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Mono- and biphasic solvent systems for the enzymatic synthesis of tyrosol fructoside: Investigation of deep eutectic solvents and organic solvents

AUTHORS

Klaudia KARKESZOVÁ / DEPARTMENT OF CHEMICAL AND BIOCHEMICAL ENGINEERING, FACULTY OF CHEMICAL AND FOOD TECHNOLOGY, INSTITUTE OF CHEMICAL AND ENVIRONMENTAL ENGINEERING, SLOVAK UNIVERSITY OF TECHNOLOGY, RADLINSKÉHO 9, BRATISLAVA
Monika ANTOŠOVÁ / DEPARTMENT OF CHEMICAL AND BIOCHEMICAL ENGINEERING, FACULTY OF CHEMICAL AND FOOD TECHNOLOGY, INSTITUTE OF CHEMICAL AND ENVIRONMENTAL ENGINEERING, SLOVAK UNIVERSITY OF TECHNOLOGY, RADLINSKÉHO 9, BRATISLAVA
Elena KARNIŠOVÁ POTOCKÁ / INSTITUTE OF CHEMISTRY, SLOVAK ACADEMY OF SCIENCES, DÚBRAVSKÁ CESTA 9, BRATISLAVA
Vladimír MASTIHUBA / INSTITUTE OF CHEMISTRY, SLOVAK ACADEMY OF SCIENCES, DÚBRAVSKÁ CESTA 9, BRATISLAVA
Milan POLAKOVIČ / DEPARTMENT OF CHEMICAL AND BIOCHEMICAL ENGINEERING, FACULTY OF CHEMICAL AND FOOD TECHNOLOGY, INSTITUTE OF CHEMICAL AND ENVIRONMENTAL ENGINEERING, SLOVAK UNIVERSITY OF TECHNOLOGY, RADLINSKÉHO 9, BRATISLAVA

PURPOSE OF THE ABSTRACT

Tyrosol and its glycosides protect cells from oxidative stress and have several health benefits. Herein, Saccharomyces cerevisiae β-fructofuranosidase catalysed synthesis of tyrosol fructoside (TyrFru) from sucrose and tyrosol is reported. The highest TyrFru yield of 20% was achieved under optimized conditions: 1.5 M sucrose, 10 g/L tyrosol, pH 6, and 40°C. Decreased product yield is caused by simultaneous fructosidase-specific reactions occurring in the reaction medium. Tyrosol, as the acceptor of fructosyl residues, competes with water in the hydrolysis reaction and even with sucrose in fructan synthesis.

The addition of organic solvents to the reaction medium can reduce the side reactions dependent on water. Also, the solvents can cause conformational changes that affect the enzyme's flexibility and change its substrate specificity. Hence, the effect of water-miscible and -immiscible organic solvents as well as of novel deep eutectic solvents (DESs) on the TyrFru biosynthesis was studied.

Monophasic organic-water systems were constructed with nine organic solvents with log P values of -1.35 to 0.5. Effects of these polar solvents considering the initial reaction rates of all three reactions and product yield were investigated. An addition of even 5 % of co-solvent had an adverse effect on the product yield. All monitored solvents caused a decrease in the yield of TyrFru from 20 % obtained in the buffer to 15–10 %.

The measurements of the initial rates of concurrent reactions revealed that, contrary to expectations, the hydrolytic activity of the enzyme was promoted. At cosolvent concentration of 15%, β-fructosidase was catalytically active only in DMSO, DMF, dioxane, and acetone, and the formation of TyrFru was even lower. However, the hydrolytic activity of the enzyme in 15 % (v/v) dioxane was still 1.38 times higher than in the cosolvent-free system.

The addition of DESs was applied in the concentration range from 5 to 70% (v/v) to investigate the possibility of shifting the reaction equilibrium towards fructoside synthesis. The effect of choline chloride- (ChCl), choline acetate- (ChAc), and betaine-based DESs on the activity and TyrFru synthesis was studied (Karkeszová et al.2023).

None of the twelve DESs investigated increased the enzyme activity. As the β-fructosidase activity may not always correlate with its efficiency in tyrosol transfructosylation, five DESs were selected as the reaction medium for TyrFru synthesis. Among them, four DESs were selected because of the retention of medium and high enzyme activity and ChAc: Urea as the “worst-performing” solvent.

The transfructosylation of tyrosol was monitored at three DES concentrations from 10% (v/v) to 40% (v/v); it decreased with the increasing concentration of DESs and completely stopped in the presence of 40% DESs. Surprisingly, among the tested DESs, ChAc: Urea had the best properties for TyrFru production.

However, strong stabilizing effect of DES was confirmed at lower concentrations (Holla et al. 2021), suggesting that the loss of activity is due to the catalyst interference and not because of enzyme denaturation. However, the destabilizing effect of DES is more pronounced than the stabilizing effect with their increasing concentration.

TyrFru synthesis in buffer/organic (70:30, v/v) biphasic systems was investigated. Tyrosol was more soluble in the organic phase formed by solvents with log P from 0.73 to 1.78. Due to the unavailability of the substrate for the enzyme, TyrFru production was low. In solvents with log P above 2.1, most of the tyrosol was in the aqueous phase and therefore the course of transfructosylation was very similar to that in water. The formed TyrFru was not soluble in any of the organic phases and therefore no shift in the reaction equilibrium was achieved by removing the product from the organic phase.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

tyrosol fructoside | Saccharomyces cerevisiae β-fructofuranosidase | deep eutectic solvents | aqueous-organic solvent systems

BIBLIOGRAPHY