Production of Phenylacetylcarbinol by Various Yeast Species

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ABSTRACT

Measurements of tolerance to the addition of benzaldehyde were carried out with six yeast species: Hansenula anomala, Brettanomyces vini Peynaud et Domercq, strain X, Saccharomyces carlsbergensis, Saccharomyces cerevisiae R XII, Saccharomyces ellipsoideus and Torula utilis. The techniques used were: comparison of evolution of carbon dioxide with and without benzaldehyde and production of phenylacetylcarbinol after a single addition of benzaldehyde (0.2%) and after four additions (total of 0.8%).

The highest decarboxylase activity in the presence of benzaldehyde was found with Hansenula anomala, Saccharomyces carlsbergensis and Saccharomyces cerevisiae. In Hansenula anomala, benzaldehyde caused a 16% inhibition of fermentation, in the other cultures inhibition lay between 35.5 and 63.2%. After a single addition of benzaldehyde the greatest amount of phenylacetylcarbinol was formed by Hansenula anomala, Saccharomyces carlsbergensis and Saccharomyces cerevisiae. The yield of phenylacetylcarbinol in these cases was between 46.0% and 51.5 weight% (calculated on added benzaldehyde). During fermentation with a higher benzaldehyde concentration, Saccharomyces carlsbergensis utilized 70% aldehyde for the formation of phenylacetylcarbinol while the rest was mostly reduced to benzylalcohol. Phenylacetylcarbinol was estimated after extraction of the fermentation medium with ether polarographically and polarimetrically, benzaldehyde was estimated polarographically directly in the medium.

The formation of optically active phenylacetylcarbinol from benzaldehyde, observed first by Neuberg (1921) in yeast fermenting a sugar substrate, depends first of all on the carboxylase activity of the yeast cells and on the tolerance of yeast to added benzaldehyde. Neuberg used mostly top brewer’s yeast (Senst). The literature contains no further data about the effect of the application of various types of yeast on the production of phenylacetylcarbinol.

The individual factors important for the production of phenylacetylcarbinol by baker’s yeast were investigated earlier (Hanč & Kakáč, 1962). In the present work we attempted to test the tolerance of further six types of yeast toward benzaldehyde and to examine the production of phenylacetylcarbinol from a single dose of benzaldehyde as compared with several divided doses, using otherwise standard conditions which had been shown earlier to be optimum for baker’s yeast.

Phenylacetylcarbinol is formed during fermentation from benzaldehyde added to the yeast suspension and from pyruvic acid in the presence of carboxylase, as was also demonstrated in a cell-free system of purely enzymic nature (Hanč & Kakáč, 1956).
PRODUCTION OF PHENYLACETYL CARBINOL

\[ \text{C}_6\text{H}_5\text{CHO} + \text{CH}_3\text{COOOH} \xrightarrow{\text{yeast carboxylase}} \text{C}_6\text{H}_5\text{CH(OH)COCH}_3 + \text{CO}_2 \]

The role of thiamine pyrophosphate (coenzyme A) during this process is not limited to decarboxylation of pyruvic acid but is of decisive importance for the decarboxylase reaction of the aldehyde component with the two-carbon residue remaining after decarboxylation of pyruvic acid just as is known from the production of acetoin (Hanč, 1960; Krampitz, Suzuki & Greuil, 1961).

**MATERIALS AND METHODS**

*Microorganisms used.* The following yeast cultures were used: *Hansenula anomala* from the collection of the Research Institute of the Brewing Industry, Prague; *Brettanomyces vini* Peynaud et Domercq, strain X, No. 416 from the collection of the Research Institute of the Wine Industry, Bratislava; *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae* R XII, *Saccharomyces ellipsoides* and *Torula utilis* from the collections of the Faculty of Food Chemistry of the College of Chemical Technology in Prague. The cultures were maintained on wort agar slopes at 0—5 °C. For *Brettanomyces vini* which forms a considerable amount of acid during growth, 3 g. of finely ground CaCO₃ was added per 100 ml. wort agar used.

*Propagation of cultures.* Cultures from agar slopes were used to inoculate 200 ml. sterile wort medium (concentration 8° Bg, pH 5.0) and the suspension incubated at 30°C. After 24—48 hrs. of growth the inoculum was used to start cultivation in 25 litres of medium of the same composition in a glass kettle; cultivation proceeded under aeration for 24—48 hrs. at 30°C. The grown culture was separated on a Sharpless centrifuge and used immediately for experiments. In each propagated culture the dry weight was also estimated.

*Test for fermenting capacity.* The fermenting capacity of a propagated culture was estimated from the volume of carbon dioxide released during 6-hours' fermentation on sucrose. For the experiment a total of 1.5 g. yeast and 50 ml. nutrient medium with 5% sucrose, 0.2% peptone (Spofa), 0.1% MgSO₄. 7H₂O and 0.2% KH₂PO₄, of pH 5.5, were used. Readings were made by the usual technique of measuring the volume of liquid displaced from a stock bottle by the carbon dioxide liberated. Fermentation vessels were placed in a thermostat at 30°C. Measurements were carried out either in simple sucrose medium or in the presence of 0.2% benzaldehyde. The amount of carbon dioxide released was calculated in the results per 100 mg. dry weight yeast introduced initially.

*Ketol fermentation.* Fermentation was carried out in 1000 ml. Philips flasks containing 500 ml. medium and placed in a thermostat at 30°C, under constant stirring. The following nutrient medium was used: 3.5% crude sugar, 6% beet molasses, 0.05% MgSO₄. 7H₂O, the pH being adjusted to 5.5 with phosphoric acid. The initial concentration of fresh yeast was 3% (the dry weight amounting to 27.5% of the fresh weight). One hour after the start of fermentation 0.2% distilled benzaldehyde was added to the mash, followed by an equal volume of acetdehyde (c. 50%). After 1, 2, and 3 hours, samples were removed which were analyzed for benzaldehyde and phenylacetylcarbinol.

In yeast forming a larger amount of ketol after a single dose of benzaldehyde, the production of phenylacetylcarbinol was investigated after adding increased amounts of benzaldehyde. The total concentration of benzaldehyde added was 0.8% (weight%). Benzaldehyde was added to the fermenting mash in four