The Effect of Urea on Degumming of Mulberry Silk with Papain

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\textbf{Abstract.} By measuring simply the degumming rate of silk, this work would know the effect of the combination of papain and urea as degumming agent on silk degumming, and the protease activity of papain would any change in the presence of urea when sericin as a solid substrate of papain. The results shows that, papain has exhibited its temperature tolerance no matter if there is urea, during the process of silk degumming, even if the temperature at 100\textdegree{}C holds the highest rate of silk degumming. To achieve the highest degumming rate, requiring temperature of silk degumming was lower in the solution containing papain and urea than in the solution only containing papain. there is the highest degumming rate using papain plus urea as degumming reagent than other three kinds of papain and urea and distilled word only separately as degumming reagent; the degumming rate with papain and urea is not the sum of the two which with papain and urea separately. Thus urea has some effect on papain when sericin as sold substrate, may be due to the expansion effect of urea on sericin.

\textbf{Introduction}

Mulberry Silk is a composite formed by two cores of fibroin surrounded by a cementing layer of sericin\textsuperscript{[1]}. Fibroin and sericin account for about 75 and 25 wt\%, respectively. Sericin might negatively impact some properties of silk fibers such as dyeing property, generally be removed before the dying process. Degumming is a key process, during which sericin is totally removed and silk fibres gain the typical shiny aspect, soft handle, and elegant drape highly appreciated by the consumers. The industrial process takes advantage of the different chemical and physical properties of the two silk components, fibroin and sericin. Sericin is readily solubilized by boiling aqueous solutions containing soap, alkali, synthetic detergents, or organic acids\textsuperscript{[2]}. Degumming of silk with chemical method is easy to bring about environmental problems, and consumes a lot of energy and water. For improving these problems, in recent years, various studies have dealt with the removal of sericin by using proteolytic enzymes, such as: acidic, neutral, and alkaline proteases as degumming agents of silk yarn\textsuperscript{[2]}. Degumming of the raw silk with protease is friendly for environment, coinciding with saving energy and sustainable development\textsuperscript{[6]}. The quality of silk degummed with enzyme is better than that with the common agent\textsuperscript{[7]}.

Papain can be activated by reducing agents\textsuperscript{[7]}, extracted from the immature fruits of papaya, and its working condition broad\textsuperscript{[8]} which as a wider range of optimum pH and temperature. Many studies have proved that papain could effectively remove sericins from raw silk\textsuperscript{[9]}, so papain should be a very good agent for raw silk degumming agent. Urea has been proved to be useful for degumming of the raw silk or silk fabrics as degumming auxiliary agent. Some papers have reported about using urea as degumming auxiliary agent\textsuperscript{[11]}. The helical content of papain molecule could be not altered by the addition of 8M urea, the proteolytic activity of papain could change with increasing concentration of urea due to different substrates\textsuperscript{[14]}. Since papain is a better degumming agent and urea could help silk degumming, so this work would like to know the effect of urea on silk papain degumming when papain and urea in the same degumming bath, and that the protease
activity of papain would any change in the present of urea when sericin as a solid substrate of papain.

Experiments

Materials

The mulberry raw silk is a kind of white steam filature, 20~22 dtex, provided by Kang Run Limited liability company, Hechuan country, Chongqing, china.

Main Instruments

Electric heating thermostat blast drying oven (101-0-B5, Shanghai leaps forward the medical instrument Limited company), electronic balance (JA2003A, Shanghai Jingtian electronic instrument limited company), constant temperature oscillator (SHA-B, Changzhou Aohua instrument limited company).

Main Agents

Papain (Beijing Aoboxing Biotech Company Ltd, activity≥500,000 unit/gram), urea (analytically pure, Chengdu Kelong Chemical industry Reagent plant).

Degumming

Four different degumming solutions which one was containing papain plus urea and other three were respectively containing papain or urea or just only distilled water, were used in the study. Urea and papain was putted into the distilled water in turn to confect the degumming solution containing papain and urea. The concentration of papain in the enzyme degumming solutions was 0.3g/L; the concentration of urea in the urea degumming solutions was 5mol/L. The degumming conditions were time 60min, pH6, temperature30℃~100℃, the bath ratio was 1:50.

The degumming method proceeded as follows: the raw silks (dried 104℃, 4h and weighed) were placed directly in degumming solution (has preheated to a given temperature) and degummed 60min under certain temperature, then samples were thoroughly washed in ddH2O, at last dried 4hours under temperature104℃.

The experiment was repeated 3 times, and the average value was taken to calculate the degumming rate.

