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Investigations on the Phosphorus Metabolism in *Rhodotorula gracilis*. I. The Influence of the Phosphate Content of the Nutrient Solution on the Formation of Fat

NIELS NIELSEN and N. G. NILSSON

Division of Food Chemistry, Royal Institute of Technology, Stockholm, Sweden

A number of microorganisms can be brought to form large quantities of fat under special cultural conditions. This fat formation takes place for example if there is a low nitrogen content in the nutrient solution, providing that plentiful quantities of sugar are present. In this way, for instance, *Rhodotorula gracilis* can be brought to contain up to 63 % fat, whereas the normal proportion of fat in this yeast is only about 8 % (Enebo, Anderson and Lundin [1]).

Fat formation can also be brought about, however, if the amounts of nutrient substances other than nitrogen are decreased below a certain level. In the case of *Rhodotorula gracilis*, Nielsen and Rojowski [2] have shown that a low sulphur or iron content can also bring about fat formation. As the fat content of the yeast increases, the protein content decreases, as is the case when the nutrient solution is nitrogen deficient. It can perhaps be assumed that the cause of fat formation is the same with low quantities of nitrogen, sulphur or iron, namely, a reduced protein formation with a correspondingly increased fat formation as a result thereof.

The investigation presented here has been made to show how fat formation in *Rhodotorula gracilis* is affected if the phosphate content of the nutrient solution is reduced. As phosphates are necessary for protein synthesis, a low amount of phosphate in the nutrient solution should cause a decrease in the protein content of the yeast, which in turn should result in an increased fat formation. At the same time, however, phosphates stimulate fat formation and therefore two different, opposing tendencies must be expected after reducing the phosphate content of the nutrient solution.

Our experiment was performed in the following way.

Rhodotorula gracilis was cultivated in a nutrient solution of varying phosphate content. The composition of the nutrient solution was as follows: 15 g asparagine — 3 g $MgSO_4$, $7H_2O$ — 1.5 g NaCl — 1.5 g $CaCl_2$, $6H_2O$ — 0.015 g $FeCl_3$ — 60 g glucose to 1 litre. Varying quantities of KH_2PO_4 were added to the solution, so that it contained per litre: 4.7 — 2.0 — 1.0 — 0.5 — 0.2 and 0.1 g KH_2PO_4. The pH was adjusted to 4.8 in all solutions.

The phosphate amount usually used for experiments with *Rh. gracilis* is 4.7 g KH_2PO_4 per litre, which is a rather excessive quantity.

The cultivation took place in 750 ml Erlenmeyer flasks each containing 300 ml of nutrient solution. The flasks were shaken at 25° C. After 5 days the protein and fat contents of the yeast were determined [3].

Table 1. Nutrient solution with 15 g asparagine per litre. Time of experiment: 5 days.

KH_2PO_4 g per litre	Fat %	Protein %	Fat + Protein %
4.7	10.9	50.6	61.5
2.0	12.2	47.5	59.7
1.0	11.8	51.3	63.1
0.5	24.2	35.6	59.8
0.2	37.8	21.3	59.1
0.1	39.2	18.8	58.0

From Table 1 it is clear that if the phosphate content of the nutrient solution falls below a certain level, the protein content of the yeast begins to decrease. This decrease in the protein content begins if the quantity of KH_2PO_4 sinks to 0.5 g per litre. At the same time as the protein content decreases, the proportion of fat in the yeast begins to rise and reaches 39 % as compared with 10 % in yeast which has been cultivated with an excess of phosphate.

A phosphate deficiency, therefore, brings about a decrease in the protein content of the yeast parallel with an increase in its fat content, as is also the case when the yeast is cultivated in a nutrient solution low in nitrogen, sulphur or iron. The fat formation is not so great, however, as when yeast is cultivated in a nitrogen-deficient nutrient solution. This question will be further discussed later. The above experiment was repeated and gave the same results.

In order to determine the effects of a low content of both phosphate and nitrogen occurring simultaneously, we have carried out an experiment in which the quantities of nitrogen and phosphate in the nutrient solution were varied at the same time.

As Table 2 indicates, if the nitrogen content of the nutrient solution (with optimal phosphate content) is decreased to 1 g of asparagine per litre the fat content of the yeast increases to 52.5 %. If the phosphate content is decreased to 0.2 g of KH_2PO_4 per litre (with optimal nitrogen content) the fat content of the yeast increases to 39.4 %. The fat content is consequently appreciably less in the latter case than with a low nitrogen content. If the

Table 2. Nutrient solution with varying quantities of asparagine and KH_2PO_4. Time of experiment: 5 days.

Aspargine g per litre	KH_2PO_4 g per litre	Fat %	Protein %	Fat + Protein %
15	4.7	11.2	48.8	60.0
15	0.2	39.4	18.8	58.2
1	4.7	52.5	14.4	66.9
1	0.2	43.2	15.6	58.8

nutrient solution is low in both nitrogen and phosphate at the same time (solution with 1 g asparagine and 0.2 g KH_2PO_4) the fat content of the yeast is lower than in a nutrient solution low in nitrogen only, namely 43.2 % fat as compared with 52.5 %. Two corresponding experiments gave the same results.

The reason why a lack of phosphate appears to decrease fat formation which has already resulted from a lack of nitrogen seems to be the fact that phosphate is essential to the formation of fat. When the maximum fat production has been reached as a result of the lack of nitrogen the low phosphate content, on the other hand, slows down the further formation of fat, and the fat content will therefore be lower than is the case with the normal quantity of phosphate. This conclusion also accounts for the fact that the fat formation brought about by a lack of phosphate is lower compared with that produced by a lack of nitrogen. The above experiments confirm the earlier results of Smedley MacLean and Hoffert [4,5] and Kleinzeller [6] concerning the significance of phosphates for the formation of fat.

SUMMARY

Fat production in *Rhodotorula gracilis* can be brought about by a low phosphate content in the nutrient solution, in the same way as by a low content of nitrogen, sulphur or iron. On account of the unfavourable effects of a lack of phosphate on the formation of fat, however, the production of fat is comparatively small.

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