyrene Resins. *Anal. Chem.*, 30: 1185. 1958.
- (16) NEILANDS, J. B., AND STRONG, F. M. The Enzymatic Liberation of Pantothenic Acid. *Arch. Biochem. Biophys.*, 19: 287. 1948.
- (17) PETERSEN, H. W. Vitamins and Minerals in Yeast, in "Yeast in Feeding—A Symposium," p. 26. 1948.
- (18) PORGES, N., PEPINSKY, J. B., AND JASEWICZ, L. Feed Yeast from Dairy Products. *J. Dairy Sci.*, 34: 615. 1951.
- (19) REISER, C. D. Food Yeast, Torula Yeast from Potato Starch Wastes. *Agr. Food Chem.*, 2: 70. 1954.
- (20) ROBERTS, R. B., ABELSON, P. H., COWIE, D. B., BOLTON, E. T., AND BRITTON, R. J. Studies of Biosynthesis in *Escherichia coli*. Carnegie Institute of Washington. Publ. No. 607, Washington, D. C. 1955.
- (21) SINGH, G., AGARWAHL, P. W., AND PETERSEN, W. H. Influence of Aeration and Agitation on the Yield, Protein, and Vitamin Content of Food Yeast. *Arch. Biochem. Biophys.*, 18: 181. 1948.
- (22) STANIER, R. Y. Some Aspects of Microbiological Research in Germany, B.I.O.S. Final Rept. No. 691, Item No. 24.
- (23) STUBBS, J. J., NOBLE, W. M., AND LEWIS, J. C. Fruit Juices Yield Food Yeast. *Food Ind.*, 16: 694. 1944.
- (24) TAYLOR, E. S. The Assimilation of Glutamic Acid by Yeast. *J. Gen. Microbiol.*, 3: 211. 1949.
- (25) TOENNIES, G., BAKAY, B., AND SHOCKMAN, G. D. Bacterial Composition and Growth Phase. *J. Biol. Chem.*, 236: 3269. 1959.
- (26) VAN LANYN, J. M., BROQUIST, H. P., JOHNSON, M. J., BALDWIN, I. L., AND PETERSON, W. H. Synthesis of Vitamin B₁ by Yeast. *Ind. Eng. Chem.*, 34: 1244. 1942.
- (27) VELDHUIS, M. K., AND GORDON, W. D. Experiments on Production of Yeast from Citrus Press Juice. *Proc. Florida State Hort. Soc.* 1947.
- (28) WASSERMAN, A. E., HAMPSON, J., ALVARE, N. J., AND ALVARE, N. F. Whey Utilization. V. Growth of *Saccharomyces fragilis* in Whey in a Pilot Plant. *J. Dairy Sci.*, 44: 387. 1961.
- (29) WASSERMAN, A. E., HOPKINS, W. J., AND PORGES, N. Whey Utilization. Growth Conditions for *Saccharomyces fragilis*. *Sewage and Industrial Wastes*, 30: 913. 1958.
- (30) WASSERMAN, A. E., HOPKINS, W. J., AND PORGES, N. Rapid Conversion of Whey to Yeast. *Proc. XVth Intern. Dairy Congr.*, 2: 1241. 1959.
- (31) WRIGHT, L. D., AND SKEGGS, H. R. Determination of Biotin with *Lactobacillus arabinosus*. *Proc. Exptl. Biol. Med.*, 56: 95. 1944.
- (32) ZACHARIUS, R. M., AND TALLEY, E. A. Identification of a Non-nitrogenous Ninhydrin-positive Compound in Hydrolysed Plant Fraction. *Proc. Ann. Meet. Am. Soc. Plant Physiol.* 1960. (In press.)