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Effect of immobilized cellulase enzyme treatment on properties of ramie fabric

Xiaoyan Ni[1], Yuanyuan Yu[1], Qiang Wang[1,a], Xuerong Fan[1], Artur Cavaco-Paulo[2] & Jiugang Yuan[1]

[1] Key Laboratory of Science and Technology of Eco-Textile, Ministry of Education, Jiangnan University, Wuxi, Jiangsu 214122, PR China[2] Center of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

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In this study, Eudragit S-100 has been covalently bound to the cellulase enzyme to form immobilized cellulase enzyme and then the effect of the treatment on ramie fabric properties is studied. The ramie fabrics treated with immobilized cellulase enzyme show lower quantities of reducing sugar, weight loss, and higher tensile strength than native cellulase enzyme-treated fabrics. Scanning electron microscopic analysis shows that the surface of ramie fabrics treated with cellulase enzyme is smoother than that of the untreated sample. Furthermore, treatment by the immobilized cellulase enzyme is less damaging to the fibres. X-ray diffraction studies show that there is hardly any loss in the crystallinity of ramie fabrics. Low-stress mechanical properties evaluated by the Kawabata Evaluation System for Fabric indicate that immobilized cellulase enzyme treatment improves the softness, flexibility, and elastic recovery of the ramie fabrics.

Keywords: Cellulase enzyme, Eudragit S-100, Immobilization, Ramie fabric, Textile-related properties

1 Introduction

Recently, with the increasing interest in ecological values and renewable materials, cellulosic materials have aroused extensive concern on the basis of their quality merits, including environmental quality[1]. There is a growing urgency to develop novel bio-based products and other innovative technologies[2]. Cellulosic fibres, as a category of renewable, recyclable, sustainable, and triggered biodegradable natural materials, can make a difference in the environment. Ramie, a perennial fibre plant belonging to the Urticaceae family, is one of the strongest plant fibres. It contains cellulose (65–75%), hemi-cellulose (14–15%), pectin (4–5%), and lignin (0.5–1.5%)[3]. Ramie is used for the production of textiles and ropes because it is extremely absorbent, dries quickly, resists shrinkage, and is unusually antibacterial, mildew resistant, and insect resistant[4]. However, ramie has failed to become a major textile material because of its poor elasticity, rigidity, and prickle sensation to human skin.

Cellulase enzyme treatment, i.e. treatment with enzymes that catalyze the hydrolysis of cellulose, is a widely used technique in the textile industry. For example, cellulase enzyme, together with pectinase and xylanase, is selected as a degumming agent for the removal of non-cellulosic impurities in jute fibres[5]. Cellulase enzyme treatment can change the dyeing behavior of cellulosic fibres, resulting in an enhancement of the dyeing efficiency with reactive dyes[6,7]. Cellulase enzyme is specially used on denim fabrics to achieve the fascinating worn look as well as soft handle[8,9]. Cellulase enzyme is also used in bio-finishing, which can improve the appearance and handle properties of cellulosic fabrics including cotton, ramie, and linen[10-12].

Although cellulase enzyme treatment can improve the performance of cellulosic fabrics, it inevitably results in serious damage to the fibres[13-15]. Enlarging the molecular size of cellulase enzyme by chemical modification can significantly inhibit the diffusion of the enlarged enzyme molecule into the interior of the cellulosic fibres. Thus, the attack of the modified cellulase enzyme is restricted to the surface of the cellulosic fibre, leading to minimal loss in the tensile strength[16,17].

In our previous study, a commercially available cellulase enzyme was covalently immobilized onto Eudragit S-100 (a copolymer of methacrylic acid and methyl methacrylate), and a Eudragit–cellulase enzyme conjugate with a higher molecular weight, henceforth ~~–i~~mmobilized cellulase enzyme" was obtained[18]. Previously, the properties imparted to cotton yarns and fabrics after the enzymatic treatment with native or immobilized cellulase enzyme were evaluated[19]. In the present study, the properties of

[a] Corresponding author.
E-mail: qiang_wang@163.com

native and immobilized cellulase enzyme-treated ramie fabrics including softness, handle, retention of weight, and strength are evaluated.

