HIGH MOLECULAR COMPOUNDS FROM BREWER'S YEAST

II. CARBOHYDRATES

by

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As main carbohydrate constituents of yeast are known: 1. Yeast glucan[1-4], the resistant building material of the cell wall, 2. Yeast mannan[1,5,6], soluble polymeric carbohydrate of the cell wall, 3. Glycogen[3,7,8], and trehalose, reserve carbohydrates stored in the cytoplasm, 4. D-Ribose, occurring as constituent of yeast nucleic acids, and 5. Yeast mucilage[9-11], the occurrence of which is not yet properly elucidated. The extraction of a carbohydrate by weak sodium bicarbonate solutions has been described by VENDRELY AND SARCIRON[12]. Microchemical, electron microscope and X-ray studies on the composition and structure of the cell wall have been made by HOUWINK, KREGER AND ROELOFSEN[13], investigations on fractionation and microdetermination of yeast carbohydrates by TREVELYAN AND HARRISON[14].

In this work the principal carbohydrates were investigated by means of sedimentation analysis, diffusion measurements, and electrophoresis.

EXPERIMENTAL AND RESULTS

For the investigations fresh bottom yeast was used from Upsala Bayerska Bryggeri A.B. The yeast was cleaned before using it by sifting and decantation with running tap water. In some cases parallel experiments were made with top yeast from A.B. Gronwall's Bryggeri, Stockholm.

Yeast glucan. At treatment with boiling 3 % sodium hydroxide according to the method of SALKOWSKI[1], all of the yeast substance was dissolved, but yeast glucan. 8.6–9.2 % from dry yeast were obtained, at nitrogen contents of 0.5–1.2 %.

Yeast mannan. From the alkaline extract yeast mannan was precipitated with Fehling's solution. The precipitate was dissolved in dilute hydrochloric acid, and reprecipitated three times with an equal volume of alcohol. The substance was washed with alcohol, dried, and weighed in the centrifuge tube. 5.3 % were obtained from dry yeast. Almost the same amount (4.8 %) was obtained from ground yeast, after the soluble cell contents had been extracted with cold water. Elementary analysis showed the following constitution: 43.5 % C, 6.48 % H, 1.0 % N, 0.2 % P, 0.03 % minerals, and 48.7 % C*.

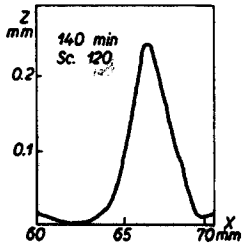

Fig. 1.
Yeast mannan

Sedimentation analysis demonstrated a high monodispersity of the substance (Fig. 1). The experimental technique was the

* According to analysis, made by the Micro-analytical Laboratory, Dept. of Medicine, Upsala University.

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same as in Part I of this work. The construction and operation of the ultracentrifuge employed is described in the monograph of SVEDBERG AND PEDERSEN[15]. Results of determinations of the sedimentation constants for different preparations are given inthe graph Fig. 7. By extrapolation to zero concentration the value $s^0_{20} = 4.1\ S$ was obtained. Determinations of the diffusion constant according to the Lamm Scale method[16] were made, using the diffusion cell designed by CLAESSON[17]. From the results in Table I the mean value 3.92 is obtained.

TABLE I

DIFFUSION CONSTANTS OF YEAST MANNAN AT 20° C, IN UNITS OF 10^{-7} $cm^2\ sec^{-1}$

Solvent: 0.1 *M* sodium phosphate, pH 6.8.

	1	*2*	*3*	*4*	*Average*
D_m	3.79	3.66	4.14	4.14	3.93
D_A	3.59	3.97	4.11	3.92	3.90
D_m/D_A	1.06	0.92	1.01	1.06	1.01
Concentration	0.62%	0.42%	0.40%	0.43%	

The partial specific volume at 20° C was determined by Pro . C. DRUCKER as 0.658. From these data, using Svedberg's formula $M = RTs/D(1-V\varrho)$, the molecular weight 74,000 is calculated. The frictional coefficient, according to SVEDBERG[15] expressed by the formula $f/f_0 = 10^{-8}\left(\frac{1-V\varrho}{D^2\,s\,V}\right)^{1/3}$, was calculated to 2.02.

