EFFECT OF THIAMINE ON THE RATE OF FERMENTATION OF ZYMOHEXOSES AND OF MALTOSE BY BAKER'S YEAST

by

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INTRODUCTION

The studies of SCHULTZ, ATKIN AND FREY were the chief ones to make known that thiamine stimulates to a notable extent fermentation by baker's yeast low in thiamine. They treated the problem from the point of view of thiamine determination[2,11], using glucose for substrate. In passing, they mention that an addition of thiamine which in glucose fermentation produces an increase in carbon dioxide yield, causes an additional gas production also when sucrose or maltose replaces glucose[9].

In connection with certain other studies on fermentation, we came to test to what extent the activating effect of thiamine on the glucose fermentation by baker's yeast is extended to other zymohexoses, fructose and mannose, and in particular, how far it tends to abolish the known induction period of maltose fermentation by commercial baker's yeast.

EXPERIMENTAL

Material

The baker's yeast used was commercial ware, produced from beet molasses by the Rajamäki Factories of the State Alcohol Monopoly, Rajamäki. In some individual cases experiments were also made with precommercial culture stages, apparently richer in thiamine. They are produced by lesser aeration from molasses containing green malt wort and malt sprout extract. The brewer's yeast originated from the brewery of Oy P. Sinebrychoff Ab, Helsinki. The baker's yeast was obtained from the factory washed and pressed to yeast cake and was used as such while the brewer's yeast was washed by centrifugation, filtered in a Büchner funnel and sucked to the same degree of dryness as the commercial baker's yeast, containing about 25% dry matter. The dry yeast preparations were made from the commercial baker's yeast by crumbling and drying on a layer of filter paper in a thermostat room at 30° C.

Of the sugar preparations used, both glucose and maltose were Kerfoots' "pure bacteriological", fructose was "cryst., for scientific purposes" from Schering-Kahlbaum and mannose C.P. from Hema Drug Co. Thiamine was a product of Hoffmann-La Roche. Other chemicals used were of pure or C.P. grade.

Experimental procedure

The fermentation experiments were made in thermostat room at 30° C with a fermentometer in principle similar to that introduced by NILSSON[7]. Pyrex Erlenmeyer flasks of 50 ml were used as fermentation vessels; in the side of each flask was fitted with grindings a turnable 10 ml bulb (*cf.* NILSSON[7], Fig. 5d). The shaker made 115 oscillations (double swings) per min. As sealing liquid for the gas burettes 10% solution of calcium chloride was used (SCHULTZ, ATKIN AND FREY[11]), slightly colored with small amount of cupric chloride.

As shown by ATKIN, SCHULTZ AND FREY[8], thiamine is without significant effect upon the fermentation rate when added to a pure sugar-water solution but the effect is dependent on the

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simultaneous presence in the medium of magnesium, phosphate and assimilable nitrogen. Therefore, the experiments were made using their method[11], however, on half the quantities. Thus, the solution in the fermentation vessel contained in addition to buffer, salts and nicotinic acid, the amount of thiamine employed in 20 ml. To this was added the weighed amount of the particular sugar in crystals. The yeast suspension from the side bulb, containing 250 mg fresh yeast in 5 ml, was added to the fermentation solution at the start of the experiment by turning the bulb. The final fermentation solution, with a total volume of 25 ml, thus became 1 % in respect to yeast, 3 % in respect to sugar, and contained thiamine the amounts stated as hydrochloride and the following quantities per 100 ml of chemicals listed: $NH_4H_2PO_4$ 900 mg, $(NH_4)_2HPO_4$ 360 mg, $MgSO_4 \cdot 7H_2O$ 105 mg, KH_2PO_4 33 mg, KCl 25.5 mg, $CaCl_2 \cdot 2H_2O$ 7.5 mg, $FeCl_3 \cdot 6H_2O$ 0.15 mg, $MnSO_4 \cdot 4H_2O$ 0.15 mg, and nicotinic acid 1 mg. In the experiments with dry yeast the buffer-salt solution of the fermentation vessel, including the thiamine addition, contained 25 ml to which the weighed amount of the particular sugar was added in crystals and dissolved. The dry yeast, in the reported experiment 200 mg, was added at the start of the experiment by turning the side bulb.

RESULTS

The results are shown in the accompanying diagrams. In the fermentation of fructose and mannose thiamine activates the evolution of carbon dioxide in the same way

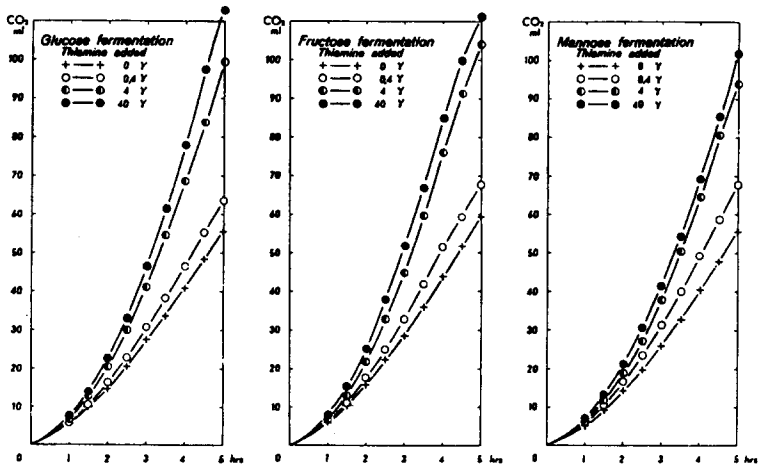

Fig. 1. Effect of thiamine addition on hexose fermentations by commercial baker's yeast. In some later experiments with commercial baker's yeast using glucose as substrate the rate of fermentation was distinctly higher.

