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Effect of Pineapple Protease on the Characteristics of Protein Fibers

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Abstract: A pineapple protease, bromelain, was used to improve the dyeing properties of protein fibers such as wool and silk. The optimal condition for the activity of the pineapple protease was about 60 °C at pH 7. The wool and silk were treated with the protease extracted from a pineapple and the K/S values of the dyed wool and silk were measured using a spectrophotometer in order to compare the dye uptake. The protease treatment enhanced the dyeing properties of protein fibers without severe changes in mechanical properties. The surface appearances of protease-treated fibers were observed by microscopy.

Keywords: Pineapple, Bromelain, Dyeing property, Wool, Silk

Introduction

The International Union of Biochemistry and Molecular Biology (1984) has recommended to use the term *peptidase* for the subset of peptide bond hydrolases (Subclass E.C 3.4.) [1]. The widely used term *protease* is synonymous with *peptidase*. *Peptidases* comprise two groups of enzymes: the endopeptidases and the exopeptidases, which cleave peptide bonds at points within the protein and remove amino acids sequentially from either N or C-terminus respectively. The term *proteinase* is also used as a synonym word for *endopeptidase* and four mechanistic classes of proteinases are recognized by the IUBMB as detailed below. Proteinases are classified according to their catalytic mechanisms. Four mechanistic classes have been recognized by the International Union of Biochemistry and Molecular Biology [2]; serine proteinases, cysteine proteinases, aspartic proteinases, and metallo proteinases.

Enzymes can be found in many different foods, both from plant and animal sources. Some plants, especially in their fruits, have high concentrations of protease enzymes. Avocados, papaya, pineapples, bananas, and mangos are all high in enzymes. Unripe papaya and pineapple are excellent sources of enzymes. The enzymes extracted from papaya and pineapple are papain and bromelain, respectively, and are proteolytic enzymes.

Bromelain is the collective term for enzymes (principally proteolytic enzymes) derived from the ripe and unripe fruit, as well as the stem and leaves, of the pineapple plant, *Ananas comosus*, a member of the Bromeliaceae family [3,4]. Pineapple has been used as a folk medicine by the natives of the tropics for centuries. It has been used as a digestive aid, as a cleansing agent to improve the texture of the skin, and to promote the healing of wounds. It is used commercially in certain cosmetics and as a meat tenderizer and dietary supplement. Bromelain may have digestant activity and there is research suggesting that it may have wound healing, anti-inflammatory, antidiarrheal and anticarcinogenic effects, as well.

The vegetal proteases are classified into two groups. One group having an active sulfydryl group has an optimized activity below pH 7.0 and has a strong milk clotting activity. The other group not having an active sulfydryl group has an optimized activity above pH 7.0 and has a weak milk coating activity. These vegetal proteases are effective for heat-resistance and have a activity at 60~70 °C [5,6]. The vegetal protease functions as tenderizer which degrades muscle protein and tissue. A picin extracted from fig rapidly degraded collagen of connective tissue. A papain extracted from papaya rapidly degraded muscular tissue. In direction of these wide substrate specificity and safety assurance, the vegetal protease is also used to make hydrolytic material. In addition, the vegetal protease could have wide application to decomposing waste product of domestic animal and the food industry as tenderizer, fermentation etc. [7,8].

Bromelain is composed of 283 amino acids and is one of basic protein of M.W 33,000. Isoelectric point of bromelain is pH 9.55 and the optimal pH about casein is pH 7.0. Bromelain has activity of benzolarginine ethylester. Bromelain, protease of a thiol type, activated by cysteine, H_2S or NaCN and deactivated by heavy metals such as Hg^{2+}, Ag^{+} etc. [9].

The structure of wool fiber is characterized by two distinct components: (i) the cuticle is responsible for the protection, luster, and elasticity of wool fibers, and increases spinning ability due to its efficient ability to combine fibers; and (ii) the cortex, which constitutes about 90 % of wool fiber, is responsible for the intensity and elasticity of the wool fiber. The quality of wool fiber is determined by crimp, thickness (regular 10~70 μm), length (the longest 20 cm), resilience, and brilliance [10].

