EFFECT OF PRETREATMENTS ON SESAME CAKE PROTEIN HYDROLYSIS BY ALCALASE

E. DEMİRHAN, D. KILIÇ APAR and B.ÖZBEK

Yıldız Technical University, Department of Chemical Engineering, Davutpaşa Campus, 34210, Esenler/Istanbul, Turkey. E-mail: bozbek@yildiz.edu.tr

Abstract—In the present work, the effects of pretreatments on hydrolysis of sesame cake protein and enzyme stability were investigated. Heat, microwave and sonic pretreatments were applied to sesame cake protein as pretreatments before the enzymatic hydrolysis reaction. The sesame cake protein was hydrolyzed by Alcalase enzyme.

Keywords—sesame cake protein; Alcalase; heat pretreatment; microwave pretreatment; sonic pretreatment.

I. INTRODUCTION

Plant proteins are increasingly being used as an alternative to proteins from animal sources to perform functional roles in food formulation. Knowledge of the kinetics of the hydrolysis reaction is essential for the optimization of enzymatic protein hydrolysis and for increasing the utilization of plant proteins in food products (Kim et al., 2004; Chabanon et al., 2007).

Pretreatments could be applied to the substrate for improving the efficiencies of the hydrolysis reactions. The rationale behind such pretreatments has been to improve the accessibility of the enzyme to the target bond in the substrate. Heat treatment is a physical technique frequently used in food industry to change the conformation of protein and to facilitate the enzymatic hydrolysis of proteins (Cui et al., 2009; Nordqvist et al., 2012). Microwave irradiation has recently been applied to accelerate chemical or enzymatic reactions due to the microwave-induced rapid heating and conformational or structural changes of the proteins along the peptide bonds (Zhong et al., 2005; Ye and Li, 2012). Ultrasound has been used to accelerate the rates of enzymatic reactions by using ultrasound as an enzymatic pretreatment to reduce particle size. In such cases, the reduction in particle size and consequent increase in the catalytic surface area are thought to reduce mass transfer limitations.

Therefore, the goal of the present study was to investigate the effect of pretreatments such as heat treatment, microwave irradiation and sonication on hydrolysis and solubilization of sesame cake as well as to test for enzyme stability.

II. METHODS

A. Materials

Sesame cake protein used in this research which contains 37.7% protein was obtained from Necdet Bükey A.Ş, Izmir. The enzyme used in this work was Alcalase 2.4L, a bacterial protease produced by Bacillus Licheniformis, obtained from Novozymes.

B. Pretreatments

For heat pretreatment, the sesame protein solution that contains 15 g protein per liter was heated up to 90°C and 100°C, and kept 30 minutes at these temperatures. Microwave pretreatment was performed in a domestic digital microwave oven (Arcelik MD 594, Turkey). For this treatment, the sesame protein solution was heated by 540 and 720 W microwave irradiation for 10 minutes.

The ultrasonic pretreatments were performed by using Bandelin Sonopuls HD 2200 sonicator. The tip of the horn was immersed about 2 cm into the 50 ml sesame cake solution and sonication was performed at constant duty cycle rate of 50% for 80 and 120 W acoustic power rates for 30 minutes. During the sonication process the temperature of the solution was kept constant at 40°C.

C. Hydrolysis reaction

Hydrolysis experiments were carried out in a 400 ml jacketed reactor with magnetic stirring with pH and temperature control. After the pretreatments, all hydrolysis reactions were carried out 120 minutes in 0.2 liter of aqueous solutions, contains 15 g protein/L sesame cake protein, at optimum conditions 50°C and pH 8.5 which obtained from previous study performed by Demirhan et al. (2011). The amount of enzyme added to these solutions was 3 ml/L.

D. Conversion of hydrolysis

The hydrolysis of the reaction was monitored by pH stat method.

E. Protein concentration

Soluble protein concentration was determined by Lowry method using bovine serum albumin as standard. Soluble background protein concentration of sesame cake protein values were given as percentage of the initial protein concentration.

F. Protease activity

For measuring the residual enzyme activity azocasein was used as a substrate.

G. Amino Acid Analysis

The amino acid composition was determined with an amino acid analyser Shimadzu UFLC LC-20 AT.

III. RESULTS AND DISCUSSIONS

The effect of pretreatments on hydrolysis and solubility of sesame cake protein and stability of Alcalase were investigated, and the results were given in Fig. 1-3. As can be seen from these figures, no significant changes were observed on the hydrolysis degrees of sesame cake protein after heat, microwave and sonic treatment.