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On the Production of Vitamins by Protein-rich and Protein-poor *Rhodotorula gracilis* Yeast

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When *Rhodotorula gracilis* was grown in nutrient solutions containing 0.1 or 1 % asparagine or ammonium sulphate as the nitrogen source, the crude protein content of the yeast varied between 8.4 and 60.7 %, calculated on a dry yeast basis. The fat content of the yeast also varied greatly, between 71.0 and 11.6 % of the yeasts dry weight. A marked correlation was noticeable, the fat content being high if the crude protein content was low and vice versa.

The sum of the contents of crude protein and fat was relatively constant, varying between 70 and 79 % of the dry yeast when asparagine was used as the nitrogen source. When ammonium sulphate was the nitrogen source this sum varied between 60 and 67 %. This difference between asparagine and ammonium sulphate media was probably caused by the increasing acidity of the latter.

The four vitamins investigated, *viz.* riboflavin, pantothenic acid, nicotinic acid and pyridoxine were present mainly in the nutrient solution (about 50 to 80 %). On calculating the vitamin contents as µg per g dry yeast, the amounts present at the end of the fermentations were much smaller for the yeast poor in crude protein. However, expressing the amounts as µg per g crude protein, no significant difference was found between the protein-poor and protein-rich types, with the exception of pantothenic acid and possibly nicotinic acid. Nicotinic acid had a tendency to be formed in higher amounts — calculated per g of protein — in the protein-poor yeast. Pantothenic acid was not formed to any appreciable extent in the protein-poor yeast, most probably due to the fact that β-alanine was not formed.

When *Rhodotorula gracilis* was cultivated in a nutrient solution low in nitrogen but containing an excess of sugar, considerable amounts of fat were produced [1]. The fat content of the yeast was found to be very high, amounting to 60 % or more calculated on a dry weight basis. Concomittantly the crude protein content decreased and a yeast with only 12 % crude protein might be obtained. If the yeast was cultivated with an excess of nitrogen a yeast with about 50 % crude protein and 10 % fat was obtained. Thus the protein content of this yeast can be varied between wide limits only by altering the nitrogen content of the nutrient solution.

Some comparative studies on the metabolism and the chemical composition of both the protein-rich and the protein-poor *Rh. gracilis* yeast have already been carried out. Nielsen, Sandegren and Ljungdahl [2] compared the amino acid content in these two yeast types and found certain differences. The protein-poor yeast had a relatively high content of glutamic acid and alanine compared with the protein-rich yeast. β-Alanine which is a component of pantothenic acid was present in the protein-rich but not in the protein-poor yeast. Later, Nielsen and Lundin [3] made quantitative determinations of some amino acids and found that whereas the amount of *iso*leucine, leucine, lysine, phenylalanine and tryptophane was practically the same in both the protein-rich and the protein-poor yeast (expressed per g of protein), the protein-poor yeast was very low in cystine and methionine. Accordingly the sulfur-content of the protein-poor yeast was also very low. Sandegren, Ekström and Nielsen [4] found that the protein-poor yeast contained fewer sulfhydryl-groups than the protein-rich yeast. The amount of phosphatides was small in the protein-poor yeast when the results were based on the weight of the yeast but no significant differences were found between the two yeast types when the phosphatide content was expressed per g of protein [5].

These investigations demonstrated important differences in the chemical composition of protein-rich and protein-poor *Rh. gracilis*. In this paper we have investigated the formation of some vitamins, *i. e.* riboflavin, pantothenic acid, nicotinic acid and pyridoxine, in these two yeast types.

METHODS

The variations in the protein content of the yeast were induced by altering the nitrogen content of the nutrient solution. The nutrient solution used for the protein-rich yeast had the following composition: 10 g asparagine or ammoniumsulphate, 4 g KH_2PO_4 — 3 g $MgSO_4$, $7H_2O$ — 1.5 g $CaCl_2$, $6H_2O$ — 0.015 g $FeCl_3$, $6H_2O$ and 40 g glucose, made up to 1 000 ml with distilled water. The nutrient solution for the protein-poor yeast had the same composition with the exception that only 1 g of asparagine or ammoniumsulphate was used.

