MECHANICAL PROPERTIES OF WET BLUE FOLLOWING ACID BATING PROCESS TREATED WITH CRUDE ENZYME FROM *Rhizopus oligosporus*

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ABSTRACT. Enzyme is one of the ingredients used in several tanning processes such as bating. Bating is generally conducted under alkaline conditions, but it can also be carried out under acidic conditions. This study aimed to evaluate mechanical and physical properties of wet blue following bating conducted under acidic conditions treated with enzymes synthesized by *R. oligosporus* compared to those of the conventional process. This study was arranged on a completely randomized design with a single factor, i.e., five levels of enzyme activity (0, 2.5, 5, 7.5, and 10 U mL^{-1}). The results showed that the use of enzymes from *R. oligosporus* influenced positively mechanical properties of the wet blue produced. Increasing levels of enzyme activity produced significantly higher tear strength and lower elongation at break values. No significant differences in tensile strength were observed following applications of both the synthesized and conventional enzymes. The synthesized enzyme was best applied at an activity level of 5 U mL^{-1} producing leather with good tear strength (55.40 ± 5.49 N mm^{-1}) and elongation at break (45.63 ± 4.17 %) values, which were significantly different from those of 0 U mL^{-1}, but not significantly different from those of 10 U mL^{-1} and the conventional process. Mechanical properties of the leather produced met the standard values for goat/sheep leather jacket as specified in Indonesian National Standard (SNI) 4593:2011. The results suggest that the synthesized enzyme from *R. oligosporus* can potentially replace the conventional enzyme commonly used in the bating process.

KEY WORDS: mechanical properties, wet blue, enzyme, *R. oligosporus*, bating

PROPRIETĂȚILE MECANICE ALE PIELII WET BLUE DUPĂ PROCESUL DE SĂMĂLUIRE CU ACIZI ȘI TRATARE CU ENZIMĂ BRUTĂ DIN *Rhizopus oligosporus*

REZUMAT. Enzima este unul dintre ingredientele folosite în mai multe procese de tăbăcire, cum ar fi sămăluirea. Sămăluirea se desfășoară în general în condiții alcaline, dar poate fi efectuată și în condiții acide. Acest studiu și-a propus să evalueze proprietățile mecanice și fizice ale pielii wet blue în urma sămăluirii efectuate în condiții acide și tratate cu enzime sintetizate din *R. oligosporus* comparativ cu cele obținute în urma procedeului convențional. Acest studiu a fost organizat ca design experimental complet randomizat cu un singur factor, adică cinci niveluri de activitate enzimatică (0, 2,5, 5, 7,5 și 10 U mL^{-1}). Rezultatele au arătat că utilizarea enzimelor din *R. oligosporus* a influențat pozitiv proprietățile mecanice ale pielii wet blue obținute. Creșterea nivelurilor de activitate enzimatică a condus la valori semnificativ mai mari ale rezistenței la sfâșiere și valori mai mici ale alungirii la rupere. Nu s-au observat diferențe semnificative în ceea ce privește rezistența la rupere în urma aplicării atât a enzimelor sintetizate, cât și a celor convenționale. Enzima sintetizată a fost aplicată cel mai bine la un nivel de activitate de 5 U mL^{-1} obținându-se piele cu o rezistență la sfâșiere (55,40 ± 5,49 N mm^{-1}) și valori de alungire la rupere (45,63 ± 4,17 %) bune, care au fost semnificativ diferite de cele obținute la 0 U mL^{-1}, dar nu semnificativ diferite de cele obținute la 10 U mL^{-1} și prin procedeul convențional. Proprietățile mecanice ale pielii obținute au îndeplinit valorile standard pentru o jachetă din piele de capră/oaie, așa cum este specificat în Standardul Național Indonezian (SNI) 4593:2011. Rezultatele sugerează că enzima sintetizată din *R. oligosporus* poate înlocui enzima convențională utilizată în mod obișnuit în procesul de sămăluire.

CUVINTE CHEIE: proprietăți mecanice, wet blue, enzimă, *R. oligosporus*, sămăluire

LES PROPRIÉTÉS MÉCANIQUES DE LA PEAU WET BLUE APRÈS LE PROCESSUS DE CONFITAGE ACIDE ET LE TRAITEMENT À L'ENZYME BRUT DE *Rhizopus oligosporus*

RÉSUMÉ. L'enzyme est l'un des ingrédients utilisés dans de nombreux processus de tannage, comme le confitage. Le confitage est généralement réalisé dans des conditions alcalines, mais peut également être réalisé dans des conditions acides. Cette étude visait à évaluer les propriétés mécaniques et physiques de la peau wet blue après acidification et traitement avec des enzymes synthétisées à partir de *R. oligosporus* par rapport à celles obtenues par la procédure conventionnelle. Cette étude a été organisée comme une conception expérimentale entièrement randomisée avec un seul facteur, soit cinq niveaux d'activité enzymatique (0, 2,5, 5, 7,5 et 10 U mL^{-1}). Les résultats ont montré que l'utilisation d'enzymes de *R. oligosporus* influence positivement les propriétés mécaniques de la peau wet blue obtenue. Des niveaux accrus d'activité enzymatique ont conduit à des valeurs significativement plus élevées de résistance à la déchirure et à des valeurs plus faibles d'allongement à la rupture. Aucune différence significative dans la résistance à la traction n'a été observée après l'application d'enzymes synthétisées et conventionnelles. L'enzyme synthétisée a été mieux appliquée à un niveau d'activité de 5 U mL^{-1} pour obtenir une peau avec une résistance à la déchirure (55,40 ± 5,49 N mm^{-1}) et des valeurs d'allongement à la rupture (45, 63 ± 4,17 %)

