An increased fat production of *Rhodotorula gracilis* yeast with a simultaneous reduction of the protein content may thus be induced not only by a deficiency of nitrogen in the nutrient solution but also by a deficiency of sulphur or iron. The agreement in composition found in yeast formed with a deficiency of nitrogen, sulphur or iron indicates that the decisive factor in the increased fat production is the reduction of the protein content of the yeast. Certain experiments which have not yet been completed show that it is possible to inhibit the growth and protein production of yeast by the addition of certain substances, in which case a simultaneous increased fat production takes place. Every decrease of the protein synthesis of the yeast seems thus to result in an increased production of fat, provided that sugar is present in sufficient amounts.

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On the Sulphur Metabolism of *Rhodotorula gracilis*. II. The Ratio between SH and SS groups *

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It is known that the protein content of *Rhodotorula gracilis*, when cultivated in a substrate poor in nitrogen and rich in sugar, decreases while the fat content increases [1,2]. The protein content may vary between 12—50 %. Thus this yeast is very suitable for investigations regarding the influence of different factors on the synthesis of proteins. It seems probable that the composition of the protein differs in yeasts with low and high protein content. The object of this communication is to describe the results of some investigations on the proportion between protein SH and SS groups in *Rhodotorula* yeast containing different amounts of protein.

The cultivation of the yeast was carried out according to Nielsen and Nilsson [3] in Erlenmeyer flasks of 750 ml with 300 ml nutritient solution.

The nutrient solution for obtaining protein rich yeast (experiments a in Table 1) contained per liter:

7.5 g	Asparagine
4.7 g	KH_2PO_4
3.0 g	$MgSO_4 + 7\ H_2O$
1.5 g	NaCl
1.5 g	$CaCl_2 + 6\ H_2O$
0.015 g	$FeCl_3 + 6\ H_2O$
60 g	Dextrose

The pH was 4.8 and the shaking time varied between 2 and 6 days. For the production of protein poor yeast (experiments b in Table 1) the quantity of asparagine was reduced to 1 g per liter and that of dextrose to 40 g per liter.

The proportion of protein SH and SS groups was determined polarographically using the technic developed by Brdicka [4]. After cultivation the yeast was centrifuged off, washed with water and plasmolyzed by mixing with 5 % solid KCl. From the plasmolysate the following two mixtures were prepared.

Sample	1	2
Plasmolysate, g	0.30	0.30
1 *N* KOH, ml	0.15	0.15
H_2O, ml	0.30	—
0.2 *M* ICH_2COOK, ml	—	0.30

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Table 1.

Experiment	Time of cultivation	Yield of yeast, dry basis	Composition of yeast: Protein dry basis	Composition of yeast: Fat dry basis	Ratio * $\frac{SH}{SH + SS}$
	days	g	%	%	
I a	2	0.69	53.0	6.9	0.71
Protein rich yeast	4	1.01	37.4	18.0	0.72
	6	1.13	38.0	18.3	0.66
I b	2	0.52	39.8	16.1	0.66
Protein poor yeast	4	0.68	20.7	36.1	0.53
	6	0.88	20.0	41.7	0.50
II a	2	0.57	54.3	7.8	0.70
Protein rich yeast	4	0.75	36.3	18.3	0.68
	6	1.12	33.6	18.1	0.73
II b	2	0.52	50.7	17.3	0.64
Protein poor yeast	4	0.60	19.0	37.8	0.52
	6	0.79	16.8	37.9	0.42

* The ratio between the polarographic currents representing SH and SH+SS groups respectively.

The samples were allowed to stand for 45 minutes. ICH_2COOK inhibits the polarographic effect of SH groups. Thus the polarographic analysis of sample 1 gives the combined effects of SH and SS groups and that of sample 2 the effect of the SS groups only.

The results of two experiments with protein rich and protein poor yeast are given in Table 1.

The protein content is reduced when the yeast is cultivated in the nitrogen poor medium of the experiments b. It is interesting that the ratio between the polarographic currents representing the SH and SH + SS groups respectively decreased when the protein content of the yeast decreased. Thus when the protein content of the yeast was varied by varying the supply of nitrogen a low protein content was accompanied by a lower value of the ratio $\frac{SH}{SH + SS}$. The metabolism of the protein poor yeast is strongly reduced compared with that of the protein rich yeast as may be seen from the reduced yield. Parallell with the decrease of protein content of the yeast a decrease of the content of the sulphydryl groups occured.

Nielsen and Rojowski [5] found that the synthesis of proteins was reduced even in a medium rich in nitrogen if the supply of sulphur was sufficiently reduced. Cultivations were carried out with the substrate containing ample quantities of nitrogen (mentioned above) for the production of protein rich yeast and further with a substrate of the same composition except for the content of $MgSO_4 + 7\ H_2O$ being 0.15 instead of 3.00 g per liter for the production of a yeast poor in protein according to Nielsen and Rojowski [5]. The results are given in Table 2.

Table 2.

Experiment	Time of cultivation days	Yield of yeast, dry basis g	Composition of yeast Protein dry basis %	Composition of yeast Fat dry basis %	Ratio * $\frac{SH}{SH + SS}$
III a	2	1.05	55.9	16.0	0.72
substrate containing	4	1.29	48.0	16.8	0.72
3 g $MgSO_4 + 7\ H_2O$	6	1.72	46.2	15.1	0.77
per lit.					
III b	2	0.78	41.8	21.0	0.73
substrate containing	4	0.81	21.8	27.3	0.92
0,15 g $MgSO_4 + 7\ H_2O$	6	1.08	17.8	28.7	1.00
per lit.					

* The ratio between the polarographic currents representing SH and SH + SS groups respectively.

In agreement with Nielsen's and Rojowski's findings the reduction of sulphur in the substrate caused a similar decrease of the protein content and increase of the fat content of the yeast as lack of nitrogen in the substrate did in the experiments of Table 1. The ratio $\frac{SH}{SH + SS}$ for the yeast cultivated on the substrate poor in sulphur was, however, after 6 days cultivation 1, *i. e.* the polarographically active sulphur existed only as SH groups.

Further experiments with different quantities of sulphur in the substrate have confirmed the results given above.

When the protein synthesis is reduced through a reduction of the supply of nitrogen in the substrate the value of the ratio $\frac{SH}{SH + SS}$ of the proteins of the yeast decreases with decreasing protein content. On the other hand when the protein synthesis is reduced through a reduction of the supply of sulphur in the substrate (the supply of nitrogen being abundant) the value of the ratio increases with decreasing protein content. If the sulphur supply is very low all the sulphur is transformed to SH groups and the value of the ratio becomes 1. The sulphydryl groups are assumed to be active parts of the protein synthesizing enzyme system of the yeast. If the nitrogen supply is low there is little need for SH groups for protein synthesis. Then the value of the ratio is low. If the supply of nitrogen and sulphur is ample there is need for sulphydryl groups for protein synthesis and the ratio is high. If the supply of sulphur (but not of nitrogen) is reduced causing a reduction of the content of protein a greater part and finally all of the sulphur is transformed into SH groups and the value of the ratio is high, finally 1, in order to promote protein synthesis from the nitrogen present and counteract the drop of the protein content of the yeast.

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