2 Materials and Methods

2.1 Materials

Cellulase enzyme Suhong B989N was kindly supplied by Novozymes (Shanghai, China), and Eudragit S-100 was supplied by Degussa-Hüls, S.A. (Shanghai, China). Carbodiimide hydrochloride and ethanolamine were purchased from Aladdin Reagent Company (Shanghai, China). All other chemicals used were of analytical grade. 100% half bleached ramie fabrics (125 g/m^2 basis weight, 29/24 yarns/cm density, 0.245 mm thickness) were obtained from Xinshen Company (Henan, China).

2.2 Immobilization of Cellulase Enzyme onto Eudragit S-100

Cellulase enzyme was covalently linked to Eudragit S-100 by carbodiimide coupling. The scheme of the immobilization of cellulase enzyme onto Eudragit S-100 is shown in Fig. 1. A carbodiimide coupling agent solution (0.2%, w/v) was added to the Eudragit S-100 (1.5%, w/v) solution and the mixture was stirred for 10 min. Then, the cellulase enzyme solution (1%, v/v) was added. The mixture was stirred at 40 °C for 6 h. Subsequently, the *p*H of the mixture was adjusted to 4.0–4.5 with acetate buffer to precipitate the Eudragit–cellulase enzyme conjugate. The precipitate was centrifuged (11,000× mg) at room temperature for 10 min and then washed by resuspending in acetate buffer (*p*H 4.5). The suspension was pelleted again by centrifugation and the supernatant separated. Finally, the Eudragit–cellulase enzyme precipitate was redissolved in acetate buffer (*p*H 5.0) for use when required[19].

2.3 Enzyme Assay

The carboxylmethyl cellulose (CMC) assay was used to determine the cellulase enzyme activity before and after the immobilization according to Mubarak[20]. The quantities of reducing sugars produced in the treatment baths were measured by the 3,5-dinitrosalicylic acid (DNS) method using glucose as the reference standard[21].

2.4 Enzymatic Treatment of Ramie Fabrics

Ramie fabrics were treated with 0.5–6% (o.w.f) native or immobilized cellulase enzyme at 30-70°C and *p*H 4.5–7.0 for 120 min with a liquor ratio of 1:20. After the cellulase enzyme treatment, the ramie fabrics were first rinsed with deionized water to wash away abraded fibres attached on the fabrics, and the residual cellulase enzyme on the fabrics was deactivated by incubation at 80°C in deionized water for 20 min. The treated fabrics were then washed once more with water and finally air dried. In the above experiments, the fabrics were treated in parallel under identical conditions with identical quantities (activity units) of the immobilized and native cellulase enzyme. Each experiment was performed in triplicate, and the results were averaged.

2.5 Weight Loss

After treatment with cellulase enzyme, the fabric samples were dried at 105 °C for 2 h and then conditioned at a standard atmosphere to reach equilibrium. The weight loss was calculated using the following equation:

$$\text{Weight loss (\%)} = (W_1 - W_2)/W_1 \quad \text{.. (1)}$$

where W_1 is the weight of the fabric before enzymatic treatment; and W_2, the weight of the fabric after enzymatic treatment.

