Protein Content and Methionine Value of Yeast Increased

YEASTS

Methionine and Cystine Contents

J. S. CHIAO and W. H. PETERSON
Department of Biochemistry, University of Wisconsin, Madison, Wis.

In view of the world shortage of dietary protein, and the fact that yeast protein can be used to replace half of other proteins in the diet of chicks and rats, the possibility of improving the nutritional value of yeast by raising its protein and methionine content was investigated. Baker's and brewer's yeasts are known to be deficient in methionine and cystine, although literature data on percentages present vary greatly. The methionine content of seven commercial yeasts was found to vary from 0.48 to 0.75% on a dry basis. A fat yeast, *Rhodototula gracilis,* contained 1.00% of methionine, which is about 20% higher than the commercial *Saccharomyces* species and nearly double that of the *Torula* type (0.54%). The protein content and methionine value of yeast were increased by adding more nitrogen salts to the medium. Theoretical precursors of methionine—choline and cystine—added to the medium did not affect the methionine content of *Rh. gracilis.*

DRIED YEAST has long been recognized as an outstanding source of the B vitamins, but as the main source of protein it has generally proved inadequate (*7, 10, 11, 14, 17, 18, 22, 25*). The inadequacies of yeast proteins, like those of other food proteins, may be largely corrected when yeast is used in combination with other foods.

Several investigators (*3, 13, 17, 32*) have shown that about one half of the protein in the ration can be replaced by yeast protein without affecting the growth of chicks or rats. However, when three fourths of the protein in the ration is replaced by yeast, growth is retarded. It is not realistic to assume that yeast will ever be used as the sole or even the main source of protein in a practical diet for humans or animals.

A well established deficiency in yeast is a lack of methionine (*10, 11, 17*). When about 0.5% of this amino acid has been added to the ration, the growth rate with yeast as the sole source of protein has usually been equal to that obtained with casein. The methionine content of yeast reported in the older literature ranges from 1 to 3% on the basis of dry matter, but most of these figures are probably too high. Recent original data (*4, 5, 9, 10, 15, 21, 28, 29*) on the methionine content of various kinds of yeast range from 0.55 to 0.85% for methionine and 0.25 to 0.50% for cystine.

Another conclusion that seems to be widespread is that the *Saccharomyces cerevisiae* yeasts are higher in methionine content and give better growth than the *Torula utilis* yeasts. Caution must be observed in drawing such a conclusion, as yeasts vary in methionine content and probably in their growth-promoting qualities, depending upon the conditions under which the yeasts were grown. Only a few reports give results with different types of yeast grown under identical conditions (*10, 11*).

Shortage of protein is a world problem, which is acute in Formosa, India, and other Asian countries. An effort is now being made to increase the consumption of yeast in this country, and it has been shown that producers of food and fodder yeast can improve its nutritional value by raising the protein and methionine content.

In view of the wide variations found in the literature, it appeared worth while to redetermine the sulfur amino acids of yeasts representative of widely used types and to look for yeasts containing more methionine and cystine than those now manufactured for food and feed purposes. The present work deals with the methionine and cystine contents of representative commercial yeasts and 20 species of yeasts grown under defined laboratory conditions. Some factors affecting the methionine and cystine contents have also been investigated.

Experimental Procedure

Yeast Strains Cultures of *Endomyces magnusii* 836, *Hansenula anomala* var. spherica 778, *Saccharomyces cerevisiae* 49, *S. cerevisiae* 53, *S. cerevisiae* "Gebruder Mayer," *S. cerevisiae* Y-30, and *Torula utilis* 3 were obtained from Elizabeth McCoy, Department of Bacteriology. Transplants of *Candida krusoides, C. pulcherrima,* and *Oidium lactis* were kindly sent by K. B. Butlin, Department of Scientific and Industrial Research, Chemical Research Laboratory, Teddington, England. In former papers from the authors' laboratory *Candida krusoides* was called *Candida arborea,* but L. J. Wickerham has identified it as *C. krusoides. S. cerevisiae* F-53 and *S. cerevisiae* F-22 were obtained from the Red Star Yeast and Products Co., Milwaukee, Wis. Cultures of *Candida guilliermondii, C. zeylanoides, Debaryomyces*

matruchoti, *Endomycopsis fibuliger*, *Hansenula saturans*, *Mycoderma cerevisiae*, and *Pichia alcoholophila* were obtained through the courtesy of L. J. Wickerham, Northern Regional Research Laboratory, Peoria, Ill. A culture of *Rhodotorula gracilis* was kindly sent by Harry Lundin, Kungliga Tekniska Hogskolan, Stockholm, Sweden.