Glycogen. After precipitation of yeast mannan with Fehling's solution, and of yeast nucleic acids with glacial acetic acid, from the supernatant solution by precipitation with alcohol (method of LING *et al.*[7]) a substance was obtained, which with IKI gave the colour reaction upon glycogen. Upon sedimentation analysis of the preparations generally no compounds were observed of the molecular weight which is assumed for glycogen. One experiment however yielded a monodisperse compound of $s_{20} \sim 65\ S$.

Yeast nucleic acids. Macromolecular yeast nucleic acids were extracted from intact yeast by a method which is detailed below.

Differential extraction of cell wall constituents. Several compounds of predominantly carbohydrate character were successively extracted by means of following treatments:

a. *Agitation at room temperature.* 4 l of a thick yeast suspension in water were stirred with a laboratory stirrer at room temperature (18° C). After two hours the yeast was centrifuged off, and the stirring repeated. The extracts obtained were concentrated by ultrafiltration, and precipitated by addition of an equal volume of alcohol, that preferably contained 1% hydrochloric acid. A third extract yielded no precipitate, by which was shown that the extraction was complete. The joint precipitates were dissolved in water. After reprecipitating twice, washing with alcohol and ether, and drying, the yield was 0.3% from dry yeast. The substance was called *cebrosan**.

Sedimentation analysis showed that the substance was monodisperse (Fig. 2a). For the sedimentation constant at infinite dilution the value 9.75 *S* was found. About the same value was determined upon crude extracts, the substance consequently being resistant against the treatment of purifying.

Determinations of the diffusion constant gave the mean value 1.96 (Table II).

* In a preceding communication[21] indicated with the symbol K_2.

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TABLE II

DIFFUSION CONSTANTS OF CEBROSAN AT 20° C, IN UNITS OF 10^{-7} cm^2 sec^{-1}

Solvent: 0.05 *M* sodium phosphate pH 6.8

	1	2	Average
D_m	1.89	2.05	1.97
D_A	1.90	1.98	1.94
D_m/D_A	1.00	1.03	1.02
Concentration	0.28%	0.40%	—

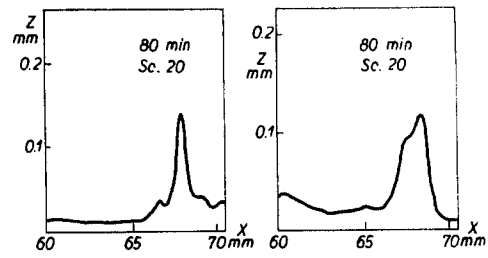

Fig. 2. a. Cebrosan; b. Cebrosan, split

The partial specific volume was determined by Prof. C. DRUCKER to 0.61 at 20° C. From these data, a molecular weight of 340,000 and a frictional coefficient of 2.47 were calculated.

The specific rotation $[\alpha]_{20}^{D}$ was determined to +84°. On hydrolysis in 0.2 *N* hydrochloric acid at 90° C the rotation decreased, and reached the stable value of + 10°*. The hydrolysate readily formed a hydrazone with phenylhydrazine.

Elementary analysis of the purified substance showed 35.5% C, 5.6% H, 1.2% N, 0.2% P, 0.70% minerals, and 56.8% O**. Titration analysis showed a consumption of 1.5 ml of 0.1 *M* sodium hydroxide per gram of the substance.

The substance was precipitable with alcohol between 40 and 45% (v/v). For precipitation was necessary the presence of electrolytes. With Fehling's solution a precipitate was formed similar to that obtained from yeast mannan. The substance was gradually split under dialysis, as was shown by sedimentation analysis (Fig. 2b). Boiling with 2% sodium hydroxide for ½ hour transferred it into a monodisperse compound of $s_{20} = 5.7\ S$.