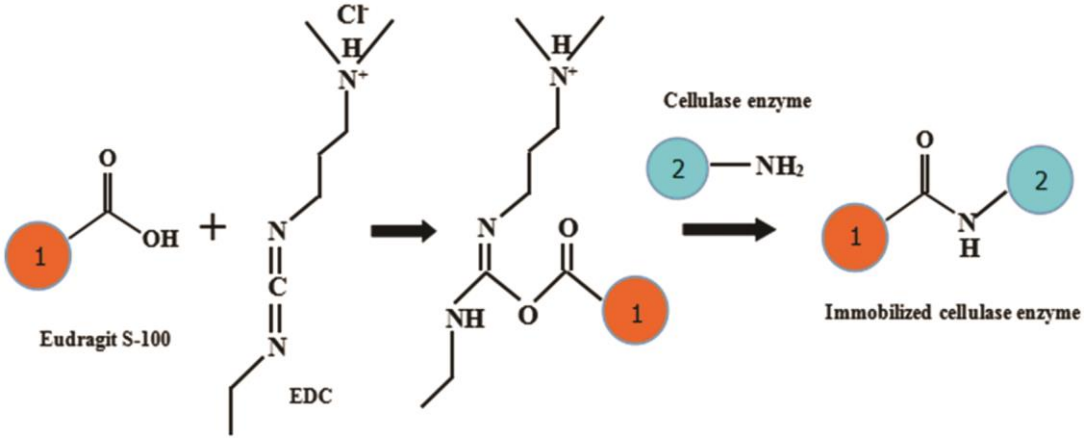

Fig. 1—Immobilization of cellulase enzyme onto Eudragit S-100

2.6 Tensile Strength Measurement

The tensile strength of the ramie yarns was measured using a YG020 tensile tester (Depu, China) with clamps spaced 500 mm apart and at a strain (bottom clamp) rate of 500 mm/min. The reported tensile strength of the yarn is the mean of 20 measurements.

2.7 Scanning Electron Microscopy

The surface morphology of the fibre sample was recorded at 100- and 1000-fold magnifications using a Quanta-200 scanning electron microscope (FEI, Netherlands). All the samples were coated with palladium before the scan.

2.8 X-ray Diffraction Method

X-ray diffraction (XRD) measurements were performed under ambient conditions on a Brucker D8 Advance X-ray diffractometer (Bruker, Germany). The scanning angles used were 5-40° at 4°/min. The crystalline indices (CrI) of the ramie samples were calculated from the XRD patterns by the following equation[22]:

$$\mathrm{CrI}(\%)=(I_{002}-I_{am})/I_{002} \quad \ldots (2)$$

where I_{002} and I_{am} are the crystalline and amorphous intensities at 2θ scale close to 22° and 18° respectively.

2.9 Fabric Handle Measurement

The Kawabata evaluation system for fabric (KES-F) was used for measuring the low-stress mechanical properties including the tensile, shearing, and bending properties of the fabric samples[23].

3 Results and Discussion

3.1 Effects of Enzymatic Treatment Parameters on Reducing Sugars

The effects of different variables of the enzymatic treatment, such as cellulase enzyme concentration, reaction temperature, and *p*H on the properties of ramie fabrics are investigated.

The variation in the released amount of reducing sugars when the ramie fabrics are treated with varying concentrations of cellulase enzyme (native and immobilized), is shown in Fig. 2. The release of reducing sugar increases with the increase in cellulase enzyme concentration up to 2.0%; variations in the concentration of cellulase enzyme above 2.0% led to a slower rate of increase in the released quantity of sugar. A reducing sugar content of 0.6260 mg/mL is achived when the ramie fabric is treated with 2.0% native cellulase enzyme. However, the treatment of the ramie fabrics with immobilized cellulase enzyme, with same activity as that of the native cellulase enzyme, results in a lower reducing sugar content (0.4749 mg/mL). In general, at the same concentration levels, native cellulase enzyme gives more reducing sugars from ramie fabrics than immobilized cellulase enzyme. It is likely that the bulky nature of the immobilized cellulase enzyme molecules inhibites their diffusion into the interior of the ramie fibres. As a result, lower quantities of reducing sugars are produced because of lower probability of contact between the modified enzymes and the fibre surface.

The trend in the released amount of reducing sugars from the ramie fabrics when treated with cellulase enzyme (native and immobilized) as a function of temperature is shown in Fig. 3. It is evident that the content of reducing sugar produced

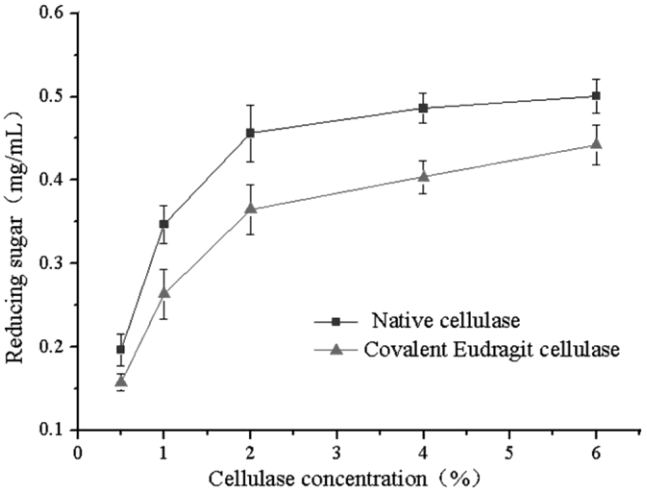

Fig. 2— Effect of cellulase enzyme concentration on released amount of reducing sugars

