YEAST MANNAN, A CELL WALL CONSTITUENT OF BAKER'S YEAST

by

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INTRODUCTION

Several authors have stated that the cell wall residue obtained by treating baker's yeast with hot alkali is a glucan[1]. Only a small quantity of chitin is present[9].

From the alkali extract of the yeast cells a mannan, so-called yeast gum, has repeatedly been isolated by precipitating with Fehling solution[3]. Since it was not known whether this mannan originated from the cell wall or from the cell content, we analyzed cell walls obtained by mechanical breakage of cells and washing away the cell contents, thereby applying a method already used[5].

However, while this investigation proceeded, the results of a similar analysis were published by NORTHCOTE AND HORNE[8]. Since these differed only slightly from ours, we decided to discontinue the investigation and to communicate briefly our findings.

METHODS

Cell wall material. About 10 g fresh baker's yeast from the Gist en Spiritusfabriek at Delft was suspended in about 50 ml water, disintegrated in a Mickle tissue disintegrator with Ballotini glass beads until at least 98% of the cell were broken and emptied. Then the suspension was repeatedly centrifuged with water until no protoplasm particles and no whole cells could be detected microscopically. After centrifugation with ethanol, absolute ethanol and ether and drying at 60° C, about 500 mg dry powder was obtained (20% of dry weight of yeast): preparation A.

Alkali-extract and residue. About 150 mg dry cell wall material was heated with 20 ml 2% NaOH during 15 min at 100° C, stirring all the while. After cooling and centrifuging, the process was repeated with fresh alkali. The residue was washed and dried by centrifuging with water, ethanol, abs. ethanol and ether: preparation B.

The alkali extract was neutralised with H-saturated cation-exchanger (Dusarit), filtered and evaporated in a current of air: preparation C.

Hydrolysis. About 100 mg of the preparations A, B and C were treated with 3 ml 72% (w/w) sulphuric acid at 20° C during 3 days. Then 30 ml water was added and the solution was boiled for 8 h. After neutralizing with $BaCO_3$ and filtration the hydrolysate was evaporated in a current of air and dissolved in 10 ml water.

Total sugar content of preparation A. In aliquots of the cell wall hydrolysate reducing sugars were determined by Scales' copper reduction method as modified by ISBELL *et al.* (see 2 page 844).

Chromatographic separation and estimation of sugar components. The hydrolysates of A, B and C were chromatographed on Whatman No. 1 paper irrigated downwards with butanol-acetic acid-water. A semi-quantitative method described by KOCH *et al.*[6] was used to determine the quantities of glucose and mannose approximately.

Chromatographic demonstration of amino acids in preparation A. The hydrolysate was chromatographed on Whatman No. 1 paper using ascending butanol-acetic acid-water and developing with ninhydrin.

Nitrogen-content of preparation A. Kjeldahl destruction in duplicate of about 200 mg and NH_3 titration.

RESULTS

The total polysaccharide content of the dry cell wall material (prep. A), expressed

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as anhydro-glucose was 68%. The only sugars detectable were glucose and mannose, occurring in approximately equal quantities. The total N-content was 1.0%. If chitin is estimated as at most 1%[7], the remaining N suggests the presence of 6% protein, either as a cell wall constituent or as adhering protoplasm. At least ten amino acids were detected.

On hydrolysis of the residue of the alkali-treatment (prep. B) only glucose was found, the amount of glucosamin apparently being too small to be detected. As determined semi-quantitatively, 3/4 of the glucose of the original cell wall was retained in the alkali residue, the rest obviously having been dissolved by the alkali. Accordingly the hydrolysate of the alkali solution (prep. C) contained glucose besides an excess of mannose.

Apparently, a 30 min treatment with hot 2% NaOH dissolves all mannan and in addition some glucan, which is in accordance with the findings of HOUWINK AND KREGER[4]. This extraction of a non-mannan constituent also appears from the data of NORTHCOTE AND HORNE[8], since their ethanol-precipitated so-called cell wall mannan contained only 85% copper-precipitatable mannan, but this was apparently disregarded. Doubtless their figure of 29% glucan is too low and, provided that the alkali treatment did not reduce part of the mannan to a residue not precipitated by ethanol, their 31% mannan is too high. The N-content of the cell wall material used by us is decidedly lower than that of theirs (1.0% against 2.1%), which may be due to a better purification of our starting material. However, it cannot be disregarded that another baker's yeast was used.

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SUMMARY

The dried cell wall of baker's yeast contains about 6% protein and 68% polysaccharide, consisting of approximately 34% glucan, 34% mannan and a small quantity of chitin. This essentially confirms the findings of others[8].

RÉSUMÉ

Les parois de cellules séchées de levure des boulangers contiennent environ 6% de protéine et 68% de polysaccharide; ce dernier ce compose de 34% de glucane, 34% de mannane et une faible quantité de chitine. Ceci confirme essentiellement les valeurs trouvées par d'autres chercheurs[8].

ZUSAMMENFASSUNG

Die getrockneten Zellwände von Bäckerhefe enthalten 6% Protein und 68% Polysaccharid, das besteht aus annähernd 34% Glukan, 34% Mannan und einer geringen Menge Chitin. Die Werte anderer Forscher werden dadurch im wesentlichen bestätigt[8].

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