HIGH MOLECULAR COMPOUNDS FROM BREWER'S YEAST

I. PROTEINS

by

WOLD. LINDQUIST

Institute of Physical Chemistry, University of Upsala, Upsala (Sweden)

The purpose of this work was to make a survey of yeast substance from the point of view of macromolecular chemistry. The isolation of the main components was attempted, proteins as well as carbohydrates, in order to characterize them by the determination of their molecular constants and other physico-chemical properties.

PART I

In the literature on the subject, interest was chiefly directed upon compounds with enzymic activity. Most of the fermentative enzymes have been isolated in the pure state. VIRTANEN[1,2,3] concluded from own investigations that the yeast does not contain reserve proteins. ROINE[4] extracted low-molecular nitrogenous compounds from yeast, after the latter had been frozen in liquid air. LJUNGDAHL AND SANDEGREN[5] determined the amino-acid composition in extracts of 8% trichloroacetic acid. Yeast nucleic acids were extensively studied by various authors[6,7,8]. STERN *et al.*[9,10] published electrophoretic studies on maceration extracts from brewer's yeast, and salt precipitated fractions. Crystalline yeast proteins were obtained by KUNITZ AND MCDONALD[11].

EXPERIMENTAL AND RESULTS

Materials and Analyses

Swedish bottom yeast, supplied by the Upsala Brewery as a 10% suspension in beer, was strained through a copper gauze, and decanted with running tap water, until an almost white product was obtained. When centrifuged to 25% dryness, the yeast could be stored in the refrigerator for 5–8 days with unimpaired freshness. It was dried in the frozen state in vacuum to preserve it.

Analyses by the micro Kjeldahl method gave an average content of 9.8% nitrogen from dry yeast. Minerals (9.0%) were determined after incineration on a Bunsen burner, fats and lipids (6.2%) by extraction of ground freeze-dried yeast with alcohol, acetone and ether. From these analyses, completed with data accessible in the literature, the following approximate chemical constitution was calculated (Table I).

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TABLE I

CHEMICAL CONSTITUTION OF BREWER'S YEAST

	% from dry yeast	*Basis % N*	*Corresponding N content in % from dry yeast*
Amino acids, peptides and proteins	49	16.0	7.8
Nucleic acids[12]	8	14.2	1.2
Fats and lipids[13]	6	4.5	0.3
Minerals	9	—	—
Carbohydrates (difference)	28	—	0.5*
Total:	100%		9.8%

* A rough approximation of the amount of nitrogen bound in mucopolysaccharides.

Extraction experiments

The yeast was treated using some of the following methods in order to obtain protein extracts.

a. *Extracts from ground freeze-dried yeast.* 20 g of freeze-dried yeast were ground in a stainless-steel ball mill from 15 min to 24 h, under partial evacuation, and cooling with ice. Portions of 1 g were extracted with 100 ml of 0.2 *M* sodium chloride, buffered with 0.05 *M* phosphate to pH 6.8**, with stirring by a stream of nitrogen. The extracts were centrifuged to clearness, and the nitrogen content in 1 ml of solution determined by the micro Kjeldahl method. The graph in Fig. 1a shows a rapidly increasing nitrogen extractibility in the first stage. After ½ hour the rise became insignificant. At microscopic observation of the extraction residue most of the cells proved to be undamaged with regard to their outer shapes. Apparently the grinding consists of two processes. During the first stage the friction of the dried cells causes the wall permeability to be sufficiently increased so that extraction of the cytoplasm proteins is assured. In the other stage mainly disintegration of the cell structure occurs, whereby additional nitrogenous material becomes slightly extractable. When the samples were ground for more than six hours, the nitrogen content of the extract decreased. A slight decrease was also found during extraction itself*** (Fig. 1b). In the following preparations, in which this method was used, the samples were ground for two hours, and extracted for the same length of time.

The extraction of ground yeast yielded nitrogenous compounds chiefly. The nitrogen content of a dialyzed extract, calculated as proteins on a basis of 16.0% *N*, corresponded to 93.5% of the observed refractive index increment of the extract, assuming an increment of 0.00180 for a 1% protein solution.