The highest grade of fabric, silk fabric, possesses a fine luster, tactile sensation, and draft property, and therefore, its dyeing characteristics are distinct from those of other fabrics. Silk is composed of two major proteins (fibroin and sericin), and it also contains trace elements, such as wax and salts [11,12].

In this study, protease was extracted from pineapple and

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applied to the protein fibers such as silk and wool. The dyeing properties, surface properties, and tensile strength of the protease treated fibers were investigated.

Experimental

Materials

A pineapple (*Ananas comosus*) produced in jeju-island, South Korea in 2003, was used for this study. Hammarsten-casein was purchased from Merck Inc. The silk (60 g/m^2) and wool fabrics (125 g/m^2) (KS K 0905) were used for protease treatment and dyeing throughout the study. All the chemicals used in this study were of laboratory-reagent grade.

Sumifix Supra Navy 3GF (C.I. Reactive Blue 222) and Itofix Navy CTR (C.I. Reactive Blue 171) used for the dyeing of wool and silk, were kindly supplied by Sumitomo.

Preparation of Culture Supernatant

A pineapple was homogenized in 0.1 M citric acid-Na_2HPO_4 buffer solution of pH 7.0 and then filtered. The filtrate was separated using a centrifugal separator (8,500 rpm) and used as the culture supernatant to investigate characteristics of protease and their effects on wool and silk samples [13].

Determination of Protease Activity

Protease activity was determined by measuring the degree of casein degradation by H. Onishi method [14]. The protease activity in a broth culture was determined by spectrophotometry (optical density at 590 nm), and total protein content was determined by a Bradford assay [15], after the removal of cells by centrifugation. One milliliter of a substrate solution (0.6 % casein in 0.1 M citric acid-Na_2HPO_4 buffer solutioin, pH 7.0) was mixed with 0.2 m*l* of the culture supernatant, which had been diluted to appropriate levels with a coenzyme solution (Na_2CO_3-Na_2HPO_4, pH 10), and the samples were incubated at 65 °C for 30 minutes. A 15 % (w/v) Trichloroacetic acid solution was added to the samples to stop the reaction, and the samples were again incubated at 37 °C for 15 minutes. The protease in the samples was precipitated by adding 2.5 m*l* of a Na_2CO_3 solution (0.55 M) with 0.5 m*l* of a phenol reagent, diluted three times with H_2O, in order to determine one unit of protease. One protease unit was determined by optical density at 660 nm, and was defined as the amount of protease necessary to produce 1 μg of tyrosine from casein.

Protease Treatment of Silk and Wool Fibers

The supernatant of the broth culture, which was diluted with the coenzyme solution, was prepared for the treatment of the silk and wool fabrics. A fresh liquid medium and a heat-treated broth culture were also prepared as a control. Forty milliliters of the prepared protease-containing broth samples, along with the control, was applied to the silk and wool fabrics (15 cm × 5 cm) in 50-m*l* conical tubes. They were then incubated at 37 °C with shaking of 130 rpm.

Microscopic Examination

Single fibers of silk and wool fabrics, which were treated with activated protease and inactivated protease(control), were observed using a SEM (scanning electron microscope, Akasi Alpha 25A) of 2,000× and 3,000× magnification in order to examine surface properties [16].

Tensile Strength Test

Tensile strength test of the fabrics was performed using a tensile strength tester (Instron, model no.4468) (KS K0520) [16]. The tensile strength (max. force, N) of protease-treated fabric, along with inactivated protease treated fabric (controls), were measured five times by the Grab method (elongation speed : 50 cm/min), and the means were generated statistically.

Dyeing Method

C.I. Reactive Blue 171 and C.I. Reactive Blue 222 were used for the dyeing of silk and wool, respectively. The fabric samples were dyed in a laboratory dyeing machine (DL-6000, Daelim starlet, Korea) at a liquor ratio of 50:1. Dyeings were carried out at the temperature of 60 °C for 30 minutes with the dye concentration of 2 %owf. The dyed fabrics were washed and dried after dyeing [17,18].