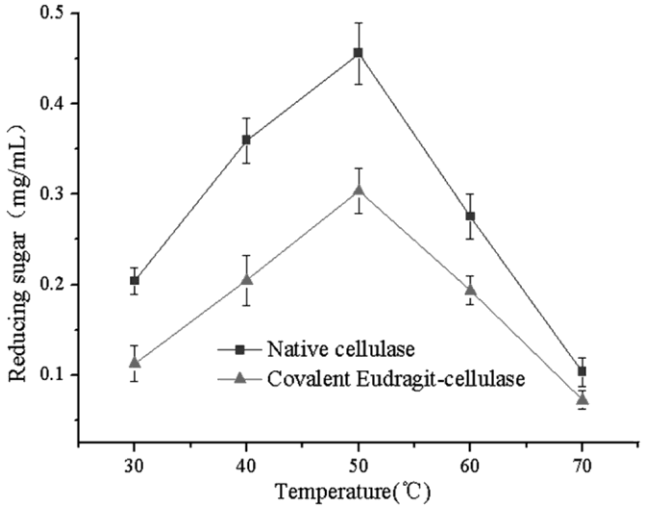

Fig. 3— Effect of temperature on released amount of reducing sugars

linearly increases from 30 °C to 50 °C and then gradually decreases with the further increase in temperature. Therefore, the optimum temperature for the release of reducing sugars from ramie fabrics in the reactions catalyzed by cellulase enzyme is found to be approximately 50 °C for both versions of the enzymes. The maximum content of reducing sugar (0.3036 mg/mL) produced by the immobilized cellulase enzyme is lower than that produced (0.4560 mg/mL) by native cellulase enzyme. It is implied that less damage is inflicted by the immobilized cellulase enzyme to the ramie fibres because of the localized hydrolytic attack on the fibre surfaces. From our previous studies, it is inferred that the immobilized cellulase enzyme preparation has better stability at temperatures above 50°C than native enzyme preparation[19]. As several long chains of Eudragit S-100 are grafted onto the surface of the enzyme, the immobilized cellulase enzyme is more rigid in the solution. Thus, the stability of the enzyme is improved, resulting in the observed difference in the rate of release of the reducing sugar.

The effect of *p*H on the released amount of reducing sugars is also investigated (Fig. 4). The results show that the released amount of reducing sugars is significantly influenced in the 4.5–7 *p*H range. The amount of reducing sugar detected increases with *p*H, and the maximum value is reached at *p*H 5.0. Further increase in *p*H (>5.0) results in a decrease in the amount of reducing sugar. The ramie fabric treated with native cellulase enzyme shows a higher amount of reducing sugar (0.4560 mg/mL) at *p*H 5.0 than that treated with immobilized cellulase enzyme (0.3036 mg/mL). This implies that the ramie fabrics treated with immobilized cellulase enzyme sufferes less damage than those treated with the native cellulase enzyme. The optimum *p*H for both the cellulase enzymes is found to be the same, indicating that the active sites of the enzyme are not affected by the modification[24].

