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PHYSICOCHEMICAL PROPERTIES AND KINETICS OF TRANSFERASE ACTIVITY OF INVERTASE

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The transferase activity of yeast invertase is found as a function of pH and reaction temperature. The degree of conversion into alkylfructosides depends on the substrate (alcohol) and enzyme concentrations and on the incubation time of the reaction mixture. The effect of ethanol concentration on transferase activity of the enzyme is determined.

Invertase (KF 3.2.1.26) is one of the most important enzymes for carbohydrate exchange in microbes and higher plants [1, 2]. Preparations of yeast invertase have the ability to hydrolyze saccharose and exhibit transferase activity. This enables them to carry out several transfructose syntheses. Thus, ethylfructoside is prepared using yeast invertase (hydrolysis of saccharose in alcohol medium) [2]. The formation of alkylfructosides in fermentation media has also been reported [1].

Our goal was to study certain properties of the transferase activity of yeast invertase in ethanol medium. Obviously the application of these investigations to several branches of fermentation production, in particular, the liquor industry, is connected to the study of the nature of higher alcohols and the provision of a criterion for developing measures to decrease the content of fusel oils in liquor products.

Studies of the kinetics of the transferase activity of invertase revealed a dependence of invertase activity on the pH of the reaction medium (Fig. 1). Increasing the pH toward the basic side in 0.1 M acetate buffer led to a 40% loss of enzyme activity. The optimal temperatures of the hydrolytic and transferase reactions are different. Thus, hydrolytic activity was practically nonexistent at 0°C for hydrolysis of saccharose by invertase [3] whereas transferase activity was 13% of the maximal value (0.191 EU/mg). Increasing the enzyme concentration in reaction medium affects on the rate of conversion of isoamyl alcohol into alkylfructoside. A high level of alcohol conversion (35%) is observed at an enzyme concentration of 3.5 mg/ml. Invertase concentrations above 3.5 mg/ml are less effective. In our opinion, this is due to enzyme aggregation, which reduces its activity.

The degree of conversion of isoamyl alcohol depends on the incubation time and reaches a maximum four hours after the start of the reaction (from 0.12 to 0.5% of isoamyl alcohol in the reaction medium). Increasing the alcohol concentration further gradually decreases the transferase activity of the enzyme. This is probably due to inhibition of the enzyme by substrate.

We studied the effect of various ethanol concentrations on the transferase activity of invertase because the ethanol concentration reaches 20-40% in liquor production. We note that a high ethanol concentration can disrupt the conformational structure of the enzymatic protein.

A test of ethanol as part of the solvent demonstrated that the rate of the transferase reaction increases with increasing concentration (30-40%). This effect is probably explained by the high solubility of isoamyl alcohol in ethanol. As a result, conditions are created for effective formation of the enzyme—substrate complex with hydrophobic isoamyl alcohol and the transferase activity of invertase is increased with a simultaneous increase of the ethanol concentration (to 40%). On the other hand, the reduced rate of isoamyl alcohol conversion at ethanol concentrations >40% (50%) may decrease the degree of hydration of the enzyme. Thus, invertase from *Saccharomyces cerevisiae* exhibits an optimum in transferase reactions at pH 4.5. The optimal temperature for enzyme activity is 25°C. The degree of alcohols conversion depends on several factors, in particular, enzyme and substrate concentrations and reaction time of the enzyme and substrate.

