$[\alpha]_D^{25} = -253.8°$. The acid is practically insoluble in water and dilute hydrochloric acid.

The preparation of optically active *disulphide-di-β-isobutyric acid* (IV) met with some difficulties. The inactive acid described by Larsson [6] is probably a mixture of racemic and *meso*-acid, and experiments with different alkaloids gave no resolution. The ethylxanthogene-*β-iso*butyric acid is liquid at room temperature and difficult to obtain in a state of purity [7], but the methylxanthogene derivative is crystallised and can be resolved by means of cinchonidine. After ten crystallisations of the cinchonidine salt from dilute (55 %) acetone, the acid showed $[\alpha]_D^{25} = -71.5°$ (acetone solution). The rotation did not change on further recrystallisation of the salt. The xanthogenic acid was decomposed with ammonia, and the resulting mercapto acid oxidised with iodine. The disulphide acid was recrystallised twice from water, in which it is sparingly soluble at room temperature, and obtained as glistening plates. M. p. 124—125°.

0.1227 g acid: 9.68 ml 0.1065 *N* NaOH:
$C_8H_{14}O_4S_2$ (238.3)
Equiv. wt. Calc. 119.2 Found 119.6

0.0220 g acid dissolved in 0.1 N hydrochloric acid to 10.00 ml: $2\alpha = -0.665°$. $[\alpha]_D^{25} = -151°$ — 0.1022 g acid dissolved in dilute ammonia to 10.00 ml: $2\alpha = -4.49°$. $[\alpha]_D^{25} = -219.7°$ — 0.1125 g acid dissolved in ethanol to 10.00 ml: $2\alpha = -0.905°$. $[\alpha]_D^{25} = -40.2°$.

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# On the Sulphur Metabolism of *Rhodotorula gracilis*. I. The Importance of Sulphur and Iron for the Formation of Protein and Fat

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It is well known that various microorganisms are able to form considerable quantities of fat if cultivated in nutrient solutions whose nitrogen content is low in comparison with their sugar content. In *Rhodotorula gracilis* [1] the fat content may thus vary between 8 % and 65 %, depending on the composition of the nutrient solution in respect of nitrogen. Parallel with the increase in the fat content there occurs a decrease in the protein content. The latter may vary between 50 % for a normal yeast poor in fat and 12 % for a pronouncedly fat yeast. Probably it is the reduction of the protein content caused by the decrease of the nitrogen content of the nutrient solution which induces fat production.

It seemed of interest to investigate whether a reduction of the protein content of the yeast might be brought about in any other way than by cultivating it in a substrate with a low nitrogen content, obtaining an increased production of fat at the same time. For this purpose we have carried out experiments in which the yeast was cultivated in nutrient solutions containing such small quantities of sulphur or iron in relation to the other nutrient substances as to be insufficient for normal metabolism.

For the cultivation of normal, not strongly fat-producing Rhodotorula gracilis yeast we use a nutrient solution of the following composition: 15 g asparagine — 4.7 g $KH_2PO_4$ — 3 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 glucose per liter. In the experiments described below we used nutrient solutions containing amounts of asparagine, $KH_2PO_4$, NaCl, $CaCl_2$ and glucose as stated above, but varying amounts of $MgSO_4$ or $FeCl_3$. 750 ml Erlenmeyer flasks containing 300 ml nutrient solution were inoculated with *Rhodotorula gracilis*. Subsequently the flasks were shaken for 5—7 days at 25° C. Then the amount of yeast formed was determined. The yeast was analyzed for fat and nitrogen content. For further details see Nielsen and Nilsson [2].

In the first experiment the sulphur content of the nutrient solution was varied. Six different solutions were used, containing per liter 3 g, 1 g, 0.3 g, 0.1 g, 0.03 g, and 0 g $MgSO_4$, 7 $H_2O$. Such amounts of $MgCl_2$ were added that the content of magnesium was constant in all solutions.

*Table 1. Cultivation of Rhodotorula gracilis in nutrient solutions with varying contents of $MgSO_4$. Time of cultivation 5 days*

| $MgSO_4$, 7$H_2O$ g/l nutrient solution | Yeast formed g/100 ml | Yeast fat % | Yeast protein % |
|---|---|---|---|
| 3 | 1.32 | 11.8 | 42.5 |
| 1 | 1.40 | 10.5 | 44.2 |
| 0.3 | 1.11 | 17.7 | 36·3 |
| 0.1 | 0.86 | 37.6 | 13.9 |
| 0.03 | 0.74 | 36.5 | 13.0 |

Table 1 shows that if the sulphur content of the nutrient solution was reduced sufficiently, the yeast entered a fattening phase while simultaneously the protein content of the yeast declined. The changes in the fat and protein content of the yeast which took place as a consequence of the reduced sulphur content, corresponded to the changes which occur when yeast is cultivated in nutrient solutions with reduced nitrogen content.

Four other experiments with nutrient solutions of varying sulphur content gave essentially the same results. It is quite evident that an increased fat production of the yeast with a corresponding reduction of its protein content can be induced by a reduction of the sulphur content of the nutrient solution in the same way as by a reduction of its nitrogen content.

In other experiments the effect of reducing the iron content of the nutrient solution was investigated. The nutrient solutions which we used contained per liter: 0.015 g, 0.005 g, 0.0015 g, 0.0005 g, and 0 g $FeCl_3$, 6 $H_2O$.

*Table 2. Cultivation of Rhodotorula gracilis in nutrient solutions with varying contents of $FeCl_3$. Time of cultivation 5 days*

| $FeCl_3$, 6 $H_2O$ g/l nutrient solution | Yeast formed g/100 ml | Yeast fat % | Yeast protein % |
|---|---|---|---|
| 0.015 | 1.40 | 12.3 | 44.6 |
| 0.005 | 1.49 | 13.2 | 45.2 |
| 0.0015 | 1.24 | 13.9 | 43.9 |
| 0.0005 | 1.01 | 19.4 | 33.5 |
| 0 | 0.71 | 27.8 | 21.9 |

Table 2 shows that if the iron content of the nutrient solution is greatly reduced, there is also formed a yeast rich in fat and poor in nitrogen. But in this case the fat content was not so high nor the nitrogen content so low as in the case of a nitrogen or sulphur defiency. As it, however, is a question of very small amounts of iron, it is probable that impurities containing traces of iron may be of some importance, and that with a nutrient solution extremely poor in iron it may be possible to obtain a higher fat content and a correspondingly lower protein content of the yeast.

*Rhodotorula* yeast rich in fat is red in colour. However, the colour tint of the yeast formed with a deficiency of iron is somewhat different from the tint of such fat yeast as has been formed with a deficiency of nitrogen or sulphur.

Three other experiments with varying amounts of iron gave the same result as the above experiments.

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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