Three further preparation methods for cebrosan are known. The substance, obtainable according to the method of VENDRELY AND SARCIRON[13]*** was found to be identical with it. JENSEN[18] found that at plasmolysis of fresh bottom yeast with solid sodium chloride a compound of $s_{20} \sim 9$ was extracted. The author showed it to be the same conpound as cebrosan. When yeast of 25% dryness was frozen, in the liquor, which separated when it was allowed to thaw, cebrosan was found as single high molecular compound.

b. *Agitation at 45° C.* The yeast remaining from treatment a. was resuspended in water, and the stirring continued for one hour at a temperature of 30–45° C. Sedimentation analysis of the extract demonstrated the presence of another monodisperse compound, for which the sedimentation constant at infinite dilution was determined to 8.2 *S* (Fig. 7). The substance was called *mycetin*. Its proportion was estimated to 1% from dry yeast. Crude preparations contained about 6% nitrogen and 0.6–1% phosphorus. In its properties it differed from cebrosan. It gave a strong reaction for glucosamine with Ehrlich's reagent, and was not precipitable with Fehling's solution. By addition of an equal volume of alcohol a precipitate was produced, which was completely soluble in water. After some hours of contact with the alcoholic solution, however, the precipi-

* *cf.* $[\alpha]_{20}^{D}$ for mannose: + 14.5°.

** Analysis made by the Micro-analytical Laboratory, Dept. of Medicine, Upsala University.

*** BERTHE DELAPORTE was the first to extract yeast with sodium bicarbonate solutions (*Rev. gén. bot.*, 51 (1939) 449).

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tate became insoluble. For precipitation the presence of electrolytes was necessary. Adpition of alcohol, containing 1% hydrochloric acid, produced no precipitation. From the solution, however, a slight precipitate was obtained at neutralization with alcoholic ammonia, which showed a sedimentation constant about 1.8 *S*. Also at dialysis against a neutral buffer mycetin was slowly decomposed.

c. *High speed agitation.* At agitation of fresh yeast in a Turmix blendor, under addition of some water, or solid sodium chloride, macromolecular yeast nucleic acids were extracted, besides cebrosan and mycetin. About one hour of agitation was needed. As the temperature was quickly rising to 50° C, the vessel had to be cooled. By exchanging the solution during the experiment, and analyzing the extracts, it was shown that cebrosan, mycetin and yeast nucleic acids were extracted successively in the named order. No cell proteins were extracted by this treatment, as addition of trichloroacetic acid produced no coagulation. Sedimentation diagrams for extracts of that kind are given in Fig. 3a and b. A pure preparation of yeast nucleic acids was obtained in the following way: The crude Turmix extract was precipitated with an equal volume of alcohol that contained 1% hydrochloric acid. The precipitate was centrifuged off, and thoroughly washed with water. The residue was dissolved in 0.1 *M* sodium carbonate,

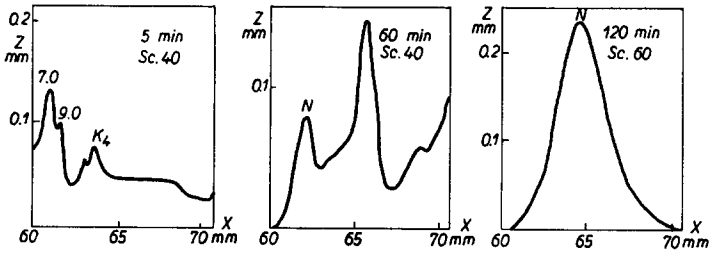

Fig. 3. a. Turmix extract; b. Turmix axtract; c. Yeast nucleic acids

and dialyzed against a neutral buffer. On sedimentation analysis a rather monodisperse compound was evident, of $s_{20} = 2.55$ *S* (Fig. 3c). Light absorption measurements showed a strong maximum at 2600 A. Analysis made in this laboratory gave 14.2% nitrogen and 7.0% phosphorus. More than 1% were obtained from dry yeast.

