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Kinetics of Enzymatic Hydrolysis of Collagen Fiber with Ammonium Chloride/Neutral Protease System

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Abstract

The kinetics of enzymatic hydrolysis of collagen fiber was studied using neutral protease and ammonium chloride(NH_4Cl)/neutral protease system. The structures of collagen fibers and neutral protease before and after the enzymatic hydrolysis were characterized by HR-XRD and FPA-FTIR to investigate the enzyme catalytic effect of NH_4Cl/neutral protease to collagen fibers. NH_4Cl/neutral protease system could accelerate the reaction between protease and collagen fibers. A simplified kinetic model for the impeded reaction is developed to describe the enzymatic hydrolysis of collagen in a complex solution system and a time-dependent decay coefficient of impeded reaction is proposed. Both the initial observed rate constants and ineffective coefficients were obtained to elucidate the interaction between neutral protease and collagen, and the applicability of the simplified model was demonstrated. A theoretical guidance is provided for the extraction and hydrolysis of collagen.

Introduction

Leather industry is an offshoot of the meat industry since the skins and hides are predominantly supplied from the meat industry; it also has economic relevance due to the offering of various consumer products. Traditional leather processing involves various unit processes and operations that require intensive use of chemicals and water. Resolving the issue of pollution coupled with operation, maintenance and treatment costs remain as a serious challenge for tanners all over the world. Conventional dehairing and fiber opening processes utilize lime and sodium sulfide along with water, which in turn release effluent with about 60–70% of total pollution load. Emission of toxic gases like H_2S as a result of the mixture of dehairing and pickling streams is another major challenge for the tanners. Advances in enzyme technology have led to the use of various enzymes in leather manufacture processes such as soaking, dehairing, bating and degreasing. Although several enzymatic dehairing and fiber opening methods have been developed, many of these processes have not yet been understandable. NH_4Cl/neutral protease system has been standardized for cow hides. However, reduced efficiency and the strict process control measures in enzyme-based fiber opening limits its commercial exploitation so far. Attempts have also been made to integrate the one-step enzymatic processes such as enzymatic dehairing and fiber opening with protease into a single-step process by successive and simultaneous enzyme application.

Methods

After lyophilization, the dehairing cowhide was crushed into pieces by ultra-centrifugal grinder and stored in a drying oven. The enzymatic hydrolysis reactions of collagen fiber with neutral protease and with NH_4Cl/neutral protease system were conducted in a glass tube at 30°C. The reaction mixture included 50 mg of collagen fiber, 0.1 g of neutral protease and 0, 1, 5, 10 mg NH_4Cl respectively. For each reaction time (0, 30, 60, 120, 180, 240, and 300 min), the samples were centrifuged for 10 min, and then the supernatant was taken to analyze with BCA kit.

Results and discussion

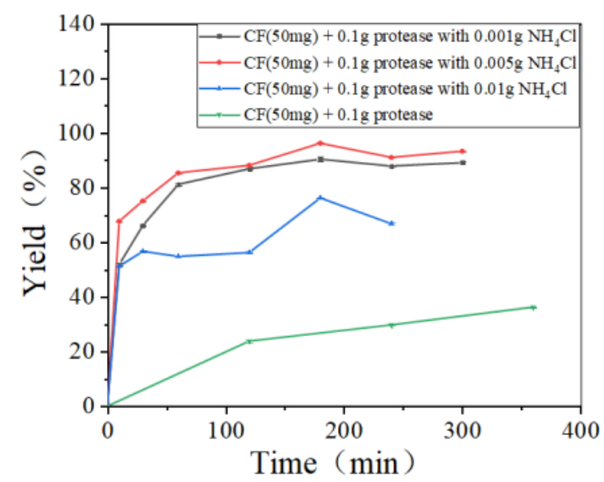

Figure 1 The decomposition rate of collagen fiber under different NH_4Cl contents.

Conclusions

In this study, the efficiencies of enzymatic hydrolysis vary with the NH_4Cl content. The NH_4^+ can combine with the negatively charged groups of the enzyme, which improves the bonding ability of enzyme with substrate and increases the activity of the enzyme. The remaining collagen fiber yields and the efficiency of enzymatic hydrolysis decreased with the increase of NH_4Cl content.

References

1. Ramamoorthi, P.; Rathinam, A.; Jonnalagadda, R. R.; Palanisamy, T., Non-aqueous green solvents improve alpha-amylase induced fiber opening in leather processing. Sci Rep 2020, 10 (1), 22274.
2. Wang, G.; Post, W. M., A note on the reverse Michaelis–Menten kinetics. Soil Biology and Biochemistry 2013, 57, 946-949.
3. Yang, C. Y.; Fang, T. J., Kinetics of enzymatic hydrolysis of rice straw by the pretreatment with a bio-based basic ionic liquid under ultrasound. Process Biochemistry 2015, 50 (4), 623-629.

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