THE INTERACTION OF ZYMOHEXOSES AND MALTOSE IN MALTOSE FERMENTATION BY BAKER'S YEAST

by

HEIKKI SUOMALAINEN, ERIK AXELSON AND ERKKI OURA

Research Laboratories of the State Alcohol Monopoly, Helsinki (Finland)

INTRODUCTION

The studies of LEIBOWITZ AND HESTRIN[2] as well as of SCHULTZ AND ATKIN[5] have revealed that an addition of a small amount of glucose is able to abolish the known induction period of maltose fermentation by commercial baker's yeast. This phenomenon is sc distinct that SCHULTZ AND ATKIN recommended it for determination of small amounts of glucose present as impurities in maltose preparations. The cause of this particular action of glucose has so far remained unsolved despite all attempts. In addition to glucose, LEIBOWITZ AND HESTRIN[3] (*cf.* also SPIEGELMAN *et al.*[9]) have, however, been able to show that other zymohexoses also, *viz.* fructose and mannose —but not galactose—exert a similar effect. On the other hand, they claimed that maltose did not act as an inhibitor in glucose fermentation by baker's yeast.

The question of the mechanism of maltose fermentation is still open (*cf.* MYRBÄCK AND RENVALL[4]). We have in this case started from the idea introduced once by SLATOR[7] with regard to the fermentation of glucose and galactose, *viz.*: "If these two reactions were quite distinct, and no interference between them took place, the rate of evolution of carbon dioxide from the fermentation of a mixture of the two sugars would be the sum of the rates of the two singly". And although the fermentation of maltose and zymohexoses were, to a slight extent only, regarded as separate reaction chains, the small amounts of zymohexoses in question are in no case sufficient for the saturation of zymase complex. Its insufficiency cannot therefore restrict the fermentation rate. On the contrary, in view of the above-mentioned accelerating effect of zymohexoses on maltose fermentation, the fermentation rate of maltose solution containing zymohexoses should still be considerably higher than the sum of the rates of the components singly.

EXPERIMENTAL

Material

The baker's yeast used was commercial material, produced from beet molasses by the Rajamäki Factories of the State Alcohol Monopoly, Rajamäki. The yeast was received from the factory washed and pressed to yeast cake containing about 25% dry matter and was used as such. The dry yeast preparations were prepared by pressing the fresh yeast through a wire net (8 mashes per cm) in thin threads upon a layer of filter paper, and air-dried at room temperature.

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.

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from Hema Drug Co. In later experiments with dry yeast and in chromatographic studies, pure maltose from Thomas Morson & Son, Ltd., London, was used. Other chemicals were of pure or C.P. grade.

Experimental procedure

The fermentation experiments were performed as mentioned previously[10]. The fermentation vessel contained 20 ml buffer-salt solution used by SCHULTZ *et al.*[6]; weighed amounts of sugar were added to it in crystalline form and dissolved. The yeast suspension, containing 250 mg fresh yeast in 5 ml, was added to the fermentation solution at the start of the experiment by turning the side bulb. The final fermentation solution, with a volume of 25 ml, thus was made 1 % in respect to yeast, 3 % in respect to sugar—the hexose additions of maltose fermentations exceeded this amount while the parallel experiments contained hexose "additions" alone in the solution—and contained per 100 ml the following amounts 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, $CaCl \cdot 2H_2O$ 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 volume of the buffer-salt solution in the fermentation vessel was 25 ml, to which weighed amounts of sugar were added in crystalline form and dissolved. The dry yeast—in the experiments reported 125 mg—was added at the start of the experiment by turning the side bulb.

The chromatograms of the fermentation experiments were run with *n*-butanol–pyridine–benzene–water (5:3:3:1) solution[1] and developed with silver nitrate solution.

RESULTS

As can be seen from the diagrams (Fig. 1) the observation of LEIBOWITZ AND HESTRIN[3] and SPIEGELMAN *et al.*[9] could be confirmed, *viz.* that all the three zymohexoses shorten the induction period of maltose fermentation by commercial baker's yeast. Maltose, on the other hand, has a distinctly retarding effect on the fermentation of small amounts of hexoses added (Fig. 2). Thus, not even in a single case does the fermentation rate of hexoses in the maltose solution reach—let alone exceed—the fermentation rate of the corresponding hexose amounts separately in the solution. It could be demonstrated by paper chromatography using a 3% suspension of baker's yeast that an 0.3% addition of glucose, which disappeared almost entirely within 1 h when added separately to the solution, required about 2 h to disappear from a 3% maltose solution. With mannose, the difference was still more pronounced: 0.3% addition of mannose separately to the solution disappeared almost completely in about 2 h, in 3% maltose solution in not less than about 4 hours.

Maltose has a distinct retarding effect on the fermentation of all zymohexoses, glucose, fructose, and mannose alike. It is of interest to note, that the abolishing

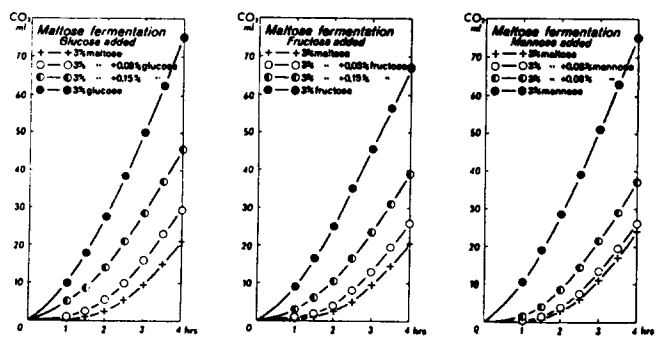

Fig. 1. Effect of small additions of zymohexoses on the induction period of maltose fermentation by commercial baker's yeast. Intervariations in the fermentation rates of zymohexoses are partly due to experiment series being run with different lots of yeast.