The above preparations were often accompanied by two heavier compounds to minor extent, denoted K_3 and K_4, with sedimentation constants about 12 and 16 *S*, respectively. They were isolated from a Turmix extract by electrophoretic separation (boundary of slowest migration, Fig. 9b), and proved to be carbohydrates of low solubility, which gave no colour reaction with iodine.

d. *Slight autolysis.* When yeast of 25% dryness was stored at 30–40° C for two days, it became liquified through excretion of water from the inner of the cells. The yeast was subsequently centrifuged off, and the extract dialyzed against distilled water. At suitable conditions of autolysis, the extracts were practically free from proteins, which was ascertained by testing with trichloroacetic acid, and contained high molecular carbohydrates in amounts exceeding 6% from dry yeast. Dialyzed extracts contained 5 to 7% nitrogen upon dry substance. Sedimentation diagrams for extracts of that kind both from bottom and top yeast (Fig. 4 and 5) made evident the occurrence of four main compounds, with sedimentation constants 2.8, 4.0, 5.8, and 7.2 *S*, respectively. The compound 5.8 *S* was isolated by fractionation with alcohol (Compound K_5, Fig. 4c).

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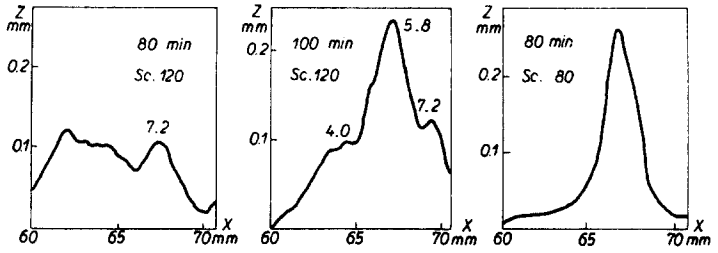

Fig. 4. a, b. Slight autolysis of bottom yeast; c. Compound K_5

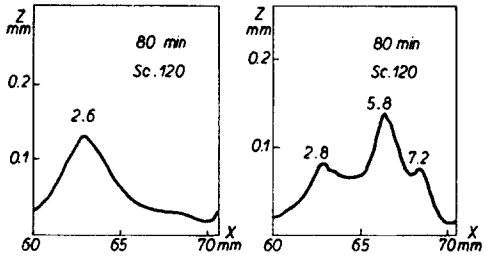

Fig. 5. a, b. Slight autolysis of top yeast

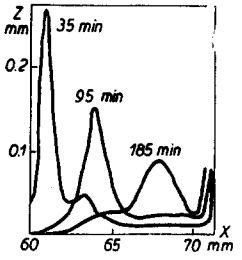

Fig. 6. Basic carbohydrate

A carbohydrate of basic properties was isolated from the extracts in following manner. Alcohol was added up to the double volume, and pH made 3–4 with trichloroacetic acid. The produced precipitate was discarded. Upon addition of alcoholic ammonia, from the supernatant solution a precipitate was obtained, which was dissolved in trichloroacetic acid, and dialyzed against a neutral buffer. A crude preparation contained 4.7% nitrogen. In Fig. 6 is given the sedimentation diagram of the substance at different stages of sedimentation. The sedimentation constant at infinite dilution was determined as 3.6 C (Fig. 7).

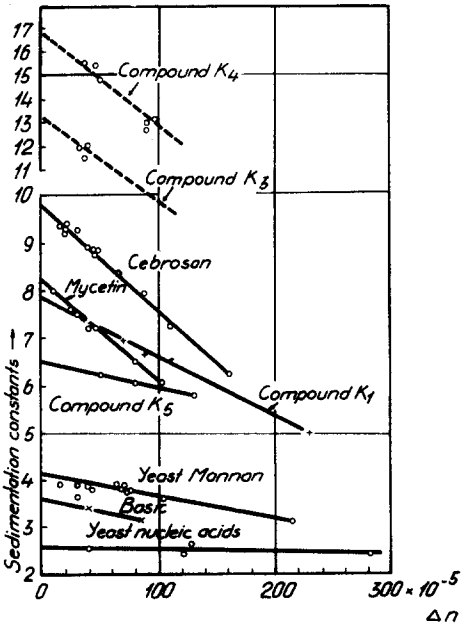

Fig. 7. Regression lines for the dependence of the sedimentation constants of the non-protein compounds on concentration, expressed by the refractive index increment (Δn) of their solutions.