The dyeing properties of the dyes on the silk and wool fabrics were investigated by measuring K/S values of dyed samples [19], defined as

$$K/S = \frac{(1-R)^2}{2R} \quad (1)$$

where, K: absorption coefficient of dyed samples
S: scattering coefficient of dyed samples
R: spectral reflectance

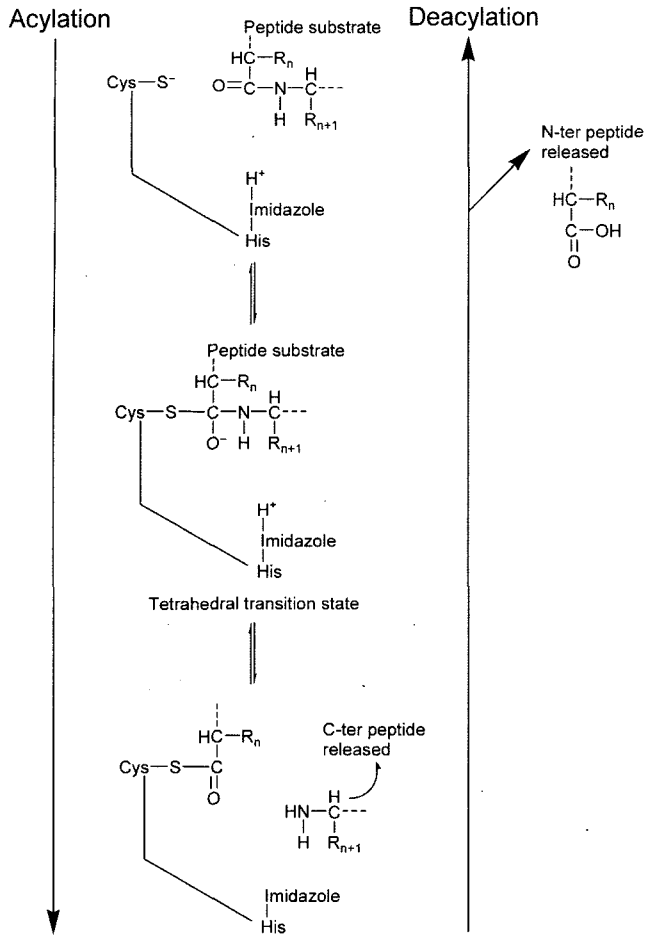
The K/S values were measured using spectrophotometer (Color-Eye 3100, Macbeth, USA).

Results and Discussion

Activity of Protease

The activity of pineapple protease was found to be optimal in the condition of 60 °C at pH 7. This result is similar to the previous report of Bai [20], Choi [7] and Suh [21]. The activity of protease dramatically decreased at acidic pH of 2~3 and the optimal condition for the activity of the pineapple protease was found to be 60 °C at pH 7.0.

Pineapple protease is a cystein protease which belongs to endopeptidase. This family includes the plant proteases such as papain, actinidin or bromelain, several mammalian lysosomal cathepsins, the cytosolic calpains (calcium-activated) as well as several parasitic proteases (e.g. Trypanosoma, Schistosoma). Like the serine proteinases, catalysis proceeds through the formation of a covalent intermediate and involves a cysteine and a histidine residue. The nucleophile is a thiolate ion rather than a hydroxyl group. The thiolate ion is stabilized through the formation of an ion pair with neighboring imidazolium



Scheme 1. Catalytic mechanism of cysteine proteinases (Cys: Cysteine, His: Histidine).

group of Histidine. The attacking nucleophile is the thiolate-imidazolium ion pair in both steps and then a water molecule is not required. Scheme 1 shows a schematic representation of the catalytic mechanism of cysteine proteinases.

Microscopic Examination of the Silk and Wool Fibers

The silk and wool fabrics were treated with the protease extracted from pineapple, and were observed by microscopy. It is known that raw wool contain 25~70 % by mass of impurities which consist of wool grease, perspiration products (suint), dirt and vegetable matter such as burrs and seeds [22]. Figure 1(b) shows the wool fiber treated with activated protease and that with inactivated protease as a control. The sample treated with activated protease (Figure 1b) was cleaner than that with inactivated protease (Figure 1a), which shows that the protease remove the impurities and scales from the wool. The results are consistent with the previous reports [23-25].