Yeast transfers were made every month to an agar stab containing 1.5% agar, 0.25% glucose, and 0.25% Difco yeast extract.

Media and Fermentation The inoculum medium contained 5.0% beet molasses, 2.0% Difco malt extract, 0.75% corn steep liquor, and 0.1% diammonium hydrogen phosphate. The solution of diammonium hydrogen phosphate was sterilized separately and was added to the clarified and sterilized solution of molasses, malt extract, and corn steep liquor just before inoculation. Hawaiian cane molasses was clarified by means of corn steep liquor as directed by Agarwal *et al.* (*2*). The medium was prepared to contain about 1% reducing sugar, 0.1% diammonium hydrogen phosphate, and 1% corn steep liquor solids treated as described by Chang and Peterson (*6*). A synthetic medium also used in some fermentations was prepared following directions of Olson and Johnson (*19*). The same synthetic medium was used for growing the inoculum and for fermentation.

The inoculum was grown for 24 hours at 30° C. in 100-ml. Erlenmeyer flasks containing 15 ml. of medium on a reciprocating shaker (84 strokes, 10-cm. length per minute). The yeast cells were centrifuged, washed once with 10 ml. of sterile water, and then made to the original volume.

The fermentations were run in 500-ml. Erlenmeyer flasks containing 50 ml. of medium per flask. To each flask 2.5 ml. of the washed inoculum, containing about 10 to 20 mg. of dry yeast, were added. The flasks were incubated at 30° C. for 24 hours on a rotary shaker describing a 2.25-inch circle at 250 r.p.m. After fermentation, the cells were separated from the medium by centrifugation and washed twice with 25 ml. of distilled water. The first washing was combined with the supernatant and the whole was used for the determination of residual sugar. The washed cells were resuspended in 25 ml. of distilled water, and aliquots were taken for determination of dry weight. The rest of the cells were saved for amino acid and nitrogen determinations.

Analytical Methods The dry weight of yeast was determined in duplicate by centrifuging 5 to 10 ml. of the homogeneous suspension (about 50 to 100 mg. of dry cells) in weighed borosilicate glass tubes, drying at 109° C. for 24 hours, and weighing. The yield of yeast was expressed as percentage of sugar fermented.

Sugar was determined after hydrolysis by the micromethod of Shaffer and Somogyi (*23*), with reagent No. 50 containing 5 grams of potassium iodide per liter. Nitrogen was determined according to Hiller *et al.* (*12*) on aliquots of the washed cells, containing about 0.6 to 6.0 mg. of nitrogen.

The twice-washed cells (about 0.1 to 0.2 gram) were hydrolyzed with 10 ml. of hydrochloric acid under the optimum conditions as given below. The hydrolyzate was filtered, neutralized with potassium hydroxide, and diluted to volume. Methionine and cystine were determined microbiologically with *Leuconostoc citrovorum* 8081 (*27*). L-Methionine and L-cystine were used as the standards. Citrovorum factor required by this organism was added in the form of a concentrate supplied by E. L. R. Stokstad, Lederle Laboratories Division, American Cyanamid Co. Sixteen hundred units were added to 50 ml. of the double-strength basal medium. The response of *L. citrovorum* to L-methionine and L-cystine was reproducible, and the assay results showed no drifting at various levels of sample. For routine assay, a dried reference yeast was assayed along with the samples as a check on the procedure. The methionine content of the reference yeast in 26 runs ranged from 0.56 to 0.66% and averaged 0.62 ± 0.02% (standard deviation of the mean) on the dry weight basis.

The optimum conditions for liberation of methionine from yeast were determined on the reference yeast. A 0.25-gram sample was autoclaved with 10 ml. of hydrochloric acid of different concentrations at 120° C. for various intervals of time. Maximum liberation was obtained when the yeast was hydrolyzed for 1 hour with 7*N* acid, 1 to 2 hours with 5*N* acid, or 3 hours with 3*N* acid. Many of the yeasts were hydrolyzed under the different conditions and assayed. In most cases the results obtained were not significantly different from one another. Because of the shorter time required, 7*N* acid was the preferred method.

