THE FERMENTABILITY OF MALTOSE BY BAKER'S YEAST CONTAINING TREHALOSE

by

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INTRODUCTION

It is known that the full-speed fermentation of maltose by baker's yeast is preceded by a relatively long induction period. The abolishment of this induction period has frequently been ascribed to adaptation to maltose fermentation[9] (*cf.* also SPIEGELMAN[11,12]). Yet it can also be assumed that the induction period is necessary in order to remove a certain factor preventing fermentation of maltose.

LEIBOWITZ AND HESTRIN[3,4,2] have observed that α-methyl glucoside prevents maltose fermentation by baker's yeast, an observation from which they have made rather far-reaching conclusions regarding the mechanism of maltose fermentation. On the other hand, MYRBÄCK's studies[7,8] in particular have revealed that baker's yeast, especially commercial baker's yeast, regularly contains relatively large amounts of trehalose, in some cases up to 14% of the dry weight. It can be presumed, that the high trehalose content of the cell blocks up a certain enzyme system necessary for the maltose fermentation, whether it be an α-glucosidase system or some other. Maltose fermentation cannot gain full speed before the blockade has been relaxed, the acceleration being promoted by small amounts of zymohexoses added to the fermentation solution.

We have attacked this problem by studying the occurrence of the induction period of maltose fermentation by precommercial culture stages of baker's yeast on the one hand and the effect of added trehalose on the fermentation of maltose, and glucose, on the other. The studies by BRANDT[1] had already revealed that very little trehalose, if any at all, is found in baker's yeast stages grown under more anaerobic conditions, its amount becoming more significant only in the more aerated precommercial yeast stage. Hence, if the trehalose content of a yeast cell has any effect on the maltose-fermenting ability, the induction period of maltose fermentation should not appear until the precommercial yeast stage and should become more pronounced at the commercial stage. On the other hand, it could be expected that if trehalose added to the fermentation solution prevents maltose fermentation as does α-methyl glucoside introduced by LEIBOWITZ AND HESTRIN, then this inhibiting action ought to be detectable also at earlier yeast stages.

EXPERIMENTAL

Material

The baker's yeast used was technical preparation produced from beet molasses by the Rajamäki Factories of the State Alcohol Monopoly, Rajamäki. The yeast cream samples of more anaerobic

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culture stages A_2 and A_3 were washed and sucked in a Büchner funnel to contain about 25% dry matter. The samples of the precommercial A_4 stage and of the commercial A_5 stage were obtained from the factory washed and dried containing about 25% of dry matter and were used as such. It may be stated in brief that the different yeast stages under investigation are derived from each other as follows. The A_2 stage is produced, using in factory conditions propagated yeast as seed yeast, almost anaerobically under very weak aeration. The A_3 stage again is cultured from the A_2 yeast with an aeration of about 30 m^3 per m^3 of fermentation solution per hour. The A_4 stage is cultured from the A_3 stage with a vigorous aeration, 50 $m^3/m^3/h$ and the A_5 stage in turn from the A_4 yeast under entirely aerobic conditions avoiding alcohol formation, with an aeration of 80 $m^3/m^3/h$. The A_5 stage is commercial ware. During the whole process the yeast does not come into contact with maltose-containing raw material.

The dry yeast preparations were made by pressing fresh yeast through a wire net with small meshes (8 meshes per cm) in thin threads upon a layer of filter paper, upon which it was air dried at room temperature.

The brewer's yeast originated from the brewery of Oy P. Sinebrychoff Ab, Helsinki. It was obtained from the brewery as yeast cream, washed and sucked in a Büchner funnel to contain about 25% of dry matter.

The maltose used was "pure maltose" of Thomas Morson & Son, Ltd., London. Trehalose was prepared from the fresh baker's yeast of the Rajamäki Factories according to MYRBÄCK[7,8]; it was proved by paper chromatography to be glucose-free, further, it was ascertained that it contained no heavy metal impurities. α-Methyl glucoside was likewise our own preparation[10]; determined by chromatography it contained about 0.1% glucose as impurity.

Experimental procedure

The fermentation experiments were performed as previously mentioned[14]. The fermentation vessel contained 7.5 ml yeast cream or a weighed amount of dry yeast suspended in 7.5 ml tap water; the maltose solution, 5 ml, was added to the yeast suspension at the start of the experiment by turning the side bulb. When investigating the effect of trehalose or α-methyl glucoside on the maltose fermentation, weighed amounts of these were dissolved in the fermentation vessel in a known amount of tap water, to which suspended fresh yeast or weighed dry yeast was then added; analogous to the earlier procedure, 5 ml of maltose solution was added from the side bulb at the start of the experiment to 7.5 ml of yeast suspension. The final fermentation solution with a total volume of 12.5 ml, became thus 6% with respect to maltose, 4% with respect to commercial baker's yeast, 2% with respect to brewer's yeast and 1.1% with respect to dry baker's yeast corresponding to 4% fresh yeast.