The similar trends were also observed in the case of silk. The protease-treated sample (Figure 2b) was cleaner than both the control (Figure 2a). However, the thickness of samples treated with activated protease (Figure 2b) diminished compared

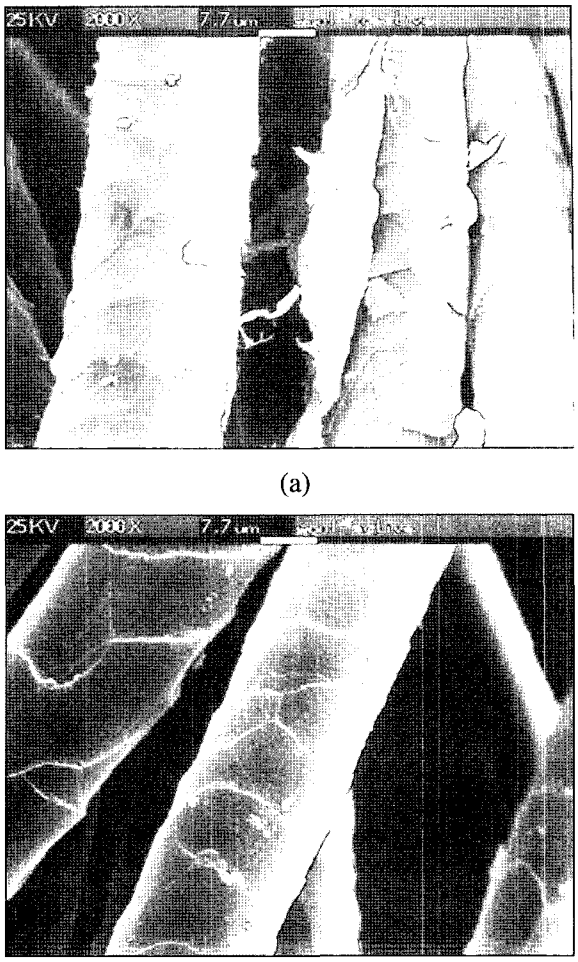
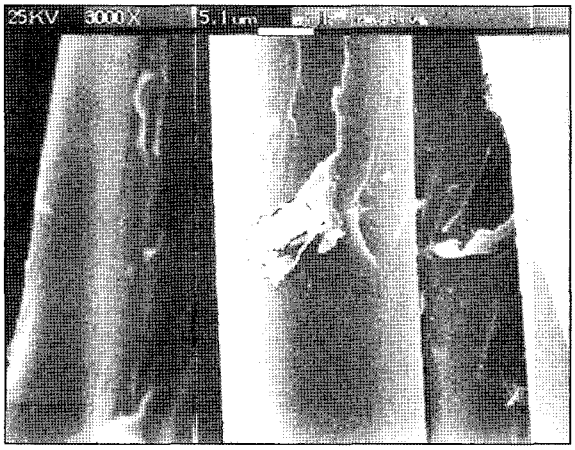


Figure 1. SEM of wool fibers (a) treated with inactivated pineapple protease for 48 h, (b) treated with activated pineapple protease for 48 h.

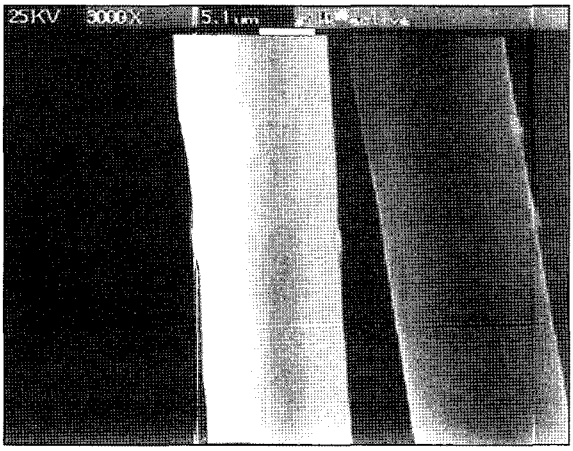
with that of inactivated protease treated samples (Figure 2a), which demonstrates that the residual sericin or fibroin of silk fiber was partially degraded by protease treatment.

