The Enzymatic Approach to Making of Alcoholic Beverages

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Abstract: *Immobilized yeast invertase was applied for treatment of alcoholic beverages with the aim of transformation of higher alcohols into alkylfructosides. Gas-liquid chromatography of treated water-alcoholic medium containing 3.0 mg/l isoamyl alcohol and 4% saccharose by immobilized invertase had shown the convertion of 40% isoamyl alcohol, which amounts to 1.8 mg/l absolute alcohol. Other parameters remained at the previous level. The high level of enzyme activity was observed when the initial concentration of sucrose in the reaction mixture attained 4.0-12.5%. Tasting of treated samples indicated the improvement of quality and degustational properties of beverages, they had softer and more harmonious taste and aroma in comparison with control sample and finished Vodka, which completed the cycle of technological processing.*

Keywords: *Yeast invertase, Immobilized invertase, Alkylfructosides, Higher alcohols, Isoamyl alcohol, Enzymatic transformation of alcohols, Fusel oil, Enzymes in non-conventional media, Transferaze activity of invertase, Invertase in organic media.*

Introduction

Improving degustational properties of alcoholic beverages by applying activated charcoal or other modern smart carbon materials has been one of the fast-developing fields of beverages technology for the last two decades and importance in it is still increasing [1]. On the other hand, both in science and technology, as well as economy and society, a strong shift towards biotechnological topics can be observed thus putting immobilized enzymes more and more into the focus of interest [2-4]. Therefore the transition from pure chemical purification to more bio-oriented research and production is currently under way. Additionally, development the quality of alcoholic beverages that meet international standard ISO 22000 is one of contemporary tasks of beverages industry. In this relation awoke the necessity of applying new ways of their treatment in a bioreactor with immobilized invertase which provide production of the high quality products with improved degustational and hygienic properties. As distinguished from adsorption, here, toxic and pungent odourant higher (fusel) alcohols represented substantially by isobutil and isoamyl alcohols are bound up with fructose to form alkylfructosides which possess pleasant fruit aroma and some biological activity. In current work it is firstly presented the application of covalently immobilized on polyamide particles yeast invertase [5] in technology of alcoholic beverages production.

Experimental

Materials and methods

Intact invertase (EC 3.2.1.26) was isolated from *Saccharomyces cerevisiae* [6]. The enzyme was covalently immobilized on polyamide particles by the method described in [5]. High purified ethanol (purity grade "Prima") was provided by the public corporation "Combinat Tashkentvino". Sorting (a term used to define 40 vol% aqueous ethanol prior to the

technological processing that converts it to Vodka) possessed following parameters: etanol-40 vol%, precipitate – transparent, alcality (0.1M HCL, ml) in 100 ml of water – alcoholic mixture – 0.5 mg/l, isoamyl alcohol content – 3 mg/l, aldehydes content – 8 mg/l, esters content – 18 mg/l scaled of absolute alcohol. Sucrose served as the substrate. The activity of invertase in the aqueous medium was determined by the method described in [7]; the quantity of yielded (generated) glucose in the reaction mixture was measured by the method described in [8]. One unit of transferase activity of invertase corresponded to conversion of 1μM isoamyl alcohol into alkylfructosid per 1 h under optimal conditions, the activity was calculated per 1 mg protein. Gas-liquid chromatography of alcohols was performed using a 5.8 m stainless steel column with an internal diameter of 3-4 mm, packed with charged spherochrom-2 (UNZ-600), in a Chrom-5 chromatograph (Czech Republic) equipped with a flame-ionization detector as specified by the appropriate State Standard [9]. The content of admixtures in alcohols was determined by using an internal standard (n-octane).

Results and discussion

It was demonstrated that the initial concentration of substrate is a limiting factor in the enzyme-substrate interactions. The dependence of the enzyme activity in hydrolytic reactions on the initial concentration of sucrose in the medium is shown in Fig. 1. The high level of enzyme activity (114 Unit) was observed when the initial concentration of sucrose in the reaction mixture attained 7.5%. It is also found that enzyme activity remains high enough within a broad range of sucrose concentrations (5.0-12.5%). At concentrations as high as 12.5%, the immobilized enzyme was, as rule, less efficient in catalyzing the reaction, likely sue to substrate inhibition; the enzyme activity decreased up to 10%.

The data are of applied significance for the enzymatic treatment of alcoholic beverages, which is performed with the aim of decreasing the content of higher alcohols and improving thereby their quality.

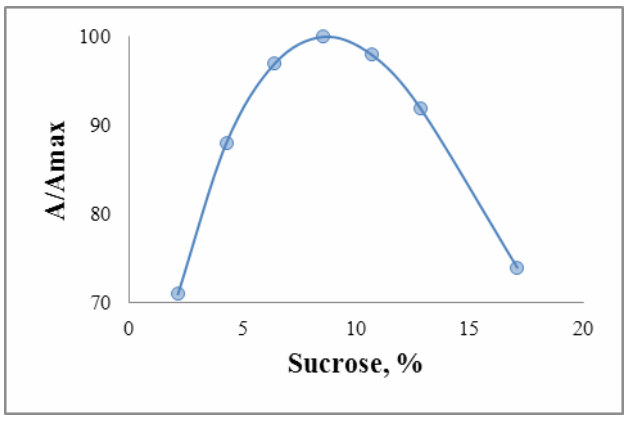

Fig. 1 The dependence of the initial rate of sucrose hydrolysis from sucrose concentration in the medium. A_{max} – 114 mM/min, 25°C. A/A_{max} is relative enzyme activity.