Carbohydrates in extracts from ground yeast. After precipitation of the proteins with ammonium sulphate at saturation, the supernatant solution contained a main compound of $s^0_{20} = 7.9\,S$ (Compound K_1, Fig. 7). It contained 1.2% nitrogen and 0.2% phosphorus. If the proteins were precipitated by acidification, or heating, in the solution were found still other compounds, of $s^0_{20} = 2.0$ and 5.1 S, respectively.

Total autolysis. When yeast of 25% dryness was stored for about one month at 30° C, the cell structure was destroyed by autolysis, and the proteins split. From the dialyzed extract by precipitation with alcohol were obtained 5–6% carbohydrates from dry yeast, for which the

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following properties were determined: mean sedimentation constant at infinite dilution: 3.3 S, mean mobility at pH 7.2: $-1.1 \cdot 10^{-5}$, nitrogen content: 1.2%, and specific rotation $[\alpha]_{20}^{D}$: $+82°$.

ELECTROPHORETIC MEASUREMENTS

The compounds obtained were investigated in the Tiselius apparatus[19], with the Philpot-Svensson optical system[20]. Citrate, acetate, phosphate and barbiturate (Veronal) buffers were used, covering the pH interval from 2 to 11. The compounds showed well-defined boundaries at electrophoresis. In the following their electrophoretic behaviour is briefly described.

Yeast mannan was found to be of anodic migration at all pH values, the mobility slowly rising from zero. In some experiments was observed a minor second boundary, which often showed cathodic migration.

Cebrosan showed up to pH 7 the same mobility as yeast mannan. The mobility declined continuously on the alkaline side.

TABLE III

MOBILITY × 10^5

Yeast mannan				*Cebrosan*				*Mycetin (Ionic strength* 0.1		
pH	*Buffer*	μ	$u \times 10^5$	*pH*	*Buffer*	μ	$u \times 10^5$	*pH*	*Buffer*	$u \times 10^5$
3.0	Citrate	0.1	−0.46 and −0.12	2.4	Citrate	0.2	−0.32	4.8	Acetate	+1.19
5.1	Acetate	0.1	−0.77	4.6	Citrate	0.2	−0.71	5.1	Acetate	−0.05
7.2	Phosphate	0.2	−1.16	6.0	Phosphate	0.2	−0.98	5.6	Acetate	−0.36
7.8	Phosphate	0.2	−1.2	7.1	Phosphate	0.2	−1.2	6.1	Acetate	−0.75
9.3	Veronal	0.1	−1.38 and +0.19	7.2	Phosphate	0.2	−1.3	7.1	Phosphate	−1.3
9.5	Veronal	0.1	−1.46 and +0.28	7.8	Phosphate	0.2	−1.2 and −1.4	8.1	Veronal	−1.53
				9.0	Veronal	0.2	−0.90			
				9.3	Veronal	0.2	−0.90			
				9.7	Veronal	0.2	−0.80			
				10.1	Veronal	0.1	−0.73			

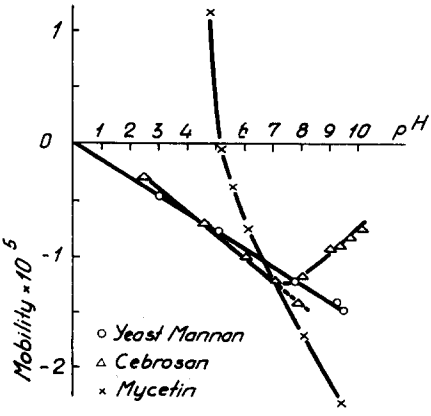

Fig. 8. Relation between pH and electrophoretic mobility for yeast mannan, cebrosan and mycetin.

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For mycetin the isoelectric point 5.1 was determined. Detailed results are given in Table III, and Fig. 8.

The extracts obtained by Turmix-stirring of fresh yeast showed boundaries for yeast nucleic acids, mycetin and cebrosan (which were of the same mobility at pH 7.2), and the compounds K_3 and K_4 (Fig. 9b). The electrophoretic pattern of yeast nucleic acids, isolated from a Turmix extract, is given in Fig. 9c.