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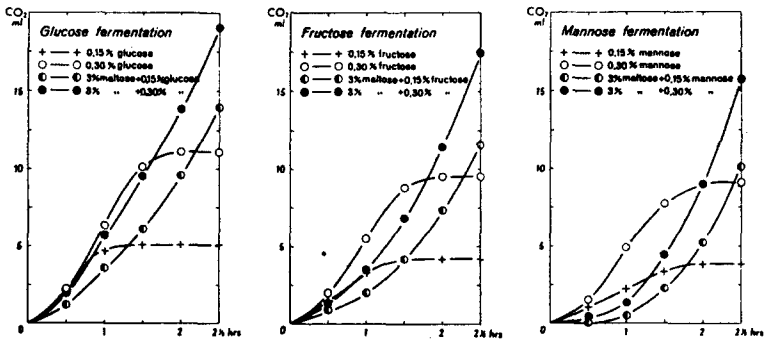

Fig. 2. Fermentation rates of small amounts of zymohexoses by commercial baker's yeast as such and in maltose solution.

effect of zymohexoses on the induction period of maltose fermentation and the retarding effect of maltose on the fermentation of zymohexoses are inversely proportional. Thus, the effect of maltose on the mannose fermentation is most retarding and the effect of mannose on the maltose fermentation least activating.

The retarding effect of maltose being detectable also with dry yeast (Fig. 3) it cannot be thought that the entrance of sugar molecules into the cells is prevented by the effect of unfermentable sugar—*i.e.* maltose during its induction period—as SOBOTKA, HOLZMAN AND REINER[8] assumed with regard to the "pentose effect" they noted with brewer's yeast. There is presumably a competition for a certain factor connected with the fermentation enzyme complex.

If maltose were fermented by baker's yeast after preceding hydrolysis to glucose, this ought to be detectable in the fermentation solution unless the activity of α-glucosidase (maltase) were the limiting factor for fermentation. It has not been possible, however, to prove the appearance of glucose in the fermentation solution of maltose fermentation by baker's yeast as has been done with brewer's yeast[11]. If again, the α-glucosidatic activity of baker's yeast were the limiting factor of maltose fermentation, glucose added to the fermentation solution ought to disappear rather quickly, its rate of fermentation being greater than the rate of formation. Yet, if maltose itself—as has been shown—acts as an inhibitor for glucose fermentation, maltose might also retard its own fermentation, especially with increasing concen-

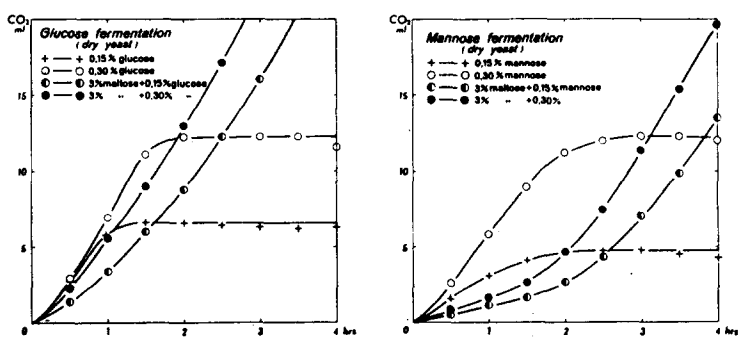

Fig. 3. Fermentation rates of small amounts of zymohexoses by dried commercial baker's yeast as such and in maltose solution.

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trations. It can at least be assumed that the more concentrated the maltose solution, the more effective the competitive effect in respect to glucose fermentation.

LEIBOWITZ AND HESTRIN[3] reported that high concentrations of maltose may inhibit the rate at which maltose is fermented by aged baker's yeast. We have noted that high concentrations of maltose retard its own fermentation by fresh—and dry—baker's yeast, whereas glucose fermentation in glucose solutions of even twice the same molar concentration is not notably retarded[12]. Yet, after glucose which followed maltose as an impurity had fermented away we were unable to show by paper chromatography the presence of glucose in the maltose fermentation solution, to say nothing of its enrichment. The problem of the inhibitory effect of maltose on its own fermentation by baker's yeast will be returned to in another paper. Since the presence of maltose, as already stated, retards the fermentation of hexoses, the absence of glucose from the fermentation solution suggests that maltose fermentation is retarded for some other reason than the depressing action of maltose on the fermentation of glucose formed through hydrolysis or phosphorolysis.

SUMMARY

Zymohexoses activate maltose fermentation by commercial baker's yeast, glucose in the highest degree, mannose in the least. Maltose on the other hand, retards the fermentation of small amounts of zymohexoses to the extent that the rate of carbon dioxide formation is distinctly higher when zymohexoses are present alone in the solution than when maltose is also present. The phenomenon is most pronounced with regard to mannose, least so with glucose. It is also detectable with dried yeast.

The retarding effect of maltose on the fermentation of zymohexoses has also been proved by paper chromatography.

Higher concentrations of maltose retard its own fermentation but it has not been possible to show the occurrence of glucose in the fermentation solutions by paper chromatography. This suggests that the retardation would be ascribable to some other reason than the depressing effect of maltose on the fermentation of glucose formed either by hydrolysis or phosphorolysis.

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