The efficiency of each degumming solution was quantitatively assessed by simply measuring a loss in weight using JA2003A electronic balance. Therefore, sample’s respective weight losses are expressed as a percentage of the initial weight and the weights were recorded until successive weightings agreed within 0.1 mg.

$$\text{degumming rate/\%} = \frac{m_a - m_b}{m_0} \times 100$$

(1)

Results and Analysis

Comparison of the Silk Degumming in Four Kinds of Solution

The degumming results were significant differences with different degumming solutions (Figure 1 to 4). When other conditions, such as the temperature and the pH value and the time, are the same, the degumming rate of the raw silk with papain in presence of urea is the highest than other three occasions which with only papain or urea or distilled water; the lowest degumming rate appeared in the occasion which degummed with distilled water only. Sequence the results of degumming in different solutions is obvious: papain plus urea > papain only> just urea > water only.

The Degumming Result in Distilled Water

The silk degumming rate is very bad when silk degumming in distilled water, and trends to increase
with the increase of temperature, but the highest is only the 1.68% even if the temperature reached 100°C. The result indicated that just using distilled water as degumming solution, sericins could not be removed off from the raw silk, the weight loss of silk was less, when the degumming temperature ≤ 100°C (Fig.1).

**The Degumming Result in Urea Solution**

In 5mol/L urea solution, the loss of the raw silk changed with changing temperature (Fig.2). When the temperature ≤ 70°C, the degumming rate is less, but still higher than that degummed in the distilled water only, similar about its 2 times; elevating temperature from 70°C to 90°C, the degumming rate increased a little, similar about 3~4 times of that degummed in the distilled water; more increasing temperature from 90°C to 100°C, the degumming rate jumped from 3.36% to 11.96%, which at 100°C is similar to 3.5 times of that at 90°C, and similar 7.2 times of that which in the distilled water.

![Figure 1. Degumming in distilled water.](image1)

![Figure 2. Degumming with urea.](image2)

When the degumming temperature at 100°C, the expansion of sericin of the silk has been enough, under the combined action of high temperature and urea, sericin dissolved more into degumming solution from the silk which thus appears its weight reduction.

**The Degumming Result in Enzyme Solution**

In 0.3g/L papain solution, the weight of silk reduced about 5% when the degumming temperature ≤ 50°C, and then the weight loss of silk increased with increasing temperature from 50°C to 80°C, the degumming rate is a marked rise (from 5.43% to 15.83%), but when the degumming temperature from 80°C to 100°C, the degumming rate keeps on higher plane that rise more slowly (Figure 3). Thus, from this point of view, papain has exhibited stronger heat tolerance during the degumming process. The papain optimum temperature range in the degumming process is from 80 to 100°C, higher than the optimum temperature parameter given by the manufacturers, and also higher than which when the others as papain substrates, such as casein as substrate its optimal temperature is 60°C-80°C[15].

**The Degumming Result in the Solution of Enzyme PLUS Urea**

The results, silk degummed in the solution of 0.3g/L papain and 5mol/L urea, are plotted in Figure 4. The first, no matter what at temperature conditions, degumming in papain solution contained urea, the weight loss of silk is higher than those which in papain or urea or distilled water solution, its degumming rate is about to 1.4~3.5 times of which in only papain solution, and about to 2~34 times in just urea solution.

The second, the curve change of degumming rate with the temperature is similar to which just in papain solution; from 50°C to 60°C, the degumming rate increased from 14.5% to 24.0%, reached at higher level at 60°C, and then has kept at high plane within 25±0.33%, is near to the sericin percent of the raw silk which within (24.87±0.24)% confirmed by degumming in alkali solution. The degree of the weight loss of the raw silk just achieved at the highest level (24.0%) with papain
plus urea when the temperature at 60°C; but only with papain, when the temperature was up to 80°C the degumming rate was just at 15.8% of the highest level which was lower 8.2% than that with papain plus urea.

The third, the sum of the degumming rates of which with papain and with urea separately, is less about 6~14% than that with papain plus urea. The effect of papain and urea on silk degumming is not simply the addition of their work by oneself, and that papain is seemingly affected by urea.

Summary

Although at 100°C, sericin could not be removed effectively from the raw silk in distilled water. Just in urea solution, sericin could be removed some from the raw silk when the temperature at 100°C. In papain solution, sericin could be removed effectively from the raw silk under certain conditions.

Papain shows high temperature resistance at degumming process. Especially in the presence of urea, the ability of papain degumming rises, to achieve the largest degumming rate requires temperature reduction. The degumming rate with papain plus urea is not the sum of both which with papain and urea separately, probably is the expansion effect of urea on sericin favors the diffusion of enzymes and their degradation products.

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