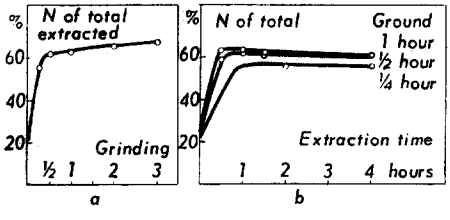

Fig. 1. Nitrogen extractibility of freeze-dried yeast in dependence on grinding time (a), and extraction time (b).

b. *Extracts from fresh yeast.* Fresh yeast of 25% dryness was frozen, and ground

** A solution of this composition is referred to where the designation "Standard buffer" is used in the following.

*** Caused by coagulation of proteins in the phase boundary gas/liquid.

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with CO_2 ice in a mortar. The amount of nitrogen extracted was the same as for ground freeze-dried yeast (Table II).

TABLE II

NITROGEN EXTRACTIBILITY OF FRESH YEAST GROUND WITH CO_2 ICE

g Yeast of 25% dryness	*Grinding time hrs*	*Additions*	*Extracted N in % from total*
3.0	½	None	58
2.5	1	Glass powder	71

c. *Maceration extracts.* By using the method described by LEBEDEV[14], fresh yeast was dried in air of 30° C for two days. 1 g of the resulting brownish granulated substance, extracted with 100 ml standard buffer for two hours at 37° C, showed an extractibility of 47% from total nitrogen. At maximum 70–80% from total nitrogen were extracted within two days.

d. *Extracts from unground freeze-dried yeast.* Generally the nitrogen extractibility of unground freeze-dried yeast with water, or neutral buffers, was but slightly greater than that obtained with 4% trichloracetic acid. Some preparations showed variations in the nitrogen extractibility from 21 to 43% from total. The contents of dialyzable nitrogenous compounds was found to be constant (Table III). On dialysis of the above extracts against distilled water no precipitation was produced.

Splitting of yeast proteins in solutions

The protein contents of yeast extracts decreased continuously by the action of proteolytic enzymes, as was shown by nitrogen determinations during dialysis. A decrease of about 5% a day at 4° C, and of 10% at 20° C, respectively, was determined at pH 6.8, both in crude extracts, and in pure protein fractions. Mercurium chloride at a concentration of 0.1% inhibited the splitting to 85%. Because of its interaction with proteins, however, it could not be used for stabilization. Sodium bromate, or fluoride, respectively, had no influence.

Sedimentation measurements

The obtained extracts were dialyzed for two days at 4° C against standard buffer, and analyzed in the Svedberg oil turbine ultracentrifuge[15]. A rotation radius of 6.5 cm, and a rotor speed of 60,000 r.p.m. were used, whereby a centrifugal force of some 300,000 times gravity was attained. The measurements were made by the LAMM[16] Scale method, and the sedimentation constants s_{20} expressed in units of 10^{-13} cm sec^{-1} $dyne^{-1}$ (Svedberg units, *S*).

Runs with extracts from ground yeast, freeze-dried as well as fresh, and with maceration extracts, showed in accordance two dominating compounds, with sedimentation constants of 3.9 *S* and 6.2 *S*, respectively. Three further compounds of 3.9, 5.9 and 8.1 *S* respectively were indicated. A typical sedimentation diagram is reproduced in Fig. 2b. Minor compounds with sedimentation constants about 7, 9, 13, 24 and 40 *S*, respectively, have often been found. Some of these are detailed in Part II of this paper (Carbohydrates).