As amino acids may be destroyed during acid hydrolysis of protein, recovery of methionine added to the reference yeast was determined; under the conditions of maximum liberation of methionine, recovery was 96% or better. Therefore no correction has been made in presenting the methionine content of yeasts.

Smith and Schlenk (*26*) studied the metabolic relationship between methionine and adenine thiomethylriboside in yeasts. The accumulation of the latter was found to depend on the presence of the former in the medium. Shapiro (*24*) found that adenine thiomethylriboside can replace methionine for some mutants of *Aerobacter*. The presence of adenine thiomethylriboside in the basal medium containing no methionine did not promote growth of *L. citrovorun* 8081 and its addition to yeast hydrolyzates did not affect the methionine assay. The adenine thiomethylriboside used was kindly supplied by F. Schlenk.

Optimal conditions of hydrolysis for the release of cystine were not studied. The yeast hydrolyzates prepared for methionine assay were used also for the determination of cystine.

Table I. Methionine and Cystine in Commercial Yeast Samples

(Dry weight basis)

Sample	Methionine, %	Cystine, %
Active dry yeast	0.63	0.25
Brewer's yeast	0.67	0.39
Baker's yeast	0.66	0.45
Nondebittered brewer's yeast	0.56	0.31
Debittered brewer's yeast	0.75	0.21
Baker's foil yeast	0.54	0.28
T. utilis yeast from sulfite waste liquor	0.48	0.33

Methionine And Cystine in Commercial Yeasts Seven samples of commercial yeasts were analyzed with the results given in Table I. The range for methionine, 0.48 to 0.75%, is in good agreement with recent data (0.55 to 0.85%) and the figures for cystine, 0.21 to 0.45%, agree well with those (0.25 to 0.50%) reported in recent publications (*4*, *5*, *9*, *10*, *15*, *21*, *28*, *29*). The *S.*

Table II. Methionine and Cystine in Yeasts Grown on Hawaiian Molasses Medium

Yeast	Yield, Sugar Fermented, %	Methionine of Dry Yeast, %	Cystine of Dry Yeast, %
C. krusoides	80.0	0.66	0.28
C. guilliermondii	67.2	0.74	0.37
C. pulcherrima	54.7	0.58	0.31
C. zeylanoides	69.0	0.82	0.37
D. matruchoti	83.4	0.42	0.19
E. fibuliger	63.8	0.17	0.20
E. magnusii	69.1	0.58	0.38
H. anomala	72.0	0.50	0.32
H. saturans	59.6	0.61	0.58
M. cerevisiae	75.9	0.83	0.32
O. lactis	54.0	0.30	0.26
P. alcoholophila	66.5	0.48	0.36
Rh. gracilis	73.3	1.00	0.27
S. cerevisiae Y-30	66.2	0.75	0.30
S. cerevisiae 53	63.8	0.71	0.38
S. cerevisiae 49	64.1	0.67	0.25
S. cerevisiae G. M.	63.5	0.78	0.27
S. cerevisiae F-53	67.7	0.85	0.31
S. cerevisiae F-22	60.1	0.73	0.21
T. utilis 3	68.3	0.54	0.21

cerevisiae yeasts were all higher in methionine than the *T. utilis* sample.

In Yeasts Grown In Hawaiian Molasses Medium

Table II gives the yield of cells and methionine and cystine figures for 20 yeasts representing 11 genera. Five strains gave yields of 70% or better, two gave 80% or more, and the others gave yields ranging from 50 to 70%. The high yields obtained in this medium were probably due to the utilization of nonsugar carbon from corn steep liquor, as reported by Agarwal and Peterson (*1*). In all cases the utilization of sugar was from 90 to 100% at the time of harvesting.

Methionine figures varied from 0.17 to 1.0% and cystine from 0.19 to 0.58% on the basis of dry weight. With the exception of *E. fibuliger*, the methionine figure was always higher than that of cystine.

The methionine content of *S. cerevisiae* yeasts ranged from 0.71 to 0.85% with an average of 0.75%. One of them, F-53, was notably high in both yield and methionine content. *T. utilis* gave slightly higher yields than the *S. cerevisiae* yeasts, but was considerably lower in both methionine and cystine. The methionine figures are in agreement with the general belief that *S. cerevisiae* yeasts are superior to *T. utilis* in nutritive value when both are produced under the same conditions.