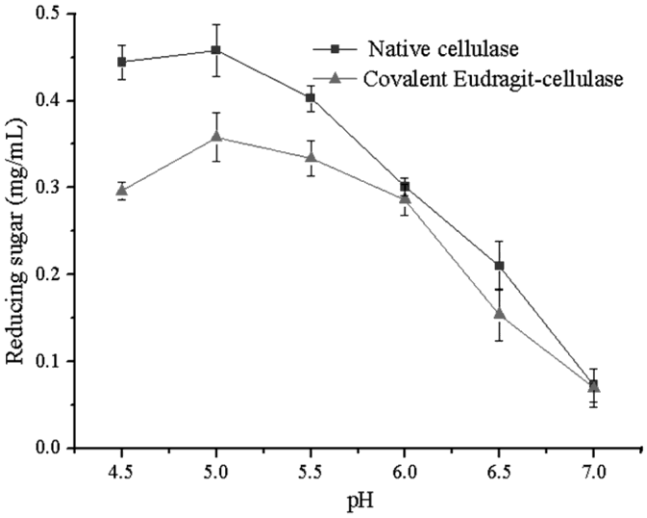

Fig. 4 —Effect of *p*H on released amount of reducing sugars

3.2 Weight Loss and Tensile Strength

Cellulase enzyme treatment often results in weight loss and deterioration in the strength of the cellulosic fabric, because of the ruptures in the enzymatically weakened fibres. Therefore, the loss in weight and tensile strength after enzymatic treatment can be used as measurable characteristics of the extent of the enzyme-catalyzed hydrolysis[25].

The trend in magnitude of weight loss in the ramie fabrics after treatment with varying concentrations of cellulase enzyme (native and immobilized) is shown in Fig. 5. It can be observed that the magnitude of weight loss gradually increases with the concentrations of native and immobilized cellulase enzymes from 0.5% to 2% (o.w.f); at higher concentrations, any additional increase in the concentration leads to only a slight increase in the weight loss. The weight losses of the ramie fabrics treated with native and immobilized cellulase enzymes (at 2% o.w.f) are found to be 2.175% and 1.975% respectively. As shown in Fig. 6, the tensile strength of the ramie yarns continues to decrease after treatment with increasing dosages of native and immobilized cellulase enzymes. At a cellulase enzyme concentration level of 2% (o.w.f), the tensile strength of ramie yarns treated with the immobilized

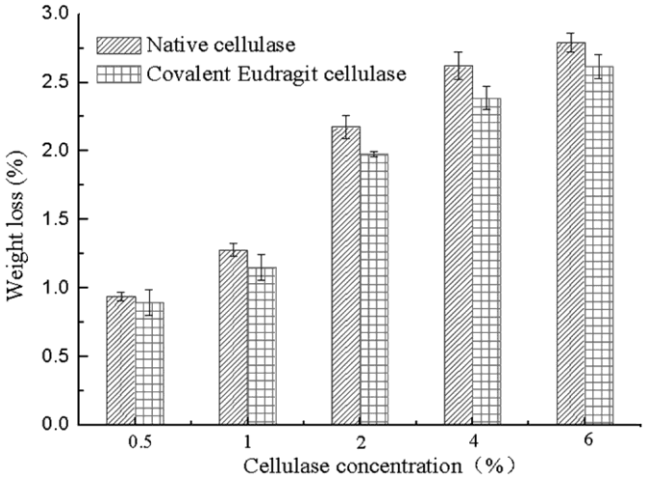

Fig. 5 —Weight loss of ramie as a function of cellulase enzyme concentration

cellulase enzyme (141cN) is found remarkably higher than that treated with native cellulase enzyme (118cN).

In general, the ramie fabrics treated with immobilized cellulase enzyme show higher tensile strength and lower weight loss than those treated with the native cellulase enzyme. On the basis of these results, it can be concluded that immobilized cellulase enzyme treatment causes limited damage to the ramie fabrics. This can again be attributed to the fact that the hydrolytic attack of the immobilized cellulase enzyme is restricted to the surface of the ramie fibres, thus, enabling a control over the hydrolysis process.

3.3 X-ray Diffraction Study

The crystalline nature and supramolecular structure of the cellulose substrate play a key role in their reactions with cellulase enzyme. These factors need to be considered to gain a better understanding on the effect of cellulase treatment on ramie fabrics. The changes in the crystallinity parameters of the ramie fibres after treatment with native and immobilized cellulase enzymes are shown in Table 1. There is a negligible decrease in the percentage of crystallinity of the ramie fibres after the cellulase enzyme treatment. These findings are found consistent with Schurz's theory, i.e. cellulase enzyme attacks the accessible termini of cellulose chain on the crystallite surface[26].