as in the glucose fermentation (Fig. 1). On the other hand, it does not accelerate maltose fermentation at its start phases, *i.e.* it does not shorten the induction period of maltose fermentation by commercial baker's yeast (Fig. 2); indirectly this already appears from the studies of SCHULTZ *et al.*[8,10]. But once the maltose fermentation has started thiamine does increase the rate of carbon dioxide production as it does with zymohexoses (*cf.* SCHULTZ *et al.*[9]).

According to the latest studies of the WESTENBRINK school, made by MAESEN[6] (*cf.* LEIJNSE AND TERPSTRA[5])

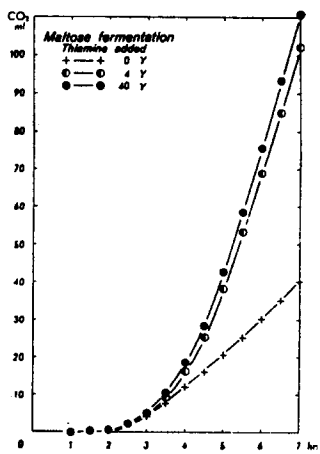

Fig. 2. Effect of thiamine addition on maltose fermentation by commercial baker's yeast.

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the effect of thiamine upon the glucose fermentation under these conditions depends quantitatively upon the formation of carboxylase, this enzyme being the limiting factor of fermentation of commercial baker's yeast. It is noteworthy, that the induction of maltose fermentation by commercial baker's yeast is not abolished despite the presence of nitrogen—and sulphur—in the fermentation solution, the percentage of which LEIJNSE AND TERPSTRA[5] as well as MAESEN[6] have proved to be sufficient for the synthesis of the apoenzyme of carboxylase.

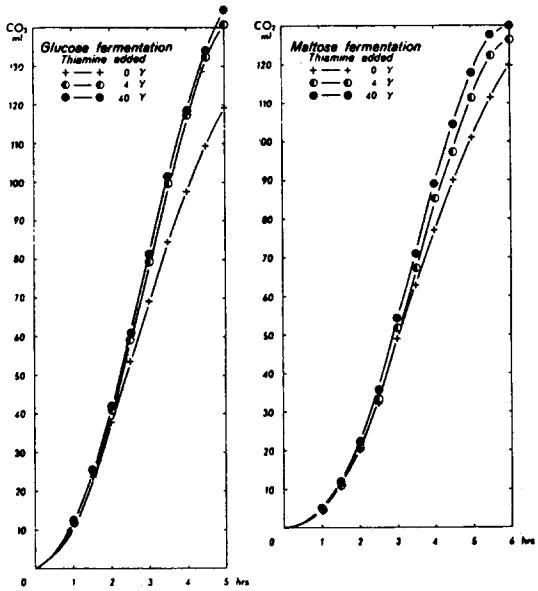

Fig. 3. Effect of thiamine addition on the fermentation of glucose and maltose by precommercial A_3 stage of baker's yeast, representing a more anaerobic industrial culture phase.

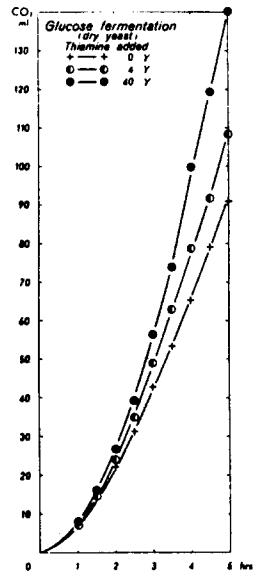

Fig. 4. Effect of thiamine addition on glucose fermentation by dried commercial baker's yeast.

Our experiments with brewer's yeast, using glucose and maltose as substrates, confirmed that added thiamine has no influence on the fermentation of brewer's yeast (*cf.* ATKIN AND GRAY[1] and MAESEN[6]). As to baker's yeast, on the other hand, the experiments with glucose and maltose as substrates with less aerated precommercial yeast stages, where the action of thiamine is slighter (Fig. 3), confirm again the common concept that the activating effect of thiamine is even with baker's yeast more pronounced with the commercial low-vitamin generation.

It may be mentioned that HEYNS[4] was unable to find any effect of thiamine in the sense of increased yield of carbon dioxide with maceration juice from dry baker's yeast. In our experiments on glucose fermentation with dry preparations of commercial baker's yeast, the effect of thiamine was distinct though less than with fresh yeast (Fig. 4).

SUMMARY

Addition of thiamine activates the evolution of carbon dioxide by commercial baker's yeast when fructose and mannose as well as glucose serve for substrates, under the conditions defined by SCHULTZ, ATKIN AND FREY.

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Addition of thiamine activates glucose fermentation by dried commercial baker's yeast but to a lesser extent than with fresh yeast.

Addition of thiamine does not bring about the abolition of the induction period characteristic of maltose fermentation by commercial baker's yeast, despite the nitrogen and sulphur content of the fermentation solution, which according to the earlier studies does afford a possibility for the synthesis of the apoenzyme of carboxylase.

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