HIGH MOLECULAR COMPOUNDS FROM YEAST

III. TOP YEAST. A COMPARATIVE STUDY

by

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In two preceeding papers[1,2] the author has described physico-chemical investigations on the main protein and carbohydrate constituents of a bottom fermentation brewing yeast. The present work deals with the application of the same methods on top fermentation yeast, in order to ascertain the most outstanding differences.

EXPERIMENTAL

For the investigations fresh yeast was used, obtained by courtesy of A. B. Grönwall's Bryggeri, Stockholm, which had been washed in the centrifuge. Analyses by the micro Kjeldahl method gave the mean contents of 9.9% nitrogen in dry substance, against 9.8%, determined in bottom yeast.

Extraction of proteins

By the maceration method of LEBEDEV[3] from bottom yeast are obtained protein extracts with less expenditure of equipment. Comparative experiments showed that this method was not employable for top yeast. Samples of top yeast, dried according to LEBEDEV, showed after 20 hours of extraction an extractibility of 27% of total nitrogen, against 66% from bottom yeast (Fig(1).

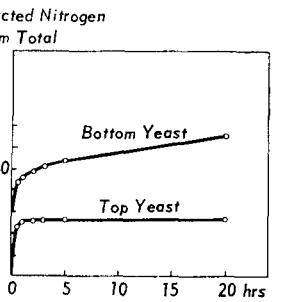

Fig. 1. Nitrogen extractibility of top and bottom yeast, dried according to LEBEDEV.

Freeze-dried preparations, ground for two hours in a ball mill, showed no difference in nitrogen extractibility, 57% of total nitrogen being extracted from top yeast, and 55% from bottom yeast, respectively.

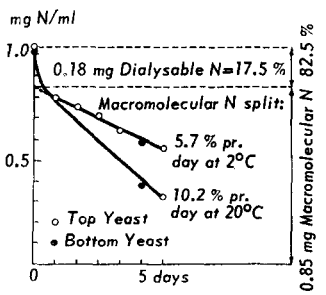

Fig. 2. Proteolytic splitting of yeast proteins extracts under dialysis against phosphate buffer of pH 6.8

Splitting of proteins in solutions

Extracts from the ground yeasts, containing 1.03 mg nitrogen per ml, were dialyzed against phosphate buffer, pH 6.8, at room temperature (20° C), and in the cool room (4° C), respectively. Nitrogen determinations were made in one ml of the solutions at intervals of 24 hours. The graph

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in Fig. 2 shows that during the first day of dialysis the low-molecular compounds were removed. Thereafter occurred a continuous decrease in the contents of macromolecular proteins, at a rate of 5.9% a day at +4° C, and 10.2% at 20° C, respectively. Both yeast kinds showed the same rate of splitting.

Sedimentation analysis of protein extracts

The extracts were dialyzed for two days at +4° C against a buffer, consisting of 0.20 *M* sodium chloride, and 0.05 *M* phosphate, pH 6.8, and analyzed in the ultracentrifuge. A rotation radius of 6.5 cm, and a rotor speed of 1000 r.p.sec were used.

By these investigations for top yeast extracts the same sedimentation constants and diagrams of similar appearance were obtained as for bottom yeast extracts. The sedimentation diagram for an extract from ground top yeast is given in Fig. 3.

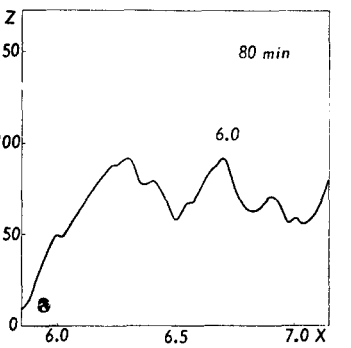

Fig. 3. Sedimentation diagram for an extract from ground top yeast.

Extraction of cell surface compounds

Cleaned top and bottom yeast were agitated by a mechanical stirrer for one hour in water (a). The yeast was thereafter centrifuged off, and the stirring continued in a 1 *M* sodium chloride solution (b). The temperature of the suspensions rose under agitation to 35° C. The extracts obtained were dialyzed against distilled water, and the amounts of undialyzable substance determined after evaporation of aliquot parts of the solutions. The results in Table I enable only an orientative conception, as the extraction was not complete in any case. In top yeast extracts (b) on dialysis occurred precipitation (of globulins), and on evaporation on the water bath coagulation was observed.

TABLE I

EXTRACTS FROM FRESH YEAST BY STIRRING FOR ONE HOUR AT 35° C

Solvent	*Top yeast*		*Bottom yeast*	
	Evaporation residue %	*Nitrogen in dried residues %*	*Evaporation residues %*	*Nitrogen in dried residues %*
a. Water	1.8	7.1	0.7	3.3
b. 1 *M* NaCl	1.7	8.8	0.6	4.3
Total:	3.5%		1.3%	

The sedimentation diagram for top yeast extract (a) indicated compounds with the sedimentation constants 6.5 and 8.5 *S*, respectively (at a refractive index increment of 000.75), which correspond to those of mycetin and cebrosan, isolated by the same treatment from bottom yeast[2]. Besides, the diagram gave evidence for containing of disperse compounds of lower sedimentation. A sedimentation experiment with an extract obtained by the same treatment from baker's yeast, showed besides cebrosan and mycetin the occurrence of several compounds of lower sedimentation (Fig. 4a and b).

