Investigation of Mass Transfer and Action Mechanism of Enzymes in Unhairing and Bating Processes using Fluorescence Tracing

Objectives

1) Fluorescently labeled unhairing and bating enzymes with both high fluorescence intensity and high biological activity will be prepared by optimizing the conditions of labeling and purification.

2) In unhairing and bating processes, skins/hides will be treated using the fluorescently labeled enzymes, and the transfer and distribution of the enzymes in skin/hide will be observed accurately using fluorescence tracking.

3) Effects of structure of skin/hide matrix and chemical pretreatment on mass transfer of enzymes will be investigated, and the relationship between the transfer of enzymes in unhairing/bating and the quality of leather products will be elucidated. These results will provide scientific data to the enhancement of mass transfer of enzymes and the development of cleaner leather production.

Literature review

Application of enzymes for replacement of polluting chemicals used in traditional leather processing and improvement of production efficiency is one of the most important trends in leather industry. For example, much research recently has focused on the development of enzymatic unhairing systems to reduce sulfide and lime pollutions. As we known, the enzymatic hydrolysis of substrates in skin/hide mainly depends on mass transfer of enzymes in skin/hide, because the skin/hide matrix is thick and complex, and the enzymatic reaction rate is extremely fast. Furthermore, the enhancement of mass transfer of enzymes is beneficial to uniform hydrolysis of substrates in skin/hide, which can reduce the damage in collagen fibers and ensure the quality of leather products. However, the mechanism of mass transfer and action of enzymes in leather processing remains unclear due to lack of an appropriate method of effectively observing the transfer of enzymes in skin/hide, which has severely restricted the wide use of enzymes in leather industry.

The lack of a direct observation of the transfer of enzyme in skin/hide is mainly due to the fact that the chemical composition of enzyme is very similar to those of skin/hide proteins. As a result, various quantitative determinations of proteins cannot be used to obtain the enzyme concentration in skin/hide or liquor. Therefore, for understanding the mechanism of transfer and action of enzymes, it is necessary to develop an accurate observation of transfer and distribution of the enzymes in skin/hide.

Fluorescence labeling is an interesting technology that can endow substance with fluorescent properties by binding it to fluorescence. Moreover, fluorescently labeled substance can be accurately located in cell or tissue by using fluorescence detector. Amino, thiol and carboxyl groups of protein are able to form covalent bond with fluorescence, which endow protein with fluorescent properties. Hence, it is reasonable to speculate that the exact location of enzyme in skin/hide can be assessed using fluorescence microscopy. In fact, the applicant has obtained fluorescently labeled trypsin with high fluorescence intensity, high purity (Figure (A) and (B)) and high protease activity (more than 80% of its original activity). Additionally, our previous study indicated that the transfer and distribution of trypsin in bated pelt could be directly observed using fluorescence microscope after the pelt was treated by the fluorescently labeled trypsin (Figure (C)-(G)). These results suggest that the fluorescence tracing is effective in investigating effects of structure of skin/hide matrix and chemical pretreatment on mass transfer of enzymes, and the relationship between the transfer of enzymes in unhairing/bating and the quality of leather products. (*References are omitted.*)

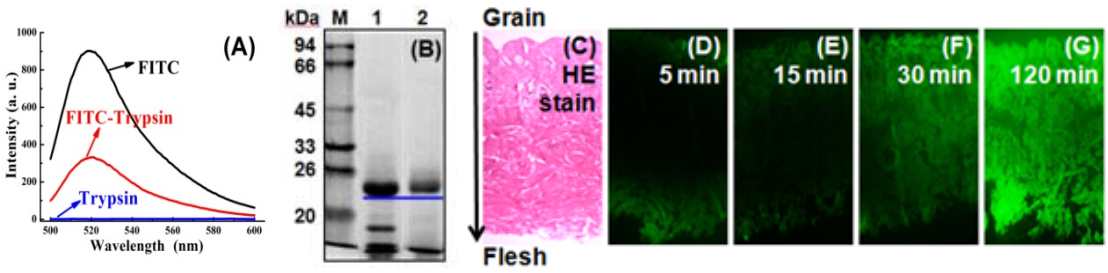

Figure (A) Fluorescence emission spectra; (B) SDS-PAGE patterns of marker(M), trypsin(1) and FITC-trypsin(2); (C) Vertical section from delimed pelt observed by biologic microscope; (D)-(G) Fluorescence micrographs of vertical sections from pelts bated with FITC-trypsin (green).

Overview of the research plan