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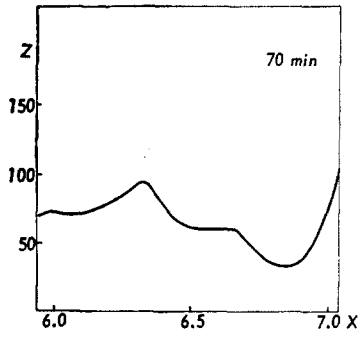
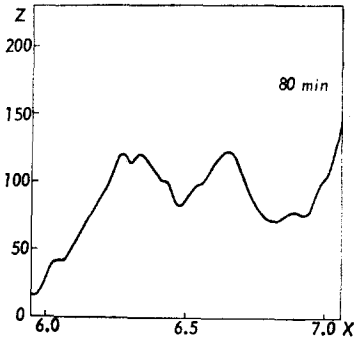
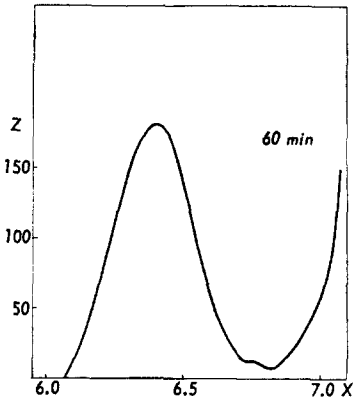


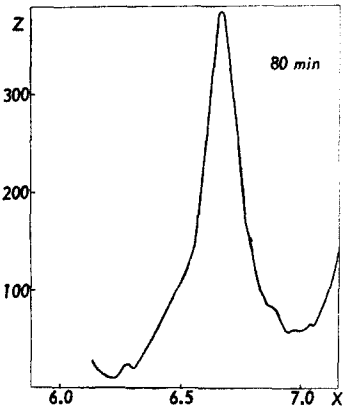
a. Extract from unground freeze-dried yeast. About 30 % from total N extracted.



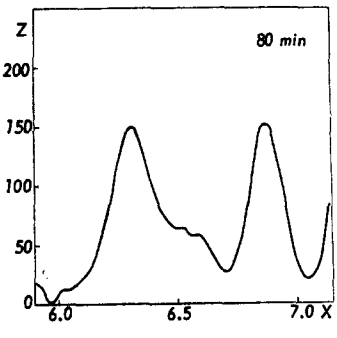
b. Extract from freeze-dried yeast, ground for 2 ½ hours. About 60 % from total N extracted.



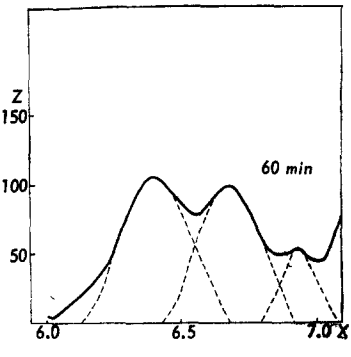
c. Fraction I.



d. Fraction II.



e. Fraction III + IV + V.



f. Maceration extract according to Lebedev.

Fig. 2. Sedimentation diagrams for yeast extracts and protein fractions.

Extracts from unground freeze-dried yeast contained up to an extractibility of 30% prevailingly disperse low-molecular compounds. The first high-molecular compound, which at rising cell wall permeability became extractable, was that of $s_{20} = 6.2\ S$. At an extractibility of 43% from total nitrogen occurred the well-defined high-molecular proteins, which are to be found in extracts from ground yeast (Table III).

When the yeast was ground for an exceedingly long time, which resulted in a decrease of nitrogen extractibility, sedimentation analysis showed the dominance of the compound $s_{20} = 3.1\ S$. This was observable both after grinding of fresh yeast with

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TABLE III

EXTRACTS FROM DIFFERENT SAMPLES OF UNGROUND FREEZE-DRIED YEAST

Extracted nitrogen % from total	*Dialyzable nitrogen % from total*	*Sedimentation constants Svedberg units*
21	18	0.9 (disperse)
30	17	1.2 (disperse)
43	17	3.2 and 6.2

With 4% trichloracetic acid extractable 15% from total N.

CO_2 ice in a mortar, or of freeze-dried yeast in a ball mill, and is presumably the result of frictional denaturation of the compound $s_{20} = 6.2\ S$.

Salt fractionation of yeast protein extracts

By choosing adequate ammonium sulphate concentrations, fractions were precipitated from crude extracts, each of which consisting of a more or less monodisperse compound, with regard to sedimentation properties.