The *Candida* yeasts, with the exception of *C. pulcherrima*, gave somewhat higher yields than the *S. cerevisiae* yeasts. The methionine contents of the best producers varied from 0.66 to 0.82% and averaged 0.74%, practically identical with the average of the *S. cerevisiae* yeasts. *Rh. gracilis*, best known for its fat-producing ability (*9*, *20*) had the highest methionine and total sulfur amino acid content among the yeasts tested. However, *O. lactis*, also a fat yeast, gave low values for methionine and cystine. Of all the yeasts tested, *H. saturans* contained the highest amount of cystine, 0.58%.

Table IV. Methionine in Yeasts Grown on Synthetic Medium

Yeast	Yield on Sugar Fermented, %	Final pH[a]	Methionine of Dry Yeast, %	
			Microbiological method	Colorimetric method
C. krusoides	29.2	3.5	0.56	...
C. guilliermondii	44.8	4.9	0.83	...
C. zeylanoides	24.0	3.5	0.62	...
D. matruchoti	45.0	5.3	0.59	...
H. anomala	41.0	6.0	0.54	...
H. saturans	44.0	5.6	0.75	...
M. cerevisiae	37.6	2.8	0.78	0.84
Rh. gracilis	45.0	5.7	1.00	1.18
S. cerevisiae Y-30	41.6	3.4	0.81	...
S. cerevisiae F-53	35.0	3.7	0.85	0.88
T. utilis 3	44.5	6.1	0.72	0.76

[a] Initial pH 5.0.

Effect of Inorganic Salts on Yield and Methionine Content

As the nitrogen content of yeast can be increased by raising the inorganic nitrogen of the medium, it appeared possible that the methionine figure would rise with the nitrogen content. The effect of such additions on methionine synthesis by *S. cerevisiae* F-53 is given in Table III.

The yield of cells and the nitrogen and methionine contents of the yeast grown on straight beet molasses medium (No. 1) were very low, but were increased by additions of diammonium hydrogen sulfate and ammonium dihydrogen phosphate up to 0.044% nitrogen (No. 4). When the added nitrogen was increased to 0.089%, the yield and methionine content dropped, although the nitrogen content of the yeast increased to 9.3%. Up to a certain limit, the yeast cell appears to convert all of the absorbed nitrogen into proteinaceous substances of fixed methionine content. Even after this limit has been reached, it continues to absorb nitrogen, but does not convert this to amino acids. In other words, its absorptive capacity is greater than its synthetic powers. It would be of interest to know if within limits the constant ratio of methionine to total nitrogen in the cell holds for other amino acids.

Addition of potassium dihydrogen phosphate had no significant effect on either the yield or methionine content, but magnesium sulfate increased the yield and maintained a high percentage of methionine in the cells. The effect of both potassium dihydrogen phosphate and magnesium sulfate was no better than of magnesium sulfate alone.

The highest yield obtained from beet molasses–salts medium was still low as compared to that obtained in the Hawaiian molasses medium. The latter medium had been clarified by adding corn steep liquor, which undoubtedly contributed an appreciable amount of nonsugar carbon and possibly also growth factors.

Methionine in Yeasts Grown on Synthetic Medium

Eleven yeasts were grown on the synthetic medium of Olson and Johnson (*19*). The results are given in Table IV.

C. zeylanoides and *C. krusoides* grew poorly in the synthetic medium. Probably they require some growth factors not present in this medium. Reasonably good, though probably not optimal, yields were obtained with the others.

S. cerevisiae F-53 and *Rh. gracilis* led all the yeasts in methionine content and contained the same percentage of methionine as when grown on Hawaiian molasses medium. Three yeasts gave lower results, but the other six yeasts showed increased methionine synthesis. The high methionine figures for *T. utilis* and *C. guilliermondii* are particularly noteworthy.

As a check on the microbiological determination of methionine, the colorimetric method of Sullivan and McCarthy (*30*) was also used. The results for four yeasts grown on the synthetic medium are included in Table IV. The colorimetric method gave figures about 10% higher than those obtained by microbiological assay, but no figures such as

Table III. Methionine Content of *S. cerevisiae* F-53 Grown on Beet Molasses Medium