In order to check for the ability of immobilized invertase to convert higher alcohols in alcoholic beverages, a series of experiments were performed. For this, blend containing 40 vol% ethanol and 4% sucrose were prepared from rectified ethanol (96.6 vol% ethanol, 4 mg/l bulk higher alcohols). The blends were treated with immobilized enzymes preparation under conditions favoring optimum conversion. Gas liquid chromatography of sorting, performed before (A) and after (B) treatment (Fig. 2) demonstrates conversion of 40% isoamyl alcohol, which amounts to 1.8 mg/l absolute alcohol. Other parameters remained at

the previous level. Tasting of treated sorting has indicated the improvement of the quality and degustation properties of beverages, the treated sample had a softer and more harmonious taste and aroma in comparison with the control sample and finished Vodka, which completed the cycle of technological processing. This is accounted for the binding of higher alcohols to alkylfructosides [4, 6]. Thus, the research provides the information on the behavior of invertase in water-ethanol medium and the Data obtained may have important practical implications, for example, for application of immobilized yeast invertase in transformation of fusel alcohols into alkylfructosides in producing of various alcoholic beverages. Gained results permit to develop bioreactor packed (filled) with immobilized yeast invertase for the fusel oils biotransformation in alcoholic beverages technologies. Immobilized invertase made good use in the production of alcoholic beverage and appreciated at its high quality taste. This was resolved by continuously passing prepared beverage through bioreactor which installed at the end of the existing flowsheet for making Vodka (Fig. 3).

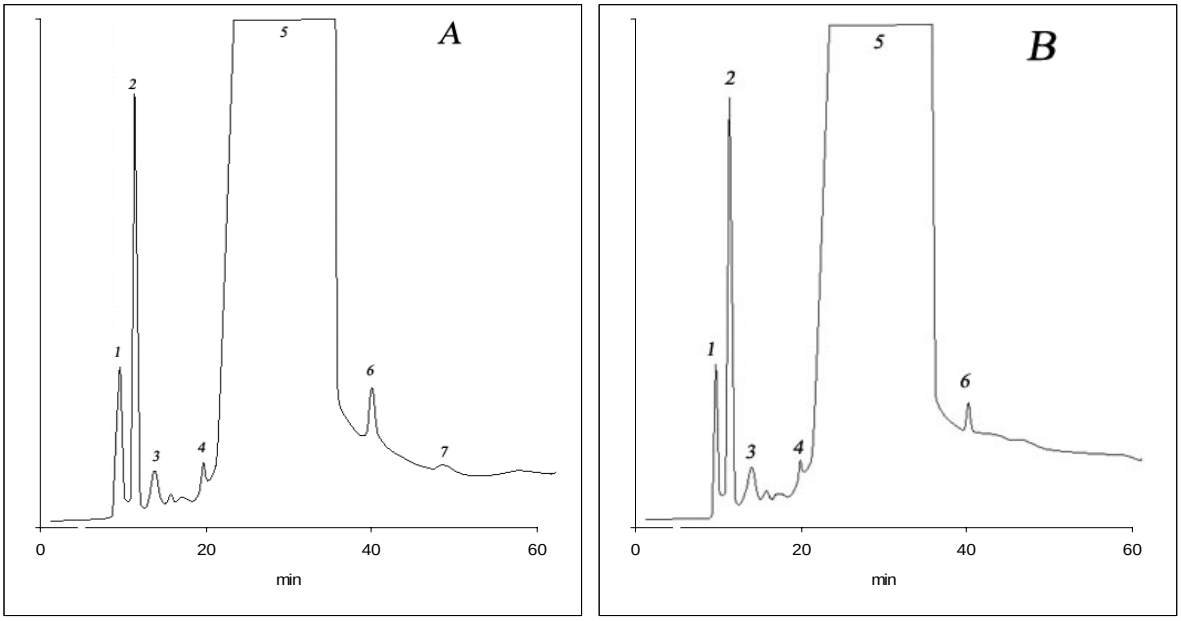

Fig. 2 Gas liquid chromatography of water-alcoholic mixtures before (A) and after treatment (B) by immobilized invertase. 1 – ethilacetat, 2 – n-octan, 3 – acetaldehyd, 4 – isopropil alcohol, 5 – ethil alcohol, 6 – isoamil alcohol.

Vodka manufacturing is put into effect in the following way: rectified spirit from spirit tank (1) comes into measuring spirit tank (2). Spirit from measuring spirit tank (2) and softened water from softened water tank (3) enter through discharge heads (4, 5) the contiguous running mixer (6). Alcohol-water mixture product first goes prefiltered (10), then runs through reactors with charcoal (12, 13, 14) and finishing filter (15), and enters the tank for purified alcohol-water mixture (16). Here are added supplements (17) and mixture treated with immobilized enzyme where fusel alcohols transformed into alkylfructosides. Further product through the tank for ready-made product (20) goes into bottling.