Fraction I was obtained by adding to a crude extract an equal volume of saturated ammonium sulphate, the pH of which was adjusted to 7. It comprehended the first of both main protein compounds, of $s_{20} = 3.1\ S$ (Fig. 2c). The greater part of the fraction became denatured, when it was dissolved in standard buffer, and reprecipitated. This occurred already at the first precipitation, when this was accomplished by dialysis against a 50% saturated ammonium sulphate solution, at pH 7. Apparently, the proteins of this fraction need the presence of the dialyzable compounds of the cytoplasm for stability.

The subsequent fractions were obtained by dialysis against ammonium sulphate solutions of 70, 75, 85, and 100% saturation. For example, the supernatant solution from fraction I was transferred into a cellophane bag, and dialyzed against a 70% saturated ammonium sulphate solution, pH 7. The abundant precipitate produced (Fraction II) comprehended the second of the main compounds, of $s_{20} = 6.2\ S$. In Fig. 2d is given the sedimentation diagram of a 1% solution after 80 min of sedimentation. The diagram indicates the containing of small amounts of other compounds. Differential precipitation experiments, carried out in analogy to the method of DERRIEN[17], made precipitation in four steps evident (Fig. 3). Individual results of the determination of sedimentation constants for the Protein Fractions I–V are evident from Fig. 4. By extrapolation to zero concentration, the values were found, given in Table IV.

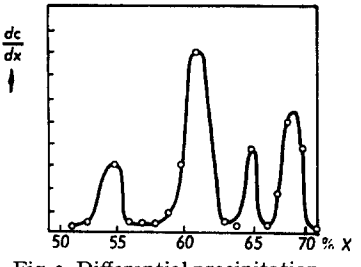

Fig. 3. Differential precipitation curve for protein fraction II. x = degree of saturation with ammonium sulphate; $\frac{dc}{dx}$ = decrease in protein concentration at the rise in saturation 1%.

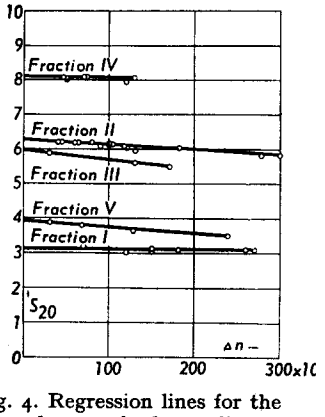

Fig. 4. Regression lines for the dependence of the sedimentation constants for protein fractions I — V on concentration, expressed by the refractive index increment (Δn) of their solutions.

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TABLE IV

YEAST PROTEIN FRACTIONS

	Range of precipitation (% saturation ammonium sulphate)	*Sedimentation constants at zero concentration*
I	40 – 50	3.1
II	50 – 70	6.2
III	70 – 75	5.9
IV	75 – 85	8.1
V	85 –100	3.9

Up to 40% saturation no significant precipitation occurred. Fraction III was a smaller one. Spectrophotometric measurements showed that a great deal of the RNA (yeast nucleic acid) content of the extract was precipitated with fraction I.

Fractionation attempts by alcohol precipitation at 0° C resulted in denaturation of the proteins. Only the compound $s_{20} = 8.1\ S$ was reproducible at an alcohol concentration of 38–40% (v/v).

Diffusion measurements

The diffusion measurements were made with the CLAESSON[18] diffusion cell, by means of the method worked out by LAMM[16]. From the experimental data, the diffusion constant was calculated by two methods using the formulae

$$D_m = \frac{2t}{\delta^2}, \text{ and } D_A = \frac{1}{4\pi t} \cdot \frac{A^2}{H^2},$$

where D_m means the diffusion constant according to the moment method, δ the standard deviation, D_A the diffusion constant according to the area method, t the time, A the area, and H the maximal ordinate or height of the diffusion curve.

As a model substance the protein fraction II was chosen, and the diffusion constant at 20° C determined for two preparations, which were obtained by precipitation three times.