No.	Additions to Medium	N in Medium, %		Yield on Sugar Fermented, %	Nitrogen of Dry Yeast, %	Methionine of Dry Yeast, %
		Total	Added			
1	None	0.026	0	19.3	5.1	0.55
2	0.05% $(NH_4)_2SO_4$ and 0.004% $NH_4H_2PO_4$	0.037	0.011	34.6	6.8	0.66
3	0.1% $(NH_4)_2SO_4$ and 0.008% $NH_4H_2PO_4$	0.047	0.022	36.6	8.2	0.75
4	0.2% $(NH_4)_2SO_4$ and 0.016% $NH_4H_2PO_4$	0.071	0.044	38.7	8.4	0.75
5	0.4% $(NH_4)_2SO_4$ and 0.032% $NH_4H_2PO_4$	0.115	0.089	27.5	9.3	0.55
6	No. 3 + 0.1% KH_2PO_4	0.047	0.022	37.7	8.3	0.82
7	No. 3 + 0.25% $MgSO_4$	0.047	0.022	49.5	8.0	0.79
8	No. 3 + 0.1% KH_2PO_4 and 0.25% $MgSO_4$	0.047	0.022	50.0	8.0	0.80

have been reported in the older literature for *S. cerevisiae* were obtained.

Effect of Intermediates on Synthesis of Methionine

As it has been reported that *Neurospora* mutants can synthesize methionine from threonine and cystine (*16*, *31*), it was thought that the same pathway might be present in *S. cerevisiae*. Threonine, cystine, and choline were added singly or in combinations to the synthetic medium in which yeast F-53 was grown, and the methionine content of the cells was determined (Table V).

Table V. Effect of Choline, Cystine, and Threonine on Synthesis of Methionine by *S. cerevisiae* F-53 in Synthetic Medium

Addition, Mg./50 Ml.	Yield on Sugar Fermented, %	Final pH[a]	Methionine of Dry Yeast, %
None	35.8	3.3	0.86
Choline, 1.42	36.9	3.3	0.80
Choline, 14.2	35.5	3.3	0.87
Cystine, 14.2	46.3	4.8	0.84
Choline, 1.42 Cystine, 1.42	36.2	3.3	0.82
Choline, 14.2 Cystine, 14.2	47.3	5.2	0.85
Threonine, 1.63	37.2	3.4	0.83
Threonine, 16.3	32.7	3.5	0.82
Choline, 1.42 Cystine, 1.42 Threonine, 1.63	37.6	3.2	0.85
Choline, 14.2 Cystine, 14.2 Threonine, 16.3	49.7	4.9	0.85

[a] Initial pH 5.0.

Cystine added at the higher level improved the yield but did not affect the percentage of methionine. As the increased weight of cells, 52.5 mg. per 50 ml., is more than 3.5 times the weight of the added cystine, the cystine must have acted in a specific way rather than merely as a source of additional carbon. Olson and Johnson (*19*) have noted such an effect for asparagine in devising their medium. Threonine and choline were without any effect on yield or methionine content. This may mean either that the synthetic pathway established in *Neurospora* mutants does not operate in this strain of *S. cerevisiae*, or that the upper limit of methionine synthesis had already been reached.

It was thought that addition of methionine to the synthetic medium might promote methionine synthesis, so *Rh. gracilis*, chosen because of its ability in this respect, was grown in synthetic medium with 1 mg. of added methionine per 50 ml. Ninety-five per cent of the added methionine was used, but the methionine content of the yeast, 1.1%, was the same as when grown without addition of methionine. Apparently *Rh. gracilis* does not take up methionine directly from the medium.

Summary

Optimum conditions for the liberation of methionine from yeast by acid hydrolysis were studied. The assay of methionine and cystine in yeast hydrolyzates with *Leuconostoc citrovorum* 8081 was reproducible and confirmed by chemical analysis. The methionine content of seven commercial yeasts varied from 0.48 to 0.75%.

Twenty yeasts, representing various species and genera, were grown on Hawaiian molasses medium. The yields ranged from 50 to 83% on the basis of sugar fermented. The high yields were probably due to utilization of nonsugar carbon of the corn steep added to the medium.

The methionine content of these yeasts varied from 0.17% for *E. fibuliger* to 1.0% *Rh. gracilis*. Cystine ranged from 0.19 for *D. matruchoti* to 0.58% for *H. saturans*. *C. zeylanoides*, *M. cerevisiae*, and *Rh. gracilis* contained more methionine and gave higher yields than *S. cerevisiae* or *T. utilis*.

Addition of ammonium salts to a beet molasses medium up to 0.045% of additional nitrogen increased the yield of yeast and the percentage of nitrogen in the cells up to a certain limit, but the methionine content remained constant. When the ammonium salts were doubled, the nitrogen content continued to increase but the methionine percentage and yield of yeast dropped. Presumably up to a certain limit the added nitrogen is converted to protein of fixed methionine content. Magnesium salts in combination with the ammonium salts further increased the yield but effected no change in the percentage of methionine.