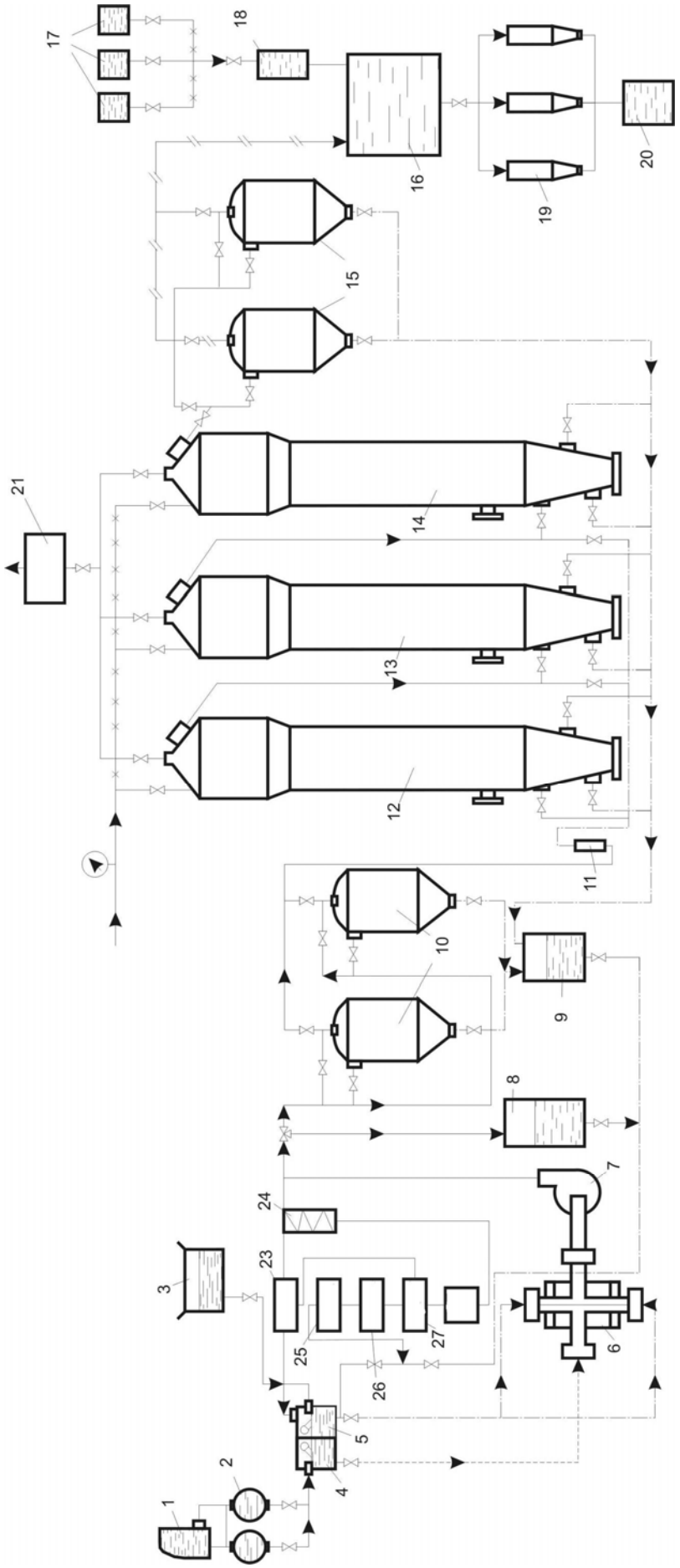

Fig. 3 Application of immobilized invertase in the Vodka manufacturing scheme:
1 – spirit tank; 2 – measuring spirit tank; 3 – softened water tank; 4 – controller of spirit discharge head; 5 – controller of softened water discharge head; 6 – running mixer; 7 – centrifugal pump; 8 – tank for defective alcohol-water mixture; 9 – tank for alcohol-water wastes after charcoal regeneration; 10 – prefilter; 11 – flow-measuring apparatus; 12, 13, 14 – reactors with activated charcoal, 15 – finishing filter; 16 – tank for purified alcohol-water mixture, 17 – tanks for additives, 18 – controller of discharge head for additives solutions, 19 – bioreactor with immobilized invertase, 20 – tank for ready-made product.

Conclusions

To summarize the above mentioned, the new approach in the technology of producing of alcoholic beverages is developed, which has numerous advantages in comparison with present-day (existing) traditional technologies. Treatment of alcoholic beverages in bioreactor filled with immobilized invertase permits:

1. to transform toxic fusel oil alcohols into alkylfructosides by providing the production of the high quality products such as Vodka, liquors, aperitifs, vermouths, brandies etc, with reduced content of high alcohols as well as with improved degustational and hygienic properties;
2. to become available the simplification of existing flow sheets;
3. to introduce a big variety of new products because of fruit adore of alkylfructozides;
4. to carry out the production of the immobilized invertase preparation directly at the wine-making plants.

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