SUCROSE ENZYMATIC HYDROLYSIS ASSESSED USING AN *IN VITRO* INTESTINAL DIGESTION MODEL

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Introduction. Some individuals with congenital deficiency of intestinal specific enzymes cannot hydrolyze disaccharides. Among these enzymes is invertase. The medical recommendation is related to avoid sugar intake or oral administration of this enzyme. Considering pH variations and the protease presence on digestive system, invertase protection is needed, which can be achieved through immobilization technology. In the present study prickly pear peel fiber and *Plantago* fiber were chosen as carriers of invertase in order to develop an alimentary supplement for sucrose intolerance regulation.

The objective of the present study was to evaluate the behavior of immobilized invertase for the hydrolysis of sucrose using an *in vitro* intestinal digestion model.

Methods. Commercial invertase (EC 3.2.1.26, Sigma Co.) was applied. Invertase was immobilized in both supports by adsorption (1) and covalent binding (2). The amount of immobilized protein was estimated as the difference between the amounts of protein (3) applied to the support and recovered in the supernatants and washings. For enzymatic sucrose hydrolysis, invertase preparations were packed into acid resistant capsules (DRcaps®). Sucrose digestion was carried out using an *in vitro* model described by Kaur *et al.* (4). Reducing sugars were measured by dinitrosalicylic acid (DNS) method (5).

Results. After 3 h of incubation a high invertase immobilization percentage (between 76 and 92%) was achieved (Fig. 1). Thus, both immobilization procedures are suitable for enzyme entrapment. Profiles of reducing sugars produced *in vitro* digestion model (Fig. 2) show that free enzyme is not active probably due to invertase inactivation by proteases presented in the reaction media. Higher reducing sugars concentrations (0.045 mg/ml at 3.5 h) were detected with applying of invertase immobilized covalently on prickly pear peel or by adsorption on the same support as well as in *Plantago* fiber. However, using invertase immobilized on *Plantago* fiber by covalent binding, lower reducing sugars concentrations were detected that might be related to the change in structural conformation during invertase immobilization.

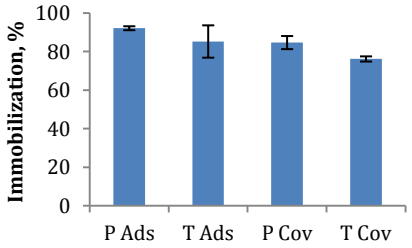

Fig.1. Percentage of invertase immobilized on *Plantago* (P) and prickly pear peel (T) fibers through adsorption (Ads) and covalent (Cov) binding.

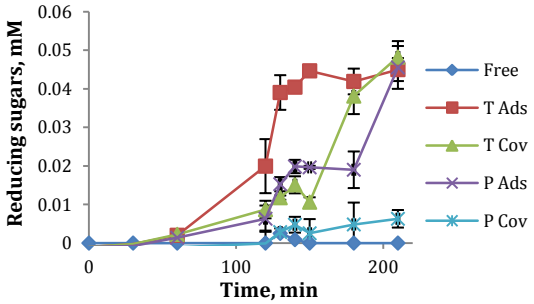

Fig. 2. Sucrose hydrolysis by invertase in free form (Free), immobilized on *Plantago* (P) and prickly pear peel (T) fibers through adsorption (Ads) and covalent (Cov) binding.

Conclusions. Immobilization protects invertase from the digestive conditions, allowing it to remain active for sucrose hydrolysis.

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