Eleven yeasts were grown on synthetic medium. The yields were lower than those obtained from Hawaiian molasses medium. Methionine contents of two of these yeasts were the same as on molasses medium, three were lower, and six were higher.

The methionine content of *S. cerevisiae* F-53 was not increased by addition of cystine, threonine, and choline to the synthetic medium. Cystine, however, greatly increased the yield, apparently in a specific manner. Addition of methionine to the synthetic medium did not increase the methionine content of *Rh. gracilis*.

References

(1) Agarwal, P. N., and Peterson, W. H., *Arch. Biochem.*, **20,** 59 (1949).
(2) Agarwal, P. N., Singh, K., King, P. S., and Peterson, W. H., *Ibid.*, **14,** 105 (1947).
(3) Axelsson, J., *Kgl. Lantbruksakad. Tidskr.*, **80,** 161 (1941).
(4) Baumgarten, W., Mather, A. N., Stone, L., *Cereal Chem.*, **23,** 135 (1946).
(5) Block, R. J., and Bolling, D., *Arch. Biochem.*, **7,** 313 (1945).
(6) Chang, W. S., and Peterson, W. H., *J. Bact.*, **58,** 33 (1949).
(7) Dunn, C. G., *Wallerstein Lab. Commun.*, **15,** 61 (1951).
(8) Enebo, L., Anderson, L. G., and Lundin, H., *Arch. Biochem.*, **11,** 383 (1946).
(9) Felix, K., and Pentl, I., *Z. physiol. Chem.*, **283,** 128 (1948).
(10) Govco, J. A., and Asenjo, C. F., *J. Nutrition*, **38,** 517 (1949).
(11) Harris, E. E., Hajny, G. J., and Johnson, M. C., *Ind. Eng. Chem.*, **43,** 1593 (1951).
(12) Hiller, A., Plazin, J., and Van Slyke, D. D., *J. Biol. Chem.*, **176,** 1401 (1948).
(13) Hock, A., *Biochem. Z.*, **311,** 385 (1942).
(14) Hock, A., and Fink, H., *Z. physiol. Chem.*, **278,** 136 (1943).
(15) Horn, M. J., Jones, D. B., and Blum, A. E., *J. Biol. Chem.*, **166,** 313, 321 (1946).
(16) Horowitz, N. H., *Ibid.*, **171,** 255 (1947).
(17) Klose, A. A., and Fevold, H. L., *J. Nutrition*, **29,** 421 (1945).
(18) *Nutrition Revs.*, **2,** 180 (1944).
(19) Olson, B. H., and Johnson, M. J., *J. Bact.*, **57,** 235 (1949).
(20) Pan, S. C., Andreasen, A. A., and Kolachov, P., *Arch. Biochem.*, **23,** 419 (1949).
(21) Riesen, W. H., Schweigert, B. S., and Elvehjem, C. A., *J. Biol. Chem.*, **165,** 347 (1946).
(22) Ringrose, R. C., *Poultry Sci.*, **28,** 75 (1949).
(23) Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, **100,** 695 (1933).
(24) Shapiro, S. K., *J. Bact.*, **65,** 310 (1953).
(25) Skinner, C. E., and Muller, A. E., *J. Nutrition*, **19,** 333 (1940).
(26) Smith, R. L., and Schlenk, F., *Arch. Biochem. Biophys.*, **38,** 167 (1951).
(27) Steele, B. F., Sauberlich, H. F., Reynolds, M. S., and Baumann, C. A., *J. Biol. Chem.*, **177,** 533 (1949).
(28) Stokes, J. L., and Gunness, M., *J. Bact.*, **52,** 195 (1946).
(29) Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C., *J. Biol. Chem.*, **160,** 35 (1945).
(30) Sullivan, M. X., and McCarthy, T. E., *Ibid.*, **133,** c (1940).
(31) Teas, H. J., Horowitz, N. H., and Fling, M., *Ibid.*, **172,** 651 (1949).
(32) Temperton, H., and Dudley, F. J., *Harper Adams Utility Poultry J.*, **26,** 172 (1941).

Received for review August 27, 1953. Accepted October 1, 1953. Published with the approval of the director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant-in-aid from the Red Star Yeast and Products Co., Milwaukee, Wis.