The extracts obtained by slight autolysis of bottom yeast showed the occurrence of a compound of cathodic migration in the pH interval 4 to 11. It was separated, and its sedimentation constant determined as 3.8 S. In Fig. 9 d–f are given electrophoretic patterns for extracts, obtained by slight autolysis of top yeast.

A communication about the results of this part was given by the author in *Nature*, 170 (1952) 544.

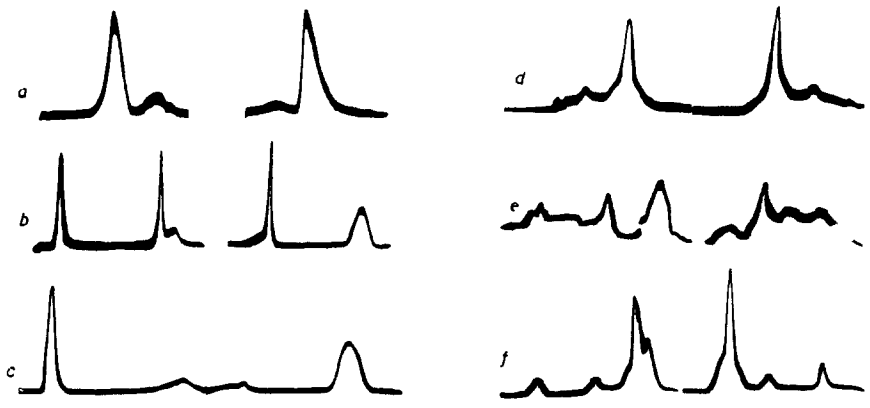

Fig. 9 a–f. Electrophoretic patterns

	pH	μ	*F*	*t*	$u \times 10^5$				
a. Cebrosan	7.2	0.2	4.7	585	— 1.29				
b. Turmix extract	7.2	0.2	4.7	140	— 0.2	1.4	14.4		
c. Yeast nucleic acids	7.2	0.2	4.5	120	—13.9				
d. Top yeast autolysis	7.2	0.1	6.0	122	— 0.83				
e. Top yeast autolysis	7.2	0.1	6.5	260	— 0.77*	2.38	3.52	4.56	15.3
f. Top yeast autolysis	11.2	0.1	6.4	215	+ 1.14	—0.35	5.8	13.5	

μ = ionic strength, F = the potential gradient (volt cm^{-1}), t = the time of electrophoresis (minutes), u = the mobility (cm^2 $volt^{-1}$ sec^{-1}). On the left side in the diagrams the ascending boundaries, on the right — the descending.

Extract d represented the first stage of autolysis, and corresponded to the sedimentation diagram in Fig. 5a, extract e to Fig. 5b. The extract f, obtained at relatively advanced autolysis, had been precipitated with alcohol, and the carbohydrates dissolved in the buffer. The compound of highest mobility represented yeast nucleic acids.

* The compound altered the direction of migration during electrophoresis, showing under the second part of the experiment cathodic migration ($u = +0.45 \times 10^{-5}$).

DISCUSSION OF RESULTS

From the compounds described in this paper chiefly those are interesting, which are obtained from fresh yeast by such treatments, as leave the cell structure intact. It was assumed that they were detached from the cell surface successively by the friction during agitation, by moderate heating, and finally by autolytical processes. Their purification was achieved by fractionation with alcohol, in some cases also by precipitation with specific reagents, as for example Fehling's solution. All compounds gave a positive Molisch test, and all but yeast nucleic acids a negative Bial test, by which a content of hexose is indicated.

The first compound, obtained by agitation of a yeast suspension, *cebrosan*, was on basis of examination of its hydrolysate characterizable as a carbohydrate of the mannan type. The elementary analysis of yeast mannan (page 580) corresponded closely to the formula $(C_6H_{10}O_5)_n$, that of cebrosan, however, showed a surplus of oxygen. Its sedimentation constant was highly dependent on concentration. The value 9.75 *S* was obtained at extrapolation to zero concentration (Fig. 7). Determinations of the diffusion

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constant for two purified preparations according to the moment method (D_m), and the area method (D_A), gave corresponding results, by which is indicated (GRALÉN[22]) that the substance was monodisperse.