Tensile Strength Test

In order to investigate the effects of the protease treatment on the physical properties of the fabric, tensile strength was measured. The components of the wool fiber are made up of the protein keratin. The preferred configuration of the keratin molecule is the helices joined by disulfide bonds, which is the relaxed form of the molecule and is known as α-keratin. The molecules can be changed into β-keratin by hot water, steam, alkali, or elongation due to the cleavage of hydrogen bonds parallel to the axes and the disulfide linkage. Also, when tension on the fiber is removed, the molecules return to the α form, and the fiber returns to its original shape and dimensions [23]. Therefore, treatment with hot water, steam,



(a)



(b)

Figure 2. SEM of silk fibers (a) SEM of silk fibers treated with inactivated pineapple protease for 48 h, (b) SEM of silk fibers treated with activated pineapple protease for 48 h.

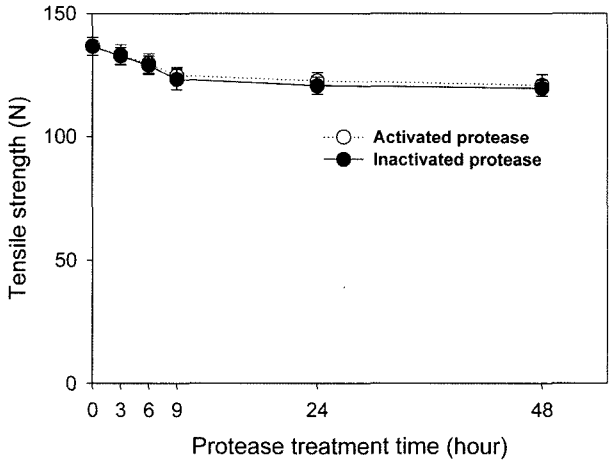


Figure 3. Tensile strength of wool fibers treated with active protease and inactivated protease.

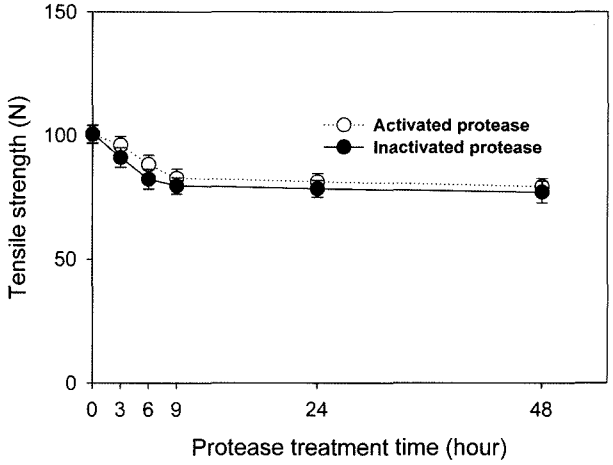


Figure 4. Tensile strength of silk fibers treated with active protease and inactivated protease.

or alkali can change the tensile strength of the wool. However, as shown in Figure 3, the results of the tensile strength analysis did not show considerable differences, after 48 hours of the protease treatment, between the treated wool samples and the control samples treated with the heat-inactivated protease. Therefore, it is considered that the protease produced by pineapple was able to remove the scales of the wool fabrics, but it did not change the internal or chemical structure of the wool. The tensile strength of wool fabrics having relatively loose structures was higher than that of silk because tighter fabric structures have more yarn interlacings and yarn crimp, contributing greater resistance and strength. (cf. wool = 125 g/m^2, silk = 60 g/m^2)) (Figures 3 and 4).

It has been established that the fibroin comprising the majority of silk yarn is composed of polypeptide chains, which themselves are composed of 17 different amino acids [26]. The majority of these amino acids, however, are glycine and alanine, which possess shorter side chains than others and, therefore, hydrogen bonds can be more easily formed between their polypeptide chains. However, >C=O and -NH- in the polypeptide chains of silk fiber contribute to the structural stability of silk against various stress conditions. The tensile strength difference between the protease treated silk samples and the control samples were negligibly small as shown in Figure 4. Consequently, this result demonstrated that the protease partially degraded the residual sericin or fibroin of silk, but it did not alter dramatically their tensile properties.

Dyeing Properties of Protease Treated Wool and Silk Fibers

The fabrics which had been protease-treated at intervals of 3, 9, 24, and 48 hours were dyed, washed, and dried. In order to compare the dyeing properties of protease treated fabrics, K/S values were measured using spectrophotometer interfaced with a personal computer.