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bonnes, significativement différents de ceux obtenus à 0 U mL^{-1}, mais pas significativement différents de ceux obtenus à 10 U mL^{-1} et par la procédure conventionnelle. Les propriétés mécaniques du cuir obtenu ont respecté les valeurs standard pour une veste en cuir de chèvre/mouton, telles que spécifiées dans la Norme Nationale Indonésienne (SNI) 4593 : 2011. Les résultats suggèrent que l'enzyme synthétisée à partir de *R. oligosporus* peut remplacer l'enzyme conventionnelle couramment utilisée dans le processus de confitage.
MOTS CLÉS : propriétés mécaniques, peau wet blue, enzyme, *R. oligosporus*, confitage

INTRODUCTION

Tanning is one of the oldest technologies that can improve physical, chemical, biological, and mechanical properties of skin. The skin quality improvement technology involves many stages, which are grouped into beamhouse/pre-tanning, tanning, and post-tanning processes [1]. In each process, there are several stages that need to be passed to obtain tanned leather, some of which are deliming and bating [2]. Deliming and bating are different, but they are integrated processes. Deliming is a process that aims to remove lime left on the skin after the liming process is carried out [1]. It needs to be done so that the resulting skin is not hard and stiff.

According to [3, 4], deliming can be carried out in two conditions, namely slightly acidic and slightly alkaline. The bating stage will adjust the conditions carried out at the time of deliming. This shows the connection between the deliming and bating processes. In general, both deliming and bating processes are carried out under alkaline conditions. Some studies have shown that deliming can also be carried out under acidic conditions. However, only few studies have shown bating in acidic conditions.

Bating aims to remove non-collagen components remaining in the skin [2]. In addition, it helps perfect the opening of collagen fibers [5]. To achieve this goal, a bating agent, which contains enzymes, is required in the process. There are many different types and brands of bating agents (enzymes) that could be used for leather tanning. They could be obtained from bacteria, fungi, or yeast. This certainly provides an opportunity to synthesize enzymes from certain species that can also be used in bating, such as *Rhizopus oligosporus*.

R. oligosporus is a mold used in the production of tempeh (a traditional Indonesian food made from fermented soybeans). *R. oligosporus* is an enzyme-producing mold that can work optimally in acidic conditions. Enzymes from *R. oligosporus* can be synthesized at pH 3-7 and temperatures of 25-30℃ [6, 7]. Previous studies have shown that *R. oligosporus* can synthesize proteases and lipases [6–9]. According to [7], the resulting enzyme can work optimally at pH 5.5. This condition is in line with our previous findings [10], where the deliming process works optimally at the same pH. Therefore, in this study, bating was carried out under acidic conditions using enzymes produced from *R. oligosporus* and the characteristics of the resulting skin was evaluated.

EXPERIMENTAL

Materials and Methods

The study was conducted by using a crude enzyme (acid protease) from *Rhizopus oligosporus*, liming pelt sized 30 x 15 cm from the krupon of goatskin, lime, sodium sulfide, degreasing agent, tartaric acid, sodium chloride, sulfuric acid, formic acid, chromium sulfate, and sodium bicarbonate. The main equipment used includes an autoclave and HACH UV-Vis spectrophotometer. This study was conducted in three stages; (1) enzyme production, (2) enzyme application to the pelt (as a bating agent), and (3) pelt and wet blue properties evaluation.

This study was arranged on a completely randomized design with a single factor, which was enzyme activity, and four replications. Data were analyzed using ANOVA and Duncan's advanced test with a confidence level of 5%. The research procedure, from soaking to deliming, followed [10], and then continued as described in Table 1.

Table 1: Procedure of experiment

Processes	Materials	Doses	Remarks
Bating	Alkaline bate[a] Enzyme from *R. oligosporus*[b]	0.2%	[a]enzyme that was used in the conventional process. [b]prepared enzyme that was used in the experiment. Bating was done for 45 min. Then, the pelt was drained and washed.
Pickling	Water	100%	Water and NaCl were added and mixed for 20 min in a drum. Then, other materials were added periodically with an interval of 15 min. The pH of pelt was 3.
	NaCl	10%	
	Formic acid	0.6%	
	Sulfuric acid	0.4%	
Tanning	Chromium sulphate	8%	Chromium Sulphate was added and mixed for 1 h. Then, sodium bicarbonate was added and mixed for 6 h. The wet blue was drained and washed, followed by aging and toggling.

