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Selective Immobilization of Fructosyltransferase onto Glutaraldehyde Modified Support and Its Application in the Production of Fructo-Oligosaccharides

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Abstract : In recent decades, the scientific community has recognized the growing importance of prebiotics, and therefore, numerous studies are focused on their economic production due to their low presence in natural resources. It has been confirmed that prebiotics is a source of energy for probiotics in the gastrointestinal tract (GIT) and enable their proliferation, consequently leading to the normal functioning of the intestinal microbiota. Also, products of their fermentation are short-chain fatty acids (SCFA), which play a key role in maintaining and improving the health not only of the GIT but also of the whole organism. Among several confirmed prebiotics, fructooligosaccharides (FOS) are considered interesting candidates for use in a wide range of products in the food industry. They are characterized as low-calorie and non-cariogenic substances that represent an adequate sugar substitute and can be considered suitable for use in products intended for diabetics. The subject of this research will be the production of FOS by transforming sucrose using a fructosyltransferase (FTase) present in commercial preparation Pectinex® Ultra SP-L, with special emphasis on the development of adequate FTase immobilization method that would enable selective isolation of the enzyme responsible for the synthesis of FOS from the complex enzymatic mixture. This would lead to considerable enzyme purification and allow its direct incorporation into different sucrose-based products without the fear that the action of the other hydrolytic enzymes may adversely affect the products' functional characteristics. Accordingly, the possibility of selective immobilization of the enzyme using support with primary amino groups, Purolite® A109, which was previously activated and modified using glutaraldehyde (GA), was investigated. In the initial phase of the research, the effects of individual immobilization parameters such as pH, enzyme concentration, and immobilization time were investigated to optimize the process using support chemically activated with 15% and 0.5% GA to form dimers and monomers, respectively. It was determined that highly active immobilized preparations (371.8 IU/g of support - dimer and 213.8 IU/g of support – monomer) were achieved under acidic conditions (pH 4) provided that an enzyme concentration was 50 mg/g of support after 7 h and 3 h, respectively. Bearing in mind the obtained results of the expressed activity, it is noticeable that the formation of dimers showed higher reactivity compared to the form of monomers. Also, in the case of support modification using 15% GA, the value of the ratio of FTase and pectinase (as dominant enzyme mixture component) activity immobilization yields was 16.45, indicating the high feasibility of selective immobilization of FTase on modified polystyrene resin. After obtaining immobilized preparations of satisfactory features, they were tested in a reaction of FOS synthesis under determined optimal conditions. The maximum FOS yields of approximately 50% of total carbohydrates in the reaction mixture were recorded after 21 h. Finally, it can be concluded that the examined immobilization method yielded highly active, stable and, more importantly, refined enzyme preparation that can be further utilized on a larger scale for the development of continual processes for FOS synthesis, as well as for modification of different sucrose-based mediums.

Keywords : chemical modification, fructooligosaccharides, glutaraldehyde, immobilization of fructosyltransferase
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