TABLE V

DIFFUSION CONSTANTS FOR FRACTION II AT 20° C, IN UNITS OF 10^{-7} $cm^2 \cdot sec^{-1}$

Concentration: 0.2 %. Solvent: 0.2 *M* sodium chloride + 0.05 *M* phosphate, pH 6.8.

	1	2	*Average*
D_m	7.03	6.74	—
D_A	5.96	5.82	5.89
D_m/D_A	1.18	1.16	1.17

Generally the ratio D_m/D_A can be taken as a measure of the polydispersity of a fraction[19]. In this case, however, the polydispersity was partly caused by proteolytic splitting under the diffusion experiment itself. On dialysis of a part of the solutions against the buffer at 20° C, a decrease in the nitrogen content of 11% and 8% a day, respectively, was determined. Therefore the values for D_A were regarded to come nearer

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the diffusion constant of the pure fraction, and the values for D_m, which were higher, were excluded.

The partial specific volume V was determined by Prof. C. DRUCKER to be 0.744 at 20° C. The data found were intrapolated into the formulae of SVEDBERG[15]

$$M = \frac{R\,T\,s}{D(1-V\varrho)}, \text{ and } f/f_o = 10^{-8}.\left(\frac{1-V\varrho}{D^2\,s\,V}\right)^{1/3}$$

where R means the gas constant ($8.313 \cdot 10^7$ erg $°C^{-1}mole^{-1}$), T the temperature in the absolute scale (293.2° C), ϱ the density of water at 20° C (0.998), s the sedimentation constant, and D the diffusion constant at 20° C. Thus for fraction II an average molecular weight of 98,000, and a frictional coefficient f/f_o of 1.17 were calculated.

Electrophoretic measurements

Electrophoretic experiments on yeast extracts and protein fractions were made by the moving boundaries method of TISELIUS and co-workers[20]. Buffers of $\mu = 0.2$ were employed, consisting of 0.1 M sodium fluoride and buffering substances. No optical anomalies appeared in the system. Phosphate buffer of pH 7 was used in most of the experiments, in order to ascertain the reproducibility of the determinations. According to the nomenclature introduced by TISELIUS, which was used by STERN in yeast investigations, the compounds of the highest migration were indicated with α_1, α_2, . . ., those of medium migration with β_1, β_2, . . ., and the compounds of lowest migration with γ_1, γ_2, . . .

TABLE VI

Mobility $\cdot 10^5$ at pH 7.2, and ionic strength 0:2.

Preparation		γ_1	γ_2	γ_3	β_1	β_2	β_3	α_1	α_2
1. Extract from ground freeze-dried yeast	A	+0.2	1.27	1.58	2.57	3.07	3.75	4.61	13.3
	D	—	1.13	—	2.19	—	3.85	—	13.0
2. Maceration extract according to Lebedev	A	+0.2	—	1.56	2.91	—	3.41	5.08	13.0
	D	0	0.90	1.75	2.18	—	—	—	11.0
3. Extract from fresh yeast ground with CO_2 ice	A	+0.2	0.94	—	2.29	—	4.23	8.00	13.0
	D	—	—	1.60	—	—	3.94	6.06	11.4
4. Protein fraction I	A	+0.3	1.29	1.73	2.92	—	3.53	4.9	13.3
	D	0	1.00	—	2.30	—	3.75	—	11.1
5. Protein fraction II	A	—	1.14	—	—	—	—	—	—
	D	—	1.00	—	—	—	—	—	—
6. Protein fraction III + IV + V	A	+0.1	—	1.63	2.51	3.21	3.97	4.53	12.7
	D	0	—	1.57	2.00	2.70	3.49	4.06	11.7
7. Protein fraction IV	A	—	—	—	2.50	—	—	—	—
	D	—	—	—	2.30	—	—	—	—
Average:		+0.2	1.08	1.63	2.42	2.99	3.77	4.64 and 7.03	12.5

In this table, negative mobility is understood, except where + is placed before the number. The α_1-compound alone showed a difference in the mobility with extracts from fresh, or dried yeast, respectively.