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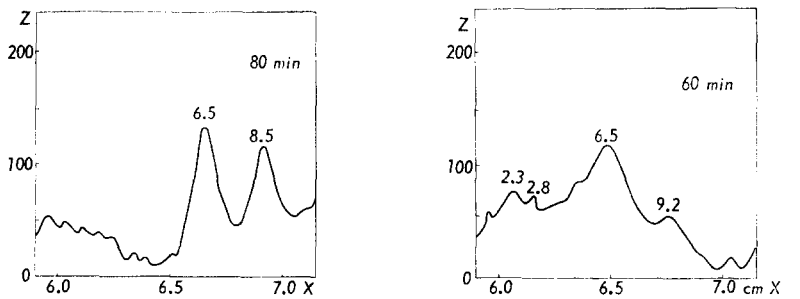

Fig. 4. Sedimentation diagrams for extracts, obtained by agitation of intact yeast. a. Top fermentation brewing yeast; b. Baker's yeast.

The extracts were subsequently compared by means of the electrophoretic method[1]. Top yeast extracts, obtained by agitation in water of 45° C, showed the containing of 4–6 compounds, most of which had isoelectric points at about 5. On dialysis against acetate buffer at pH 4.3 a precipitate was modified, apparently by coagulation of some of the substances in the solution. Bottom yeast extracts on the contrary showed two compounds only. Electrophoretic patterns are given in Fig. 5a and b.

Fig. 5. Electrophoretic patterns for extracts, obtained by agitation of intact yeast at 45°.

	pH	Time min	μ	F Volt cm^{-1}			$u \cdot 10^5$		
a. Top yeast	7.6	221	0.1	6.2	±0.10	1.12	1.80	2.85	5.68
b. Bottom yeast	7.2	286	0.1	6.2	0.14	1.30			

Some other consistuents of the cell wall loosen at the autolysis, which is induced, when storing the yeast for two days at 30–40° C. Sedimentation analysis of the extracts, obtained in this way from top and bottom yeast respectively, showed good accordance in the main compounds[2]. A carbohydrate of basic properties was precipitated from the extracts with alcoholic ammonia in both cases.

Yeast mannan, isolated from top yeast according to the method of SALKOWSKI, showed the sedimentation constant of 3.6 S at a refractive index increment of 0.00104, which is in good accordance with determinations made on preparations from bottom yeast[2].

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DISCUSSION OF THE RESULTS

Samples of bottom and top yeast of the same nitrogen contents showed the same extractibility in the ground state. Sedimentation analysis of the protein extracts made evident an equal size distribution. These results were to be expected, as the enzyme composition of both yeasts is similar.

On drying of top yeast according to the LEBEDEV method, the permeability of the cell wall was not significatively increased. From this is concluded a tighter structure of the cell wall of top yeast, compared with this of bottom yeast. It should be of interest to obtain a confirmation of this fact by other methods. The proteolytic activity of extracts from both yeast kinds showed no difference (Fig. 2).

On agitation of aqueous yeast suspensions a higher nitrogen extractibility was observed for top yeast, than for bottom yeast (Table I). The presence of proteins in top yeast extracts was made probable. By the electrophoretical investigations was confirmed the occurrence of compounds in top yeast extracts not occurring in bottom yeast, apparently proteins.

As the yeast had been thoroughly washed, it was excluded that the proteins were originating from damaged or autolyzed cells.

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SUMMARY

1. A comparative study has been made on the constituents of top and bottom fermentation brewing yeast.

2. Top yeast, dried according to the LEBEDEV method, showed an insignificative protein extractibility.

3. Ground top and bottom yeast showed an equal protein extractibility, and similar sedimentation diagrams.

4. Some compounds, isolated from intact top yeast, showed the same properties as were determined for constituents of the cell wall of bottom yeast.

5. On the cell surface of top yeast were found readily extractable proteins as permanent constituents, which did not occur in bottom fermentation yeast.

RÉSUMÉ

1. Nous avons étudié, à titre de comparaison, les constituants de la levure de bière à fermentation haute et basse.

2. La quantité de protéine que l'on peut extraire de levure à fermentation haute, séchée suivant la méthode de Lebedev, était insignifiante.

3. L'extractibilité des protéines est la même pour les deux levures; les diagrammes de sédimentation des deux levures se ressemblent.

4. Certains composés isolés à partir de levure à fermentation haute intacte ont les mêmes propriétés que certains constituants de la paroi cellulaire de la levure à fermentation basse.

5. A la surface des cellules de levure à fermentation haute nous avons trouvé, comme constituants permanents, des protéines faciles à extraire qui ne se trouvent pas dans la levure à fermentation basse.

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ZUSAMMENFASSUNG

1. Wir führten eine vergleichende Untersuchung über die Bestandteile ober- und untergäriger Bierhefe aus.

2. Obergärige Hefe, die nach der Lebedew Methode getrocknet worden war, zeigte eine unbedeutende Proteinextrahierbarkeit.

3. Gemahlene obergärige und untergärige Hefe zeigte gleiche Proteinextrahierbarkeit und ähnliche Sedimentationsdiagramme.

4. Einige aus intakter obergäriger Hefe isolierte Verbindungen zeigten die gleichen Eigenschaften, wie sie für Bestandteile der Zellwände von untergäriger Hefe bestimmt wurden.

5. Auf der Zelloberfläche obergäriger Hefe wurden leicht extrahierbare Proteine als ständige Bestandteile gefunden, welche in untergäriger Hefe nicht vorkommen.

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