RESULTS

The results show that although glucose fermentation by all examined stages of baker's yeast is more rapid than maltose fermentation, only from the precommercial A_4 stage onwards can there be any talk about the induction period of maltose fermentation. The induction period of the A_4 stage is then of nearly the same magnitude as that of the commercial A_5 stage, differing distinctly from the maltose fermenting ability of the A_2 and A_3 stages (Fig. 1). This can be regarded as being compatible with BRANDT's[1] observations on the trehalose contents of different baker's yeast

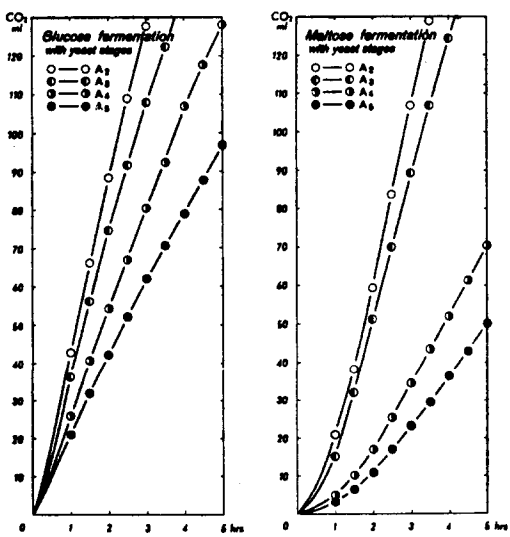

Fig. 1. Fermentability of glucose and maltose with different industrial stages of baker's yeast. A_2 stage produced nearly anaerobically, A_5 stage represents commercial product. 6% maltose, 4% fresh yeast in all experiments.

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stages, the yeasts corresponding to the two first mentioned stages both contain according to him less than 1% trehalose, those corresponding to the two latter ones contain 3.6% and about 7% trehalose of the dry matter. It should be noted that the fermentation rate of glucose is admittedly retarded from the A_2 to the A_5 stage, but the change is less striking.

Trehalose added to the fermentation solution depresses maltose fermentation by commercial baker's yeast (the rate of glucose fermentation is not affected) but not to as great an extent as α-methyl glucoside which, according to LEIBOWITZ AND HESTRIN[3], acts as an inhibitor to maltose fermentation (Fig. 2). The difference in the degree of the effect is not surprising. For instance, α-methyl glucoside is unfermentable by baker's yeast, while trehalose is fermented, though slowly. It should also be noted that the induction period of maltose fermentation—presumably due to internal trehalose of cell—is not continuous in commercial baker's yeast but is abolished with time. It is noteworthy that trehalose added to the fermentation solution depresses maltose fermentation even more strongly than α-methyl glucoside when used with the A_3 stage of baker's yeast which ferments maltose without an induction period (Fig. 3).

The added trehalose as well as α-methyl glucoside has also been found to possess a distinct retarding effect on maltose fermentation by dried commercial baker's yeast, trehalose having even a stronger effect than α-methyl glucoside (Fig. 4), a result which supports strongly the idea advanced in the outlining of the problem.

The added trehalose also impedes maltose fermentation by anaerobically grown brewer's yeast, differing distinctly from α-methyl glucoside (Fig. 5), which LEIBOWITZ AND HESTRIN[3] had found to have practically no effect on maltose fermentation by brewer's yeast. Both the glucosides are slightly fermentable

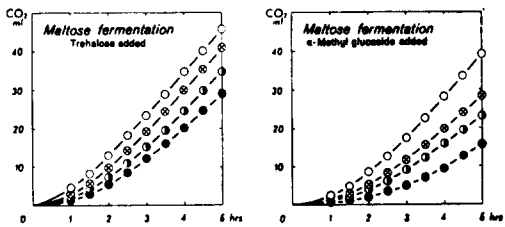

Fig. 2. Effects of added trehalose and α-methyl glucoside on the fermentation of maltose by commercial baker's yeast. O——O 6% maltose only, ⊗——⊗ with 0.1 *M*, ◑——◑ with 0.2 *M*, and ●——● with 0.3 *M* added sugar. 4% fresh yeast in all experiments.

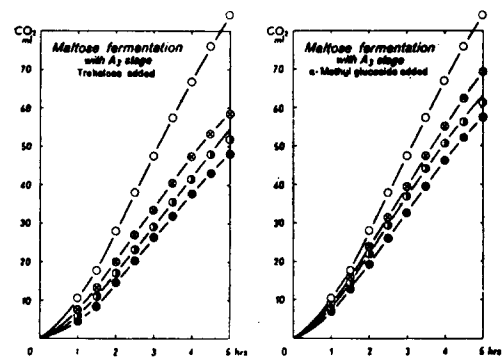

Fig. 3. Effects of added trehalose and α-methyl glucoside on the fermentation of maltose by the industrial A_3 stage of baker's yeast produced with limited aeration. O——O 6% maltose only, ⊗——⊗ with 0.1 *M*, ◑——◑ with 0.2 *M*, and ●——● with 0.3 *M* added sugar. 2% fresh yeast in all experiments.