The yeast was cultivated in 5 litre fermentors containing 3 litres of nutrient solution. The fermentors were kept at 25° and were aerated with 0.5 litre air per 1 litre solution per min. Each fermentor was seeded with 250 ml of a culture grown for 3 days in 250 ml Erlenmeyer flasks each containing 50 ml of the nutrient solution used for the production of protein-rich yeast. This amount of yeast corresponded to about 0.4 g of dry material.

During the experiments the pH of the nutrient solution and the amount of yeast present was determined daily. After the yeast had been removed by centrifuging, the amount of nitrogen was determined in the yeast-free solution, *i. e.* the amount of nitrogen not assimilated. From these analysis the crude protein content of the yeast could be calculated. At the end of the fermentations the yeasts was collected by centrifuging and washed twice with water. The yeast was then dried at 45° and a part was used for the determination of nitrogen and fat. Another part was dried at 105° in order to express the contents of nitrogen and fat on a dry weight basis. By the fat analysis [6] the total lipid content was determined. From the nitrogen analysis the crude protein was calculated as N × 6.25.

The vitamins were determined microbiologically using the methods described by Barton-Wright [7]. The yeast was separated by centrifuging and the vitamin content determined on the nutrient solution directly and on the yeast after treatment according to Ref. [7].

RESULTS

Five experiments with a nitrogen-rich and five with a nitrogen-poor nutrient solution were carried out. Four of these experiments (2 with a nitrogen-rich and 2 with a nitrogen-poor nutrient solution) were made with ammonium sulphate as the nitrogen source and in the others asparagine was used.

At the beginning of the fermentations growth was as good in the nutrient solution low in nitrogen as in that containing excess nitrogen. After about 2 days, however, the nitrogen in the nitrogen-poor medium was practically consumed and the growth in these fermentors became slower. This reduction in growth rate becames apparent first when the crude protein content of the yeast is below 20 %. The growth rate of a yeast with 20 or 60 % crude protein is almost the same which was found to be the case in all experiments independent of the nitrogen source used.

A remarkable and important difference between the fermentations with ammonium sulphate as the nitrogen source and those with asparagine, is the resulting pH of the nutrient solution. Whereas the pH remains about 5 during the fermentations with asparagine the final pH is about 2 in the fermentations with ammonium sulphate as the nitrogen source. The acidity of the latter nutrient solution is due to the well known fact that the ammonium ions are taken up and the solution becomes more acid. This acidity is no doubt the reason why the growth is somewhat slower when ammonium sulphate is the nitrogen source. Most probably it is also the reason why the fat production is slightly less and the production of nicotinic acid is higher (see below).

The values obtained for the crude protein and fat content of the yeast at the end of the fermentations are summarized in Table 1. In this table the fermentations are arranged according to the crude protein content of the yeast.

The values given in Table 1 clearly show that both the crude protein content and the fat content of the yeast can be varied greatly by altering the nitrogen content of the nutrient solution. The crude protein content varied between 8.4 and 60.7 % and the fat content between 11.6 and 71.0 %. When the crude protein content is low the fat content is high and vice versa, which shows an inter-relationship between the two.

Table 1. Crude protein and fat content of the yeast at the end of the fermentation.

Expt. No.	Crude protein, % of dry weight	Fat, % of dry weight	Crude protein + fat, % of dry weight	Nitrogen source
10	8.4	71.0	79.4	asparagine
5	10.0	64.0	74.0	asparagine
6	10.2	63.2	73.4	asparagine
1	18.4	47.1	65.5	am. sulphate
2	25.4	41.2	66.6	am. sulphate
3	43.2	17.5	60.7	am. sulphate
4	43.8	15.7	59.5	am. sulphate
9	50.8	19.4	70.2	asparagine
7	53.5	16.7	70.2	asparagine
8	60.7	11.6	72.3	asparagine

The sum of the contents of crude protein and of fat is relatively constant, varying between 70 and 79 % of dry yeast, when the yeast is grown in the nutrient solutions containing asparagine as the nitrogen source. When the yeast is grown with ammonium sulphate as the nitrogen source this value is appreciably lower, ranging between 59 and 65 % of the dry yeast. Relatively little fat is formed when ammonium sulphate is the nitrogen source. The difference between the composition of the yeast grown with asparagine and with ammonium sulphate is probably caused by the different pH values during the fermentation, the nutrient solution being more acid when ammonium sulphate is employed. It must, however, be remembered that no determinations of protein *per se* were made but that only the nitrogen content multiplied by 6.25 was taken as a measure of the protein content of the yeast. If the yeast contains a large number of non protein compounds this would be included in the figures for crude protein.