The sedimentation constant for purified *yeast mannan* showed a slight regress with increase of concentration, the tendency was however not pronounced at low concentrations (Fig. 7). Diffusion measurements showed good accordance between D_m and D_A (Table I). In one case, in which a yeast mannan solution was dialyzed against the buffer for two weeks, diffusion constants of $D_m = 6.35$ and $D_A = 4.88$ were determined, which points to degradation during dialysis. Remarkable is that preparation 4, which was obtained by the action of cold 75% sulphuric acid according to the method of GARZULY-JANKE[23], showed the same values for the sedimentation and diffusion constants as the other preparations, which were obtained by the alkaline extraction method.

Mycetin appeared homogeneous at sedimentation analysis and electrophoresis. Its glucosamine content, and its property to become insoluble at prolonged contact with alcohol, make it appear a mucopolysaccharide according to the definition of STACEY. It was easily hydrolyzed by strong acids.

Yeast nucleic acids in considerable amounts were extracted by Turmix-stirring of the yeast, in minor extent also at slight autolysis. It is assumed, that they are present in deeper layers, or pores of the cell surface.

The conditions of extraction of yeast carbohydrates upon beginning autolysis were not studied in detail. The method described in this paper gave reproducible results. Among the extracted compounds yeast mannan apparently was present. The basic polysaccharide, precipitable with alcoholic ammonia, was isolated also by electrophoretic separation, which latter method is a careful one. In this connection the investigations of ROELOFSEN AND HOETTE[24] are of great interest, as they give new evidence for the occurrence of chitin in the yeast cell wall.

ACKNOWLEDGEMENTS

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SUMMARY

1. A study has been made on the carbohydrates of yeast. A method is described by which was obtained a differential extraction of several constituents of the cell wall. The compounds were characterized by their sedimentation constants, electrophoretic mobility, and nitrogen contents.

2. On stirring of a yeast suspension in water were extracted: A high molecular mannan (9.75 *S*), called *cebrosan*, 0.3% from dry yeast; a mucopolysaccharide (8.2 *S*) with high contents of nitrogen and phosphorus, called *mycetin*, about 1% from dry yeast; macromolecular *yeast nucleic acids* (2.55 *S*), in about the same proportion, and two high molecular carbohydrates (about 13 and 16 *S*) in minor proportions.

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On beginning autolysis several high molecular carbohydrates were extractable (2.8, 4.0, 6.5, and 7.2 *S*), besides a *carbohydrate of basic properties* (3.6 *S*), which contained about 5% nitrogen.

The sedimentation constant of *yeast mannan* was determined as 4.1 *S* at infinite dilution.

3. For yeast mannan and cebrosan were determined the diffusion constants of 3.92 and $1.96 \cdot 10^{-7}$ cm^2 sec^{-1}, and relative specific volumes of 0.658 and 0.61, respectively. From these data, the molecular weight 74,000 was calculated for yeast mannan, and 340,000 for cebrosan.

4. Yeast mannan showed anodic migration in the pH interval 3 to 9, the mobility being $-1.16 \cdot 10^{-5}$ cm^2 $volt^{-1}$ sec^{-1} at pH 7.2, ionic strength 0.2. About the same value, $-1.3 \cdot 10^{-5}$, was determined both for cebrosan and mycetin, under same conditions. For the latter compound the isoelectric point 5.1 was determined.

RÉSUMÉ

1. Nous avons étudié les hydrates de carbone de la levure. Nous décrivons une méthode d'extraction différentielle de plusieurs constituants de la paroi cellulaire. Les composés ont été caractérisés par leurs constantes de sédimentation, leur mobilités électrophorétiques et leurs teneurs en azote.

