**Preparation, isolation and characterization of enzymes for the production of terpenes**

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**Highlights**

* Adsorption properties of various types of chromatographic adsorbents were tested.
* Good selectivity of a strong multimodal anion exchanger has been achieved.
* Influence of pH and ionic strength on adsorption was studied.

**1. Introduction**

Monoterpene glycosides play a fundamental role as significant aroma precursors of volatile monoterpenes. They contribute to some of the most important aroma characteristics of wine. These flavor compounds can be released by enzymatic hydrolysis catalyzed by glycosidases to enhance beverage aroma and flavor. The hydrolysis firstly requires the action of a particular enzyme (α-L-arabinosidase, β-D-apiosidase, α-L-rutinosidas, β-D-xylosidase), and secondly the liberated monoglucoside is than hydrolysed by a β-D-glucosidase. Even though one of the most abundant glycosides in fruit juice are apiosylglycosides, there is no pure β-D-apiosidase on the market. This enzyme is often present as a part of commercial enzyme preparations produced by fungi [1].

**2. Methods**

Static adsorption experiments were performed with six different chromatographic adsorbents. Among the examined adsorbents was one weak anion exchanger (SEPABEDAS-FPDA), three strong anion exchangers (ESHMUNO Q, CAPTO Q, PRAESTO Q), one salt-tolerant anion exchanger (TOYOPEARL NH2-750F) and one strong multimodal anion exchanger (CAPTO Adhere). Column experiments were carried out with the selected adsorbents using the FPLC system ÄKTA Purifier (GE Healthcare, Uppsala, Sweden). Tricorn 5/50 columns were used for the flow experiments.

**3. Results and discussion**

The purpose of this work was to purify β-apiosidase from an enzyme preparation produced by fungi. The aim of the batch experiments was to observe the behavior of each adsorbent during the adsorption. The effect of pH and salt concentration was examined too. Based on the obtained selectivities, which gave information about the purification of apiosidase from proteins and contaminating glucosidase, three chromatographic adsorbents were selected for flow experiments. Column experiments were carried out under optimized condition with strong anion exchanger ESHMUNO Q, salt-resistant anion exchanger TOYOPEARL NH2-750F and multimodal anion exchanger CAPTO Adhere. The most promising results were obtained using the multimodal adsorbent CAPTO Adhere. The separation was performed in 25 mM Tris-HCl buffer with pH 9 and 300 mM NaCl and the desorption was conducted by a linear gradient of this solution and a salt-free citrate phosphate buffer with pH 5. Compared to other adsorbents, CAPTO Adhere offered the highest purification factors from proteins (15.6) and glucosidase (5.9).

**4. Conclusions**

The aim of the work was to study the equilibrium characteristics of adsorbents for chromatographic purification and further characterization of glycoside used in production of terpenes.

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**References**

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