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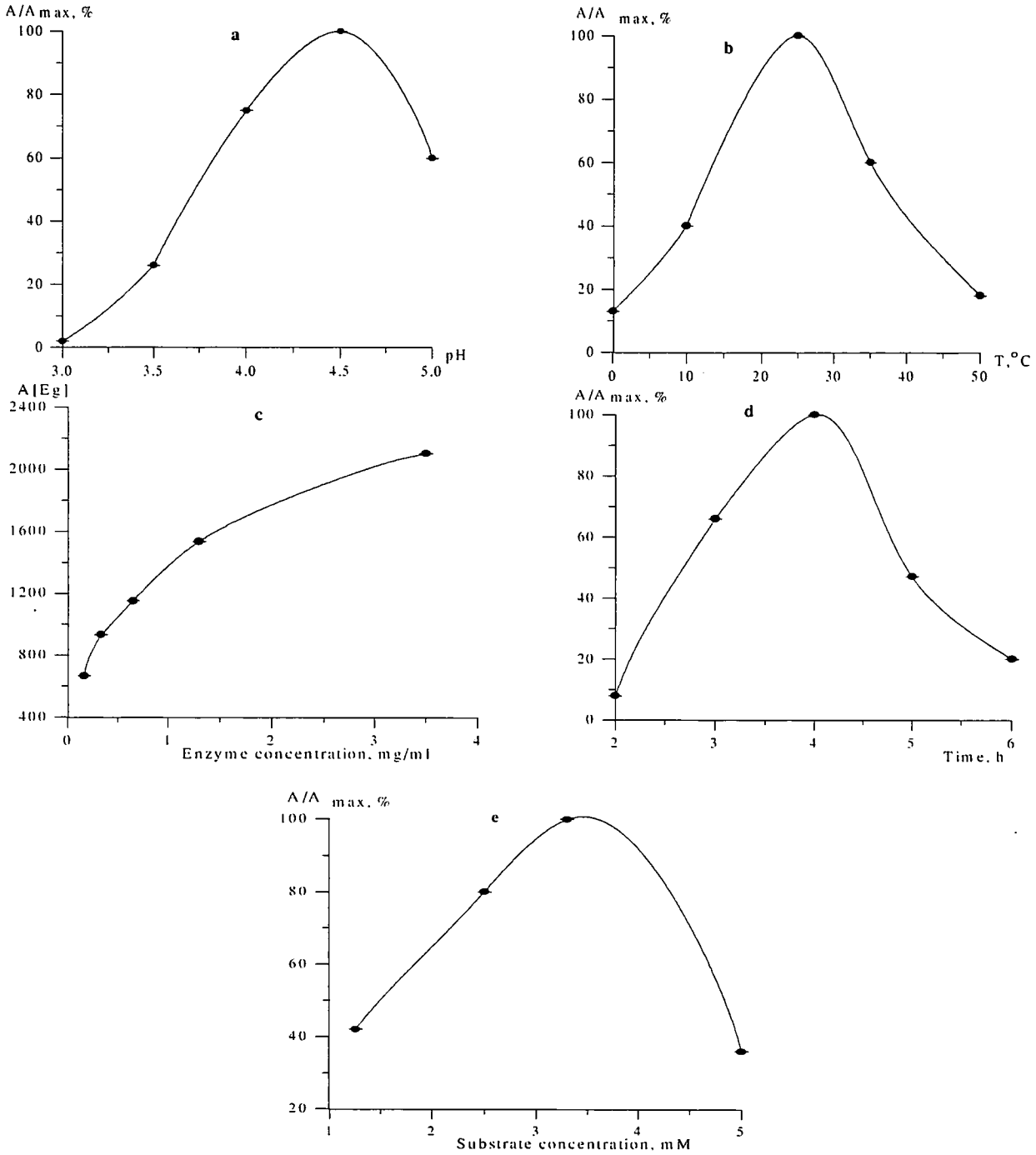

Fig. 1. Transferase activity of invertase as a function of incubation-medium pH (a), reaction temperature (b); enzyme concentration in the medium in the presence of isoamyl alcohol (c), reaction medium incubation time (d), and isoamyl alcohol concentration (e).

EXPERIMENTAL

Invertase (KF 3.2.1.26) isolated from baker's yeast *Saccharomyces cerevisiae* was used. **The invertase was isolated**

and purified as follows:

1. Baker's yeast was subjected to two freeze—thaw cycles and extracted with 0.1 M acetate buffer at pH 5 (1:1) for 2 h at 4°C. The extract was centrifuged at 6000 rpm at 4°C for 30 min.

2. The yeast extract was treated with ammonium sulfate (up to 70% saturated). Extraneous proteins were separated by centrifugation at 15,000 rpm for 1 h.

3. Gel-filtration of the desalted extract was performed on a Sephadex G-50 (Serva) column (1.5 × 60 cm) equilibrated with 0.01 M Tris-HCl buffer at pH 7.5.

4. Ion-exchange chromatography was performed on a column (1 × 10 cm) filled with DEAE-TOYOPEARL 650M (Toyo Soda Manuf. Co. Ltd., Japan) equilibrated with 0.01 M Tris-HCl buffer at pH 7.5.

Enzyme activity of invertase was determined as follows. A solution containing saccharose (10%) and ethanol (40% by volume) was divided into two equal portions. One of these was treated with enzyme solution (50 µl, 0.65 mg). The other was a control containing the same amount of enzyme inactivated by heating. Transferase activity was determined by the decrease in the amount of isoamyl alcohol in the medium, which was quantitatively determined by GLC on a Chrom-5 instrument [4]. The solvent phase for isoamyl alcohol was 40% ethanol; for K_m determination, 20% acetonitrile. Transferase activity of invertase was calculated using the formula:

$$A = (S_k - S_u)m/S_k t,$$

where m is the amount of isobutanol or isoamyl alcohol in the incubation medium (mM), S_u is the area under the peak of isoamyl alcohol (or other alcohol) calculated from the GLC analysis, S_k is the area under the peak of isoamyl alcohol (or other alcohol) in the control, and t is the incubation time (min).

The unit transferase activity was taken as the amount of isoamyl alcohol (mM) converted to alkylfructoside after 1 h at 25°C and pH 4.5. Protein in the reaction medium was determined by the Lowry method [5].

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