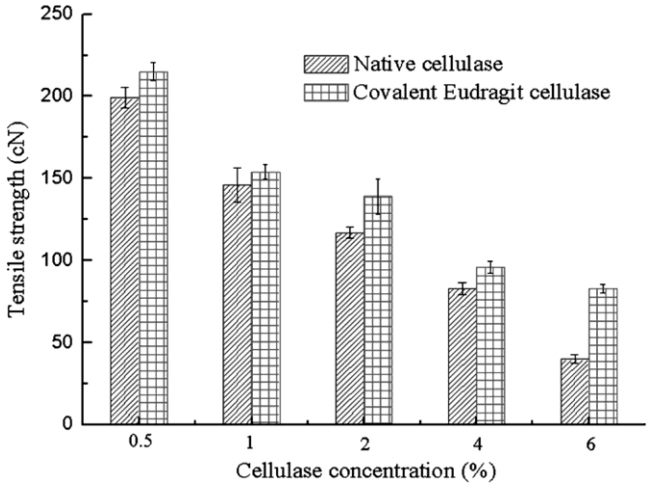

Fig. 6 —Tensile strength of ramie as a function of cellulase enzyme concentration

Table 1—X-ray diffraction for ramie fibres

Ramie sample	2θ, deg		CrI %
	I_{002} 2θ = 22.9°	I_{18} 2θ = 18.5°	
Untreated	576	93	83.85
Native cellulase enzyme treated	486	93	80.86
Immobilized cellulase enzyme treated	428	81	81.07

3.4 SEM Studies

Scanning electron micrographs in [Fig. 7 (A–C)] show the morphologies of cellulase enzyme-treated and untreated ramie fabrics. Comparison of the SEM images reveals a clear-cut distinction between untreated and cellulase enzyme-treated ramie fibres. Rough surface, fibrillation, and surface pilling were observed on the surfaces of the untreated fabrics. In contrast, the surface of the ramie fabric treated with immobilized cellulase enzyme is smoother and polished. However, ramie fabrics treated with native cellulase enzyme present high levels of peeling and cracking, which leads to significant loss in their weight and tensile strength. The SEM images reveal that the fibres treated with immobilized cellulase enzyme suffer much less damage than those treated with native cellulase enzyme.

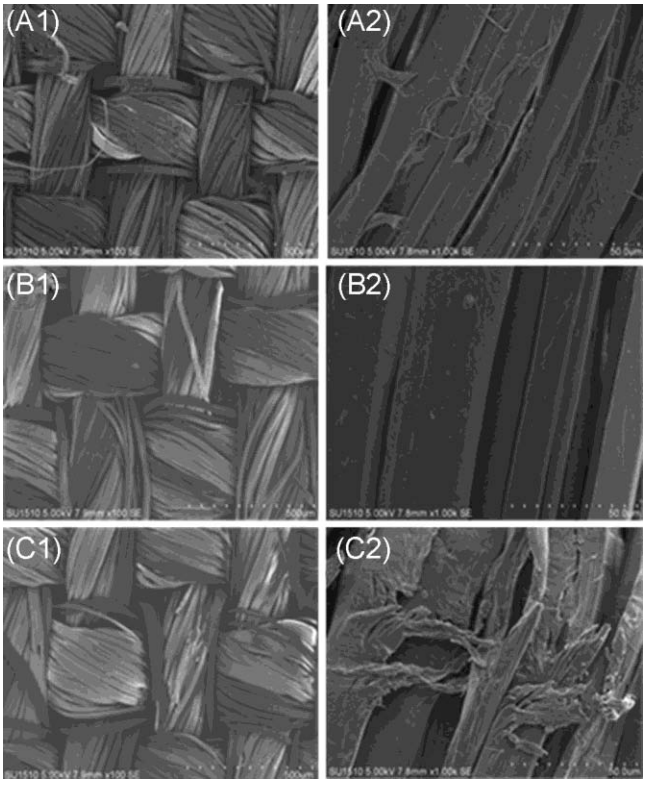

Fig. 7 —SEM morphology of ramie fabrics treated with (A) buffer (blank), (B) immobilized cellulase enzyme, and (C) native cellulase enzyme. [left: ×100 and right: ×1000]