The vitamins riboflavin, pantothenic acid, nicotinic acid and pyridoxine were determined both in the cell-free nutrient solution and in the yeast both during fermentation and at the end of the fermentation. It was found that the largest quantity of the vitamins was present in the nutrient solution, the yeast only contained some 20—50 % of the total amount.

Table 2 gives the amounts of the vitamins found at the end of the fermentations. The values are calculated both per g dry yeast and per g of protein. The 10 fermentations are arranged according to the crude protein content of the yeast.

Table 2. Vitamin content of protein-rich and protein-poor yeast at the end of the fermentation.

Expt. No. Experiment	10	5	6	1	2	3	4	9	7	8
Nitrogen source	asp.	asp.	asp.	ams.	ams.	ams.	ams.	asp.	asp.	asp.
Crude protein, %	8.4	10.0	10.2	18.4	25.4	43.2	43.8	50.8	53.5	60.7
Riboflavin										
μg/g yeast	28	61	65	100	124	161	187	250	140	150
μg/g protein	333	610	637	543	488	373	427	492	262	247
Pantothenic acid										
μg/g yeast	0	4	5	0	0	35	45	150	420	420
μg/g protein	0	40	49	0	0	81	103	295	785	692
Nicotinic acid										
μg/g yeast	30	97	86	190	210	465	421	105	77	124
μg/g protein	357	970	843	1 033	827	1 076	961	207	144	204
Pyridoxine										
μg/g yeast	4	3	4	12	14	16	17	10	13	16
μg/g protein	48	30	39	65	55	37	39	20	24	26

As can be seen from Table 2, the amounts of pantothenic acid present depended on the crude protein content of the yeast. If the crude protein content was low, no or very little pantothenic acid was present. The reason for the low pantothenic acid content in the protein-poor yeast may be that one of the components of this vitamin, β-alanine, is not formed when the nutrient solution is deficient in nitrogen. Nielsen, Sandegren and Ljungdahl [2] found β-alanine to be absent in protein-poor *Rh. gracilis* yeast whereas it was present in the protein-rich yeast.

With regard to the other vitamins the differences between the amounts present in the protein-rich and protein-poor yeast were not so striking. The amounts, calculated per g of yeast are somewhat smaller for the protein-poor yeast compared with the protein-rich one; for nicotinic acid this difference is rather insignificant. If calculated on g of protein, however, the amounts present are at least as high for the protein-poor yeast as for the protein-rich. There seems to be a tendency for the protein-poor yeast to be somewhat richer in the vitamins estimated than the protein-rich one, when calculated on a protein basis, which especially holds true for nicotinic acid. With regard to pantothenic acid the amounts present were, as mentioned above, very small in the protein-poor yeast.

The nicotinic acid appeared to be produced in higher amounts when the yeast was cultivated in the ammonium sulphate medium which was probably due to the low pH of the nutrient solution.

The vitamin contents of the protein-poor yeast, when calculated per litre nutrient solution, were of course much smaller than for the protein-rich one, due to the fact that the production of yeast was much less.

The amounts of vitamins calculated as μg per g did not vary much during the fermentation in the case of the protein-rich yeast. With regard to the protein-poor type a decrease could be noticed due to the fact that the yeast was growing poorer in protein, but if the vitamin content is expressed as μg per g protein the values were relatively constant during the fermentation. Calculated per litre nutrient solution a marked increase naturally occurs during the fermentation because of the increase in the amount of yeast.

In earlier investigations [8] it was found that the addition of thiamine and pantothenic acid stimulated the growth of *Rh. gracilis*, whereas the addition of nicotinic acid, riboflavin or pyridoxine had no effect. This is in good agreement with the fact that pantothenic acid under certain conditions is not formed at all or only in small amounts.

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