2. En remuant une suspension de levure dans l'eau, nous avons extrait les substances suivantes: un mannane à poids moléculaire élevé (9.75 *S*) appelé *cébrosane*, 0.3% de la levure sèche; un mucopolysaccharide (8.2 *S*), à teneurs en azote et phosphore élevées, appelé *mycétine*, environ 1% de la levure sèche; des acides nucléiques macromoléculaires de levure (2.55 *S*) en proportions environ égales et deux hydrates de carbone à poids moléculaire élevé (environ 13 et 16 *S*) en moindres proportions.

Une fois que l'autolyse avait commencé, plusieurs hydrates de carbone à poids moléculaire élevé (2.8, 4.0, 6.5 et 7.2 *S*) et un *hydrate de carbone à propriétés basiques* qui contenait environ 5% d'azote pouvaient être extraits.

Pour la constante de sédimentation du *mannane de levure* nous avons trouvé une valeur de 4.1 *S* à une dilution infinie.

3. Pour le mannane et le cébrosane de levure nous avons trouvé des constantes de diffusion de 3.92 et $1.96 \cdot 10^{-7}$ cm^2 sec^{-1} et des volumes spécifiques relatifs de 0.658 et 0.61. A partir de ces valeurs nous avons calculé le poids moléculaire du mannane, 74,000 et celui du cébrosane, 340,000.

4. Le mannane de levure montrait une migration anodique dans l'intervalle de pH 3 à 9, la mobilité étant de $-1.16 \cdot 10^{-5}$ cm^2 $volt^{-1}$ sec^{-1} à un pH de 7.2 et une force ionique de 0.2. Pour le cébrosane et la mycétine on trouve, dans les mêmes conditions, environ la même valeur, c.à.d. $-1.3 \cdot 10^{-5}$. Pour le point isoélectrique de la mycétine nous avons trouvé la valeur 5.1.

ZUSAMMENFASSUNG

1. Es wurden die Kohlenhydrate der Hefe untersucht. Eine Methode wurde beschrieben, mit deren Hilfe die differentielle Extraktion von mehreren Bestandteilen der Zellwände erreicht wurde. Die Verbindungen wurden durch ihre Sedimentationskonstanten, ihre elektrophoretische Beweglichkeit und ihren Stickstoffgehalt gekennzeichnet.

2. Beim Rühren einer Hefesuspension in Wasser wurden extrahiert: ein hochmolekulares Mannan (9.75 *S*), genannt *Cebrosan*, 0.3% von trockener Hefe; ein Mucopolysaccharid (8.2 *S*) mit hohem Stickstoff- und Phosphorgehalt, genannt *Mycetin*, ungefähr 1% aus trockener Hefe; makromolekulare *Hefenukleinsäure* (2.55 *S*), zu ungefähr den gleichen Anteilen und zwei hochmolekulare Kohlenhydrate (*ca.* 13 und 16 *S*) zu kleineren Anteilen.

Zu Beginn der Autolyse konnten mehrere hochmolekulare Kohlenhydrate extrahiert werden (2.8, 4.0, 6.5 und 7.2 *S*), sowie ein *Kohlenhydrat mit basischen Eigenschaften* (3.6 *S*), welches ungefähr 5% Stickstoff enthielt.

Die Sedimentationskonstante von *Hefemannan* wurde zu 4.1 *S* bei unendlicher Verdünnung bestimmt.

3. Von Hefemannan und Cebrosan wurde die Diffusionskonstante zu 3.92 bzw. $1.96 \cdot 10^{-7}$ cm^2 sec^{-1} und das relative spezifische Volumen zu 0.658 bzw. 0.61 bestimmt. Aus diesen Daten wurde das Molekulargewicht 74,000 für Hefemannan und 340,000 für Cebrosan bestimmt.

4. Hefemannan zeigte anodische Wanderung in einem pH-Intervall von 3 bis 9, die Beweglichkeit ist $-1.16 \cdot 10^{-5}$ $volt^{-1}$ sec^{-1} bei pH 7.2, Ionenstärke 0.2. Ungefähr derselbe Wert, $-1.3 \cdot 10^{-5}$, wurde unter den gleichen Bedingungen sowohl für Cebrosan wie Mycetin bestimmt. Von der letzten Verbindung wurde der isoelektrische Punkt zu 5.1 bestimmt.

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