Table 2 —Low-stress mechanical properties of ramie fabric

Properties	Untreated		Native cellulase enzyme treated		Immobilized cellulase enzyme treated	
Tensile	Warp	Weft	Warp	Weft	Warp	Weft
WT	6.45	16.25	5.65	16.1	6.05	15.3
G	0.44	0.42	0.35	0.35	0.39	0.37
Shearing						
2HG	0.25	0.28	0.23	0.28	0.2	0.25
2HG5	1.68	1.53	1.25	1.25	1.18	1.43
Bending						
B	0.1596	0.1191	0.1337	0.0811	0.1272	0.0995
2HB	0.0589	0.0484	0.0507	0.035	0.0428	0.031

3.5 Low-stress Mechanical Properties of Ramie Fabrics

KES-F is commonly used to determine the tactile properties (tensile, shearing, and bending) with objective data[27]. The low-stress mechanical properties of ramie fabric are summarized in Table 2.

3.5.1 Tensile Properties

The tensile properties of the fabric samples as measured by the KES-F system are shown in Table 2. Tensile energy (WT) is defined as the energy required for extending the fabric, which reveals the ability of the fabric to withstand external stress during extension. After the enzymatic treatment, a decrease in the tensile energy is observed, indicating weakening of fibres because of enzymatic hydrolysis[28]. These results are consistent with above-mentioned loss in both weight and tensile strength caused by the attack of cellulase enzymes.

3.5.2 Shearing Properties

The shearing properties include shear rigidity (G), shear stress at 0.5° (2HG), and at 5° (2HG5) shear angles respectively. A greater value of G describes attributes such as greater stiffness and resistance to shearing action. After the enzymatic treatment, there is a significant decrease in the values, representing the shearing properties of the ramie fabrics. This is mainly because both inter-fibre and inter-yarn frictions are slightly decreased during enzyme treatment[29]. The values of 2HG and 2HG5 of the untreated ramie fabrics (control) are found higher than that of the enzyme-treated ramie fabrics (Table 2), revealing that the enzyme-treated ramie fabrics have better recovery ability. Such behavior is predicted by the observation that the enzymatic treatment made the fabric surfaces smoother and reduces the friction between yarns.

3.5.3 Bending Properties

The bending properties include the bending rigidity (B) and bending moment (2HB), which have important effects on the handle and tailoring performance of the fabric. Bending rigidity (B) is defined as the ability of a fabric to resist the bending movement. Bending moment (2HB) reveals the recovery ability of the fabric after bending. The results (Table 2) indicate that the values of B and 2HB of the untreated ramie fabrics (control) are higher than that of the ramie fabrics treated with native and immobilized cellulase enzymes. Therefore, cellulase enzyme treatment of the ramie fabrics can enhance their flexibility and elastic recovery from bending.

The experimental results show that the cellulase enzyme treatment of ramie fabrics alters their low-stress mechanical properties to different extents. Cellulase enzyme treatment improves the softness, flexibility, and elastic recovery from bending of the ramie fabrics. At the same time, cellulase enzyme treatment also negatively influences the tensile strength of ramie fabrics. Fortunately, ramie fabrics treated with immobilized cellulase enzyme show limited loss in tensile strength. Therefore, it is feasible to improve the softness and handling of ramie fabrics by treatment with immobilized cellulase enzyme.

4 Conclusion

Treatment of ramie fabrics with either native or immobilized cellulase enzymes influences their performance significantly. Both the native and immobilized cellulase enzymes have the same optimum *p*H (5.0) and temperature (50°C). X-ray crystallinity of the ramie fibres is not affected by cellulase enzyme treatment. Immobilized cellulase

enzyme, which has a higher molecular weight and bulk, can improve the softness, flexibility, and elastic recovery of the ramie fabrics without causing any severe damage to the mechanical properties. Therefore, it is likely that immobilized cellulase enzyme can be used in bio-finishing of ramie and other cellulose fibres.

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