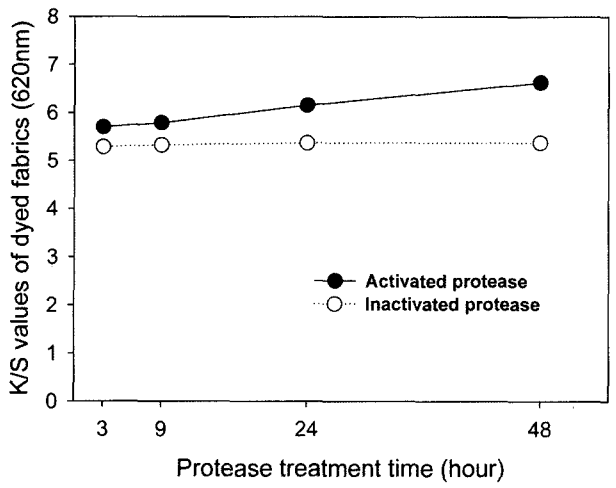


Figure 5. Dyeing properties of wool fibers at various protease treatment time.

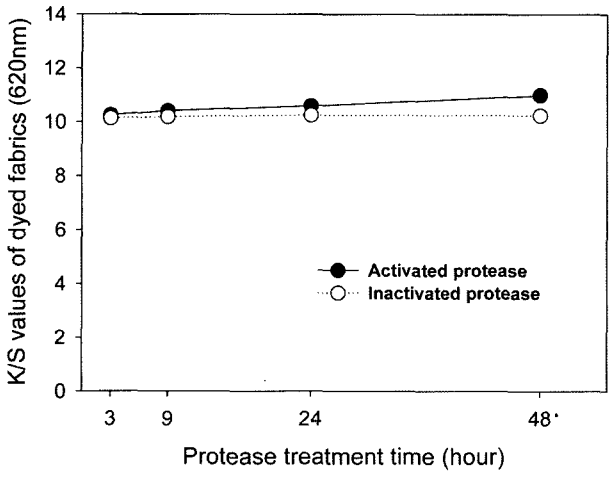


Figure 6. Dyeing properties of silk fibers at various protease treatment time.

Figure 5 shows that the K/S values of protease-treated wool fabrics gradually increased as protease treatment time increase. It is considered that the increase in dye uptake is due to the protease-induced degradation of the scales, especially the serine residues, in the wool. This result was also verified by scanning electron microscopy as shown in Figure 1. It is well known that the cuticle cells, or scales, constitute the outermost surface of the wool fiber and are responsible for important properties such as wettability, tactile properties and felting behavior [27,29]. Therefore, the result shows that the removing of scales by protease treatment can enhance the dye accessibility to the wool fibers via CMC (cell membrane complex) [31]; the K/S values of the activated protease treated wool fabrics has been improved by 8~23 % compared with the control.

In the case of silk, as shown in Figure 6, the dye uptake improvement was relatively small compared with wool fibers. The reaction partners are, on the side of the silk fiber, mainly the terminal amino groups of the lysine, especially when dyeing is carried out in the neural to weakly alkaline region. In the somewhat stronger alkaline region the phenol group of the tyrosine side chain may also react with the dye. The protease hydrolysis does not build up remarkably the dyeing site although the surface of the silk fiber became smoother compared with the wool fibers (Figure 2); the K/S values of the activated protease treated silk fabrics has been improved by 1~7 % compared with the control.

Conclusions

A pineapple-produced protease, bromelain, was used to improve the dyeing properties of protein fibers. The optimal condition for the production of the protease by a pineapple was about the temperature of 60 °C at pH 7.

The activated protease removed the impurities and scales from the wool, which was verified by microscopic examination. Also, the removing of scales by protease treatment increased the dye uptake due to the improvement of dye accessibility to the wool fibers via cell membrane complex. However, the tensile strength did not show considerable differences, for 48 hours of the protease treatment, between the treated wool samples and the control samples treated with the heat-inactivated protease. Therefore, it is considered that the protease did not degrade the serine residues in the wool fiber enough to decrease remarkably its tensile strength, although the protease did enhance the dyeing properties of wool. The similar trends were also observed in the case of silk fiber although the dyeing property improvement was not remarkable compared with wool fiber.

Acknowledgement

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