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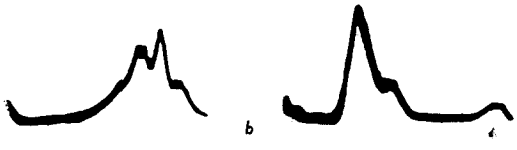
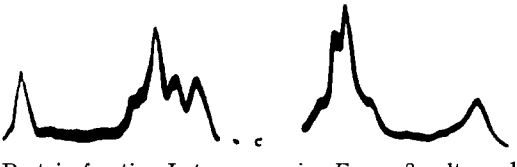
The α_2- and γ_1-components were isolated by means of electrophoretic separation. For the former the sedimentation constant 2.5 S was determined. Light absorption at 260 mμ, and phosphorus content were determined relatively to 1 mg of nitrogen. The determinations gave higher values with the separated fraction than with the original solution, by which a content of nucleic acids is indicated. For the γ_1-compound sedimentation constants about 3, 7, 8.5, and 13 S were found, which corresponded to values determined for some non-protein compounds. Typical electrophoretic patterns are reproduced in Fig. 5.

a. Extract from unground freeze-dried yeast. 43 % fr. total N extracted.
t = 232 min; F = 5.4 volt cm^{-1}.



b. Extract from ground freeze-dried yeast. 55 % fr. total N extracted.
t = 235 min; F = 5.1 volt cm^{-1}; θ = 74°.



c. Protein fraction I. t = 130 min; F = 4.8 volt cm^{-1}.

d. Protein fraction II.
t = 170 min; F = 4.9 volt cm^{-1}; θ = 60°.

e. Protein fraction III + IV + V.
t = 580 min; F = 4.0 volt cm^{-1};
θ = 85°.

Fig. 5. Electrophoretic patterns at pH 7.2, ionic strength 0.2.
t = time of electrophoresis; F = potential gradient.
Left: ascending boundaries; right: descending boundaries.

It was tried to determine the isoelectric point for protein fraction II. At pH 5.3 a considerable irreversible precipitation occurred, and the remaining part of the fraction tended to separate at electrophoresis into three components with isoelectric points 4.85, 5.0, and 5.15.

A communication of the results of this work was given by BROHULT, LINDQUIST AND SANDEGREN, in ***European Brewery Convention***, 2 (1951) 188, from which are reproduced some of the graphs with the editors' permission.

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DISCUSSIONS

It is known that at drying of yeast at 30–40° C according to the LEBEDEV[14] method, autolytic alterations are produced in the cell wall, which lead to a complete extractibility of the soluble cell contents (Fig. 2f). From Table III is concluded the occurrence of intermediate stages of permeability, at which fractional extraction of the compounds of lower molecular size is possible. These experiments made evident the following size distribution of the nitrogenous compounds: amino acids and dialyzable peptides, disperse proteins of lower molecular size, and high-molecular proteins, among which are predominant well-defined compounds with sedimentation constants of 3.1, 3.9, 5.9, 6.2, and 8.1 *S*. Occurrence of dialyzable nucleotides (co-enzymes), and of macromolecular RNA was shown by light absorption measurements, of the latter also by electrophoretic experiments. The extracts contained carbohydrates to a relatively small extent. For the compound 6.2 *S* by the extraction experiments a peripheric location in the cell is made probable.

By the produced fractionation scheme was achieved a separation of the proteins into five fractions, each being of more or less uniform sedimentation. Generally the fractions were electrophoretically not homogeneous. For example, for fraction I the containing of seven main components was made evident. However, the fractions II and IV, respectively, were found to be of rather uniform migration at pH 7.2.

Between the determinations of the electrophoretic mobility of this work and the work of STERN *et al.*[9,10], are observable differences, which are explained by the low nitrogen extractibility at the preparations of the latter authors. Some compounds were altogether absent, as for example yeast nucleic acids.

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SUMMARY

A study has been made on the proteins of yeast.