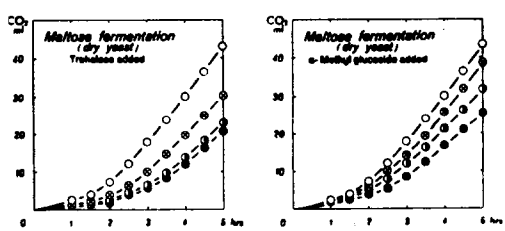

Fig. 4. Effects of added trehalose and α-methyl glucoside on the fermentation of maltose by dried commercial baker's yeast. O——O 6% maltose only, ⊗——⊗ with 0.1 *M*, ◑——◑ with 0.2 *M*, and ●——● with 0.3 *M* added sugar. Dry yeast 1.1% in all experiments, corresponding to 4% of fresh yeast.

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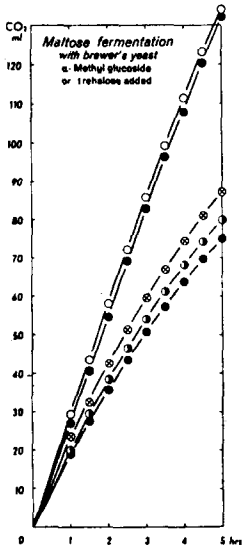

by brewer's yeast. It may be added that even the brewer's yeast which has previously been considered to lack trehalose, has by improved methods been found to contain it though in small amounts, at most 4% of the dry matter[13]. We confirmed the occurrence of a very small quantity (0.5% dry matter) of trehalose in the brewer's bottom yeast used by us[16].

Fig. 5. Effect of added trehalose and α-methyl glucoside on the fermentation of maltose by brewer's yeast. ○—○ 6% maltose only, ⊗—⊗ with 0.1 *M*, ◑—◑ with 0.2 *M*, ●—● with 0.3 *M* added trehalose, and ⫼—⫼ with 0.3 *M* added α-methyl glucoside; no noteworthy retardation of fermentation was caused even by an addition of 0.6 *M* α-methyl glucoside, as can be seen from the values representing the formation of carbon dioxide by the used 2% suspension of fresh yeast: 1 h 23.5 ml, 2 h 46.7 ml, 3 h 72.2 ml, 4 h 98.0 ml and 5 h 121.7 ml. Trehalose and α-methyl glucoside were both fermentable by brewer's yeast, although slightly, the formation of carbon dioxide in 5 h being of the order 20–25 ml.

It is typical of the industrial production of baker's yeast that during progression from anaerobic conditions to aerobic ones the nitrogen and phosphorus contents of yeast are reduced from stage to stage by limiting the supply of nitrogen and phosphorus; simultaneously the sulphur content of yeast is also reduced in spite of the sulphur excess present[15]. In many connections recently an attempt has been made to ascribe the lowered enzymic activity of low-nitrogen organism to enzyme deficiency brought about by decreased protein content. In one instance at least regarding carboxylase[5,6], the addition of nitrogen and sulphur has been proved to accelerate the enzymic activity of commercial baker's yeast—but also that of anaerobically grown brewer's yeast. We have, however, noted previously[14] that the low content of nitrogen, phosphorus and sulphur in the commercial baker's yeast cannot be an immediate cause of the induction period of maltose fermentation, for their inclusion in the fermentation solution did not abolish the induction period of fermentation.

In passing, it may be mentioned that the abolishing effect of glucose on the induction period of maltose fermentation by commercial baker's yeast is perhaps explainable by assuming that zymohexose is indispensable for starting the machinery of maltose fermentation and that the internal trehalose of the cell blocks up the α-glucosidase or other enzyme system thus preventing glucose formation.

SUMMARY

Induction period of maltose fermentation can hardly be detected at the early, rather anaerobically grown A_2 and A_3 culture stages of baker's yeast whereas at the aerobically grown precommercial A_4 and commercial A_5 stages it is distinct. According to BRANDT, the trehalose content of the corresponding yeast stages is for A_2 and A_3 a maximum of 1%, for A_4 and A_5 3.6% and about 7%, respectively, of the dry matter.

Trehalose added to the fermentation solution clearly depresses maltose but not glucose fermentation by commercial baker's yeast. The effect is not quite as distinct as with α-methyl glucoside, but still of the same magnitude. The added trehalose prevents maltose fermentation even more strongly than does α-methyl glucoside at the trehalose-low A_3 stage. The inhibitory effect of added trehalose as well as that of α-methyl glucoside on the maltose fermentation is demonstrable also with dried commercial baker's yeast.

Trehalose, contrary to α-methyl glucoside, definitely depresses maltose fermentation by anaerobically grown brewer's yeast.

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