Research article

Biotransformation of Unsaturated Aliphatic Aldehydes Using Baker's Yeast

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Actively fermenting baker's yeast (*Saccharomyces cerevisiae*) not only converts aliphatic aldehydes to the corresponding alcohols, but also may reduce certain carbon-carbon double bonds in the same molecule. Furthermore, an *in situ* acyloin condensation reaction occurs; this bioconversion reaction gives rise to relatively good yields of unsaturated 2,3-diols, which have two carbon atoms more than the corresponding aliphatic aldehyde used as substrate.

Baker's yeast has been used as a reagent in organic synthesis since the beginning of the 20th century, when fundamental studies were initiated on the mechanism of formation of fusel alcohols from the corresponding l-amino acids during the formation of ethanol.[1] Growing interest in the synthesis of chiral molecules in enantiomerically pure form has promoted considerable development in biocatalysis; such transformations mediated by baker's yeast, in particular, have attracted new interest.

The microbial hydrogenation of the carbon-carbon double bond in α,β-unsaturated aldehydes or alcohols is well-documented.[2] Baker's yeast appears to be particularly easy to use for this purpose. Furthermore, it has been known for many years that actively fermenting baker's yeast will convert benzaldehyde into optically active phenylacetyl carbinol through the acyloin condensation of acetaldehyde and benzaldehyde.[3] Under normal fermentation conditions—for example, in water and at a pH range between 5 and 7.5—yeast displays a wide range of chemical capabilities. Because most of the enzymes active in baker's yeast will display their properties under similar conditions, it is very often the nature of the substrate in the biotransformation that drives the overall reaction in one direction or the other.

A recent publication describing the generation of odoriferous acyloins by yeast and their occurrence in fermented foodstuffs, such as sherry and soy sauce, prompted us to report the results obtained quite a number of years ago during the systematic investigation of the action of baker's yeast on unsaturated aliphatic aldehydes.[4] The initial objective of this work was to study and optimize the reaction conditions under which the carbon-carbon double bond in *trans*-2-hexenal undergoes reduction.

Experimental

The reduction reactions typically were performed as follows:

d-Glucose (60 g) was dissolved in tap water (1,200 mL, pH 6.0) in a round-bottomed flask; then 120 g of fresh baker's yeast were added. The flask was fitted with an air lock and the contents stirred at room temperature using a magnetic stirrer. After approximately 45 min (rapid evolution of carbon dioxide), the substrate (1.2 g) was added. Stirring continued for 24 h. During this period, samples occasionally were drawn and analyzed.

The crude reaction product was obtained by filtering off the spent yeast, saturating the aqueous phase with salt and extracting with ethyl acetate. After drying and solvent removal, GC analyses were performed using standard equipment and procedures.

Results and Discussion

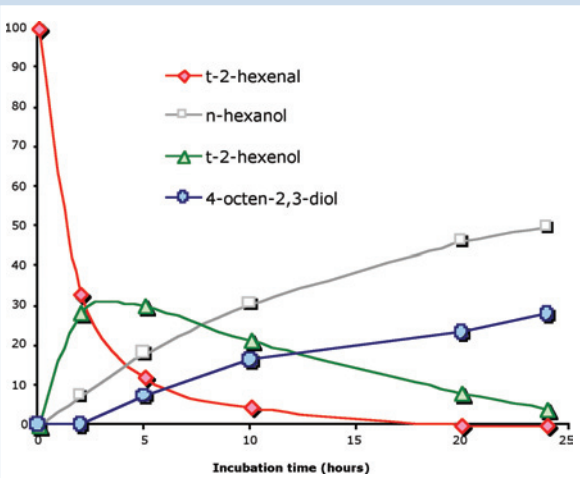
Quite a number of years ago, natural n-hexanol was in short supply and consequently was expensive. At that

time, it made economical sense to attempt to obtain natural n-hexanol via the reduction of natural *trans*-2-hexenal using baker's yeast. The results obtained from the preliminary trials were promising, and relatively good yields of n-hexanol were obtained this way. These results encouraged us to investigate the overall reaction in more detail in order to optimize the production procedure.

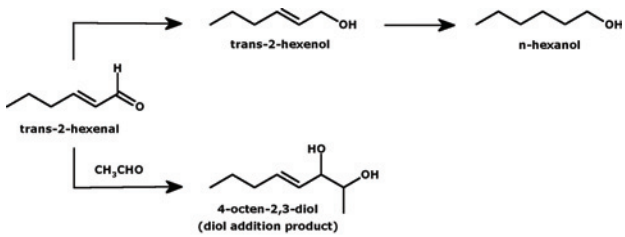
The initial results obtained indicated that during the 24-h reduction period, the *trans*-2-hexenal disappeared entirely in the reaction mixture and produced not only n-hexanol as the main product, but also two other compounds—both in reasonably good yields. Subsequently performed GC/MS studies of the crude product indicated that one of the initially unknown compounds was the corresponding alcohol, *trans*-2-hexenol; the other, 4-octen-2,3-diol, contained eight carbon atoms. Conclusive structural proof was achieved via NMR spectrometry performed on samples that had been purified via preparative gas chromatography.

