

Deep Eutectic Solvent Pretreatment of Rye Bran for The Production of *Aspergillus niger* A42 (ATCC 204447) Inulinase

Authors: [Hatice Gözde Hosta Yavuz^{1,2}](#), [Ibrahim Yavuz¹](#), [Aslı İsci Yakan³](#) and [Irfan Turhan¹](#)

Affiliation: ¹Department of Food Engineering, Akdeniz University, 07058 Antalya, Türkiye; ²Department of Nutrition and Dietetics, Akdeniz University, 07058 Antalya, Türkiye; ³Department of Food Engineering, Ankara University, 06830 Ankara, Türkiye
gozdeyavuz@akdeniz.edu.tr

The aim of this study was to produce inulinase enzyme from rye bran (RB) by deep eutectic solvent (DES) pretreatment. RB samples were mixed with choline chloride (ChCl):glycerol (Gly) at different molar ratios (1:2, 1:4, and 1:6) and pretreated at 80°C for 24 hours. The DES pretreated samples were then hydrolysed in an autoclave with 1% H₂SO₄ at 121°C for 1 minute. RB was hydrolyzed under these conditions without DES pretreatment as the control group. The levels of sugars (glucose, xylose, fructose, sucrose, maltose and arabinose) and furans (furfural and 5-hydroxymethylfurfural (HMF)) in the samples were determined. ChCl:Gly (1:2) provided the highest fermentable sugar concentration as 56,48 g/L. Therefore, hydrolysate pretreated with ChCl:Gly (1:2) was used in the fermentation of *Aspergillus niger* A42 inulinase. To determine the effect of yeast extract ratio on inulinase activity, yeast extract was added to the hydrolysates at two levels, 1% and 5%. At a yeast extract ratio of 1%, the maximum inulinase and invertase activities were 120.56 U/mL and 68.91 U/mL, respectively. When the yeast extract ratio was increased to 5%, the maximum inulinase and invertase activities also increased to 364.88 U/mL and 475.69 U/mL, respectively. The results indicate that an increase in yeast extract ratio led to an increase in inulinase and invertase activities. Fermentations at 5% yeast extract were used to calculate the kinetic parameters. The fermentation process lasted for a period of 14 days, during which the rate of sugar consumption was determined to be 96.57%. The study found that the maximum sugar consumption rate was 22.62 g/L/day, which occurred between days 2-3. Additionally, the maximum inulinase production rate was calculated to be 145.50 U/mL/day, while the maximum invertase production rate was 157.95 U/mL/day. The total dry biomass concentration at the end of fermentation was determined to be 42.40 g/L. The final pH of the fermentation broth was determined to be 6.21. The maximum consumption rate was 14.78 g/L/day, occurring between the 3rd and 4th day of fermentation. The maximum inulinase production rate was 103.16 U/mL/day, and the maximum invertase production rate was 173.67 U/mL/day. The total dry biomass concentration at the end of fermentation was 53.00 g/L and the final pH of the fermentation broth was 6.07. In conclusion, deep eutectic solvent pretreatment was a successful application in the bioconversion of RB to inulinase enzyme.

Keywords: Deep eutectic solvent pretreatment; lignocellulosic biomass; inulinase; *Aspergillus niger* A42.