Extracts from ground yeast contained nitrogenous substance chiefly, about 60% from total nitrogen. A considerable part of the extracted proteins was shown to consist of well-defined compounds with the sedimentation constants 3.1, 3.9, 5.9, 6.2, and 8.1 *S* respectively. For one of these compounds (6.2 *S*), from sedimentation and diffusion measurements the molecular weight 98,000 was calculated. Electrophoretic experiments are recorded.

RÉSUMÉ

Nous avons fait l'étude des protéines de la levure.

Des extraits de levure moulue contenaient surtout de la matière azotée, environ 60% de l'azote total. Nous avons montré qu'une partie considérable des protéines extraites étaient constituées de composés bien définis ayant respectivement les constantes de sédimentation suivantes, 3.1, 3.9, 5.9, 6.2 et 8.1 *S*. Pour l'un de ces composés (6.2 *S*) nous avons calculé un poids moléculaire de 98,000 à partir de mesures de sédimentation et de diffusion. Des expériences d'électrophorèse sont rapportées.

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ZUSAMMENFASSUNG

Es wurden die Proteine der Hefe untersucht.

Extrakte von gemahlener Hefe enthielten hauptsächlich stickstoffhaltige Substanz, ungefähr 60% des Gesamtstickstoffs. Es wurde gezeigt, dass ein beträchtlicher Teil der extrahierten Proteine aus wohl definierten Verbindungen mit den Sedimentationskonstanten 3.1 bzw. 3.9, 5.9, 6.2 und 8.1 *S* bestand. Für eine dieser Verbindungen (6.2 *S*) wurde aus Sedimentations- und Diffusionsmessungen das Molekulargewicht 98,000 berechnet. Elektrophoretische Versuche werden berichtet.

REFERENCES

1 A. J. VIRTANEN, *Suomen Kemistilehti*, 15 B (1942) 22.
2 A. J. VIRTANEN AND J. DE LEY, *Arch. Biochem.*, 16 (1948) 169.
3 A. J. VIRTANEN AND ULLA WINKLER, *Acta Chem. Scand.*, 3 (1949) 272.
4 P. ROINE, *Suomen Kemistilehti*, 19 B (1946) 40.
5 L. LJUNGDAHL AND E. SANDEGREN, *Acta Chem. Scand.*, 4 (1950) 1150.
6 R. FALCONER, J. M. GULLAND, G. J. HOBDAY AND E. M. JACKSON, *J. Chem. Soc.*, (1939) 907.
7 W. E. FLETCHER, J. M. GULLAND, D. O. JORDAN AND H. E. DIBBEN, *J. Chem. Soc.*, (1944) 30.
8 G. SCHRAMM, G. BERGOLD AND H. FRAMMERSFELD, *Z. Naturforschung*, 1 (1946) 328.
9 K. G. STERN, *J. Biol. Chem.*, 152 (1944) 345.
10 K. G. STERN, A. SCHEIN AND J. S. WALLERSTEIN, *J. Biol. Chem.*, 166 (1946) 59.
11 M. KUNITZ AND M. R. MCDONALD, *Labor. of the Rockefeller Institute of Medical Research*, 1945.
12 H. VON EULER AND L. HAHN, *Arkiv Kemi, Mineral. Geol.*, 25 A (1947) no. 11.
13 K. DIRR AND P. DECKER, *Biochem. Z.*, 316 (1943) 239.
14 A. VON LEBEDEV, *Z. physiol. Chem.*, 73 (1911) 447.
15 T. SVEDBERG AND K. O. PEDERSEN, *The Ultracentrifuge*, Oxford 1940.
16 O. LAMM, *Nova Acta Regiae Sci. Upsaliensis*, IV, 10 (1937) no. 6.
17 Y. DERRIEN, *Trans. soc. chim. biol.*, 26 (1944) 1091; *Svensk Kem. Tid.*, 59 (1947) 159.
18 S. CLAESSON, *Nature*, 158 (1946) 835.
19 N. GRALÉN, Inaugural Dissertation, Uppsala 1944.
20 H. SVENSSON, *Arkiv Kemi, Mineral. Geol.*, 22 A (1946) No 10.

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