The typical time profile obtained for the conversion of *trans*-2-hexenal into the three products is shown in F-1. It would seem quite obvious that *trans*-2-hexenol occurs as an intermediate in the reduction process and that the amount of this alcohol actually found in the final product depends upon the reaction time chosen. The reaction scheme we suggest for the overall reductive conversion of *trans*-2-hexenal in this system is shown in F-2; this scheme implies two separate bioconversions that run parallel. The formation of the diol addition product certainly implies an acyloin condensation reaction between the *trans*-2-hexenal starting material and acetaldehyde, the latter being the intermediate produced *in situ* by the actively fermenting yeast during the conversion of glucose into ethanol.

Product composition—time profile for the reduction of *trans*-2-hexenal using baker's yeast **F-1**

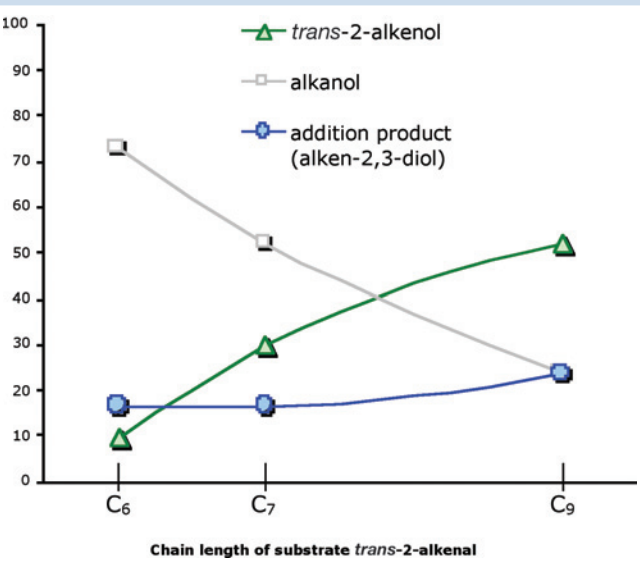


Proposed mechanism of the overall reduction reaction catalyzed by baker's yeast **F-2**



In order to learn more about this interesting reaction system, we decided to perform further trials with fermenting baker's yeast, but in the presence of other unsaturated aliphatic aldehydes as substrate. F-3 shows the accumulated data from those trials performed on *trans*-2-hexenal and its higher homologues, *trans*-2-heptenal and *trans*-2-nonenal, after a reaction period of 24 h.

Effect of chain length of *trans*-2-alkenals upon the composition of the bioconversion products obtained using baker's yeast (the alken-2,3-diols referred to here are the higher homologues of 4-octen-2,3-diol that were obtained in the *trans*-2-hexenal system) **F-3**



Obviously, the fundamental reactions originally shown for *trans*-2-hexenal also take place in F-3, whereby the extent of reduction of both the carbon-carbon double bond and the aldehyde group seems to depend upon the size of the rest of the substrate molecule. The addition reaction

between the individual aldehyde and acetaldehyde to form the acyloin, which then is converted to the diol addition product, does not seem to be influenced in the same way; the yield of the latter remains more or less constant at about 20%.

Extension of this reaction to the corresponding unsaturated alcohols and their positional isomers and other isomeric aliphatic aldehydes resulted in the following general conclusions being drawn:

1. The aldehyde group always is reduced, irrespective of whether a carbon-carbon double bond is present.
2. Nonconjugated carbon-carbon double bonds do not undergo reduction; this is the case for both the unsaturated aldehydes and alcohols tested.
3. In the case of α,β-unsaturated alcohols, the configuration of the carbon-carbon double bond plays an important role in that the *trans*-double bond is reduced well, whereas the *cis*-double bond is not. Furthermore, with *trans*-2-hexenol as substrate, approximately the same amount of the diol addition product is formed, as is the case with *trans*-2-hexenal. This may indicate that a common intermediate is involved and that the unsaturated alcohol actually first undergoes oxidation to the unsaturated aldehyde.
4. The speed at which *trans*-α,β-unsaturated aldehydes are reduced seems to depend upon the length of their alkyl chains. Thus, the shorter the chain, the more rapid the reduction, and the longer the chain, the greater the amount of the corresponding intermediate formed (the unsaturated alcohol).

The results shown here differ somewhat from those recently reported.[4] In the present system, only diols (and no acyloins) were detected as elongation products. It could be of interest to investigate this difference in more depth.

The substrate *trans*-2-hexenal actually was investigated in both studies, and the different products formed perhaps can be explained by the fact that, in the one case, a crude enzyme extract was used, whereas in this report intact baker's yeast was the biocatalyst.[4] The crude enzyme extract reportedly also was able to convert n-hexanal into the corresponding 3-hydroxy-2-octanone. This fact would indicate that the initial addition of acetaldehyde to the original aldehyde group must be more rapid than the reduction of the carbon-carbon double bond; otherwise, saturated diols also would have been detected in our studies. On the other hand, the well-known inhibitory properties of aldehydes—in particular, α,β-unsaturated aldehydes—obviously are not that strong in this system. Otherwise, no biotransformation reactions would have been observed in either of these studies.[5]

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