Enzyme Production

Rhizopus oligosporus from yeast was incubated on potato dextrose agar (PDA) media for 3 days. Then, the spores were propagated into skim media for 3 days. The propagated filtrate was used as a starter to produce the enzyme. The substrate used for enzyme production was sterilized tofu wastewater. The starter and substrate were mixed and shaken at 210 rpm, room temperature (28-30°C) for 72 h. The mixture was filtered to obtain an enzyme filtrate. Then, the filtrate was centrifuged at 4°C, 4,000 rpm for 20 min to obtain a clear enzyme solution [11].

Enzyme Application in Bating Stage

The enzyme solution (crude enzyme) obtained was applied to the pelt with different levels of enzyme activity. In general, skin, deliming, and bating processes were conducted according to the method developed by Nugraha *et al.* 2020. The crude enzyme dosages were 0 U mL^{-1}, 2.5 U mL^{-1}, 5 U mL^{-1}, 7.5 U $mL^{-1,}$ and 10 U mL^{-1} in the bating solution. Then, the pelt was tanned using chromium sulfate. The procedures are presented in Table 1.

Tear Strength

Tear strength of wet blue were tested using the INSTRON 3383 Universal Testing Machine. In this test, a hook was attached to the tool in order to pull the sample in the opposite direction so that the sample was torn. The tear strength value was measured when the sample started to tear and the needle indicated the tear strength value on the test tool stops [12]. The value of the tear strength was calculated using the following equation:

$$\text{Tear strength (kgf/mm)} = F/t \quad (1)$$

F = the value read on the test equipment (kgf)
t = skin thickness (mm)

Tensile Strength

Tensile strength test of wet blue was carried out using the INSTRON 3383 Universal Testing Machine. The sample was placed on the test equipment by means of both ends of the sample being clamped on the test equipment. The distance between the clamps was 5 cm. After the sample was ready, the tool was turned on and off when the sample broke [12]. The value of tensile strength was calculated using the following equation:

$$\text{Tensile strength (kgf/mm}^2) = F/ (t \times l) \quad (2)$$

F = value read on the device (kgf)
l = width of skin under test (mm)
t = thickness of skin under test (mm)

Elongation at Break

Elongation is a measurement of the elongation of the stretched skin from the initial condition to the final condition, i.e., when the skin is broken during tensile strength testing. Skin elongation was calculated by comparing the length of the skin before and after the test. The elongation at break was calculated using the following equation:

$$\text{Elongation at break} = (L_1 - L_0)/L_0 \quad (3)$$

L_1 = length at break time (mm)
L_0 = initial length (mm)

RESULTS AND DISCUSSIONS

Tear Strength

Tear strength is the force required to tear the resulting leather sample. Based on the analysis carried out (Table 2), enzyme activity influenced tear strength of the tanned leather produced. It is shown that increasing enzyme activity from 0 U mL^{-1} and 2.5 U mL^{-1} did not increase significantly tear strength of the tanned leather. Tear strength of the leather started increasing significantly (55.40 ± 5.49 N mm^{-1}) when the enzyme activity applied was 5 U mL^{-1}. However, further increases in enzyme activity up to 10 U mL^{-1} did not result in significantly higher tear strength values, which were also insignificantly different from that of the tanned leather produced by the conventional treatment. The tear strength values of the tanned leather produced by the all treatments complied with that of Indonesian National Standard (SNI) 4593:2011, i.e., minimum 12.5 N mm^{-1} [13].

Table 2: Mechanical wet blue properties in various enzyme activity

Enzyme Activity	Tear Strength (N mm^{-1})	Tensile Strength (N mm^{-2})	Elongation at break (%)
0 U mL^{-1}	47.66 ± 2.91^{a}	23.31 ± 2.22^{a}	51.65 ± 4.89 ^{d}
2.5 U mL^{-1}	52.20 ± 4.63^{ab}	26.13 ± 2.59^{a}	48.63 ± 2.29^{cd}
5 U mL^{-1}	55.40 ± 5.4^{9b}C	26.94 ± 2.13^{a}	45.63 ± 4.1^{7b}C
7.5 U mL^{-1}	57.74 ± 1.61^{bc}	27.85 ± 2.41^{a}	41.50 ± 1.65^{b}
10 U mL^{-1}	59.99 ± 4.13^{c}	26.63 ± 2.38^{a}	34.42 ± 2.59^{a}
Conventional	56.54 ± 3.01^{bc}	26.22 ± 2.46^{a}	46.17 ± 3.63^{bc}

Note: Means in the same column with different superscripts differ significantly ($p<0.05$)

In general, there was a consistent trend showing that the higher the enzyme activity was, the higher the tear strength of the tanned leather was. This is because the enzymes used in skin peeling degrade non-collagen compounds. This condition facilitates chromium to penetrate the skin and bind to collagen. As a result, this increases tear strength of the leather. According to [14], the amount of tanning material contained in the leather affects tear strength of the leather after treated by an enzyme. Figure 1 shows that the tear strength of the leather was influenced by the concentration of chromium oxide in the leather. It is shown that increasing concentrations of chromium oxide increased the tear strength of the leather. Meanwhile, according to [15], fiber direction, skin thickness, and the angle of collagen fibers to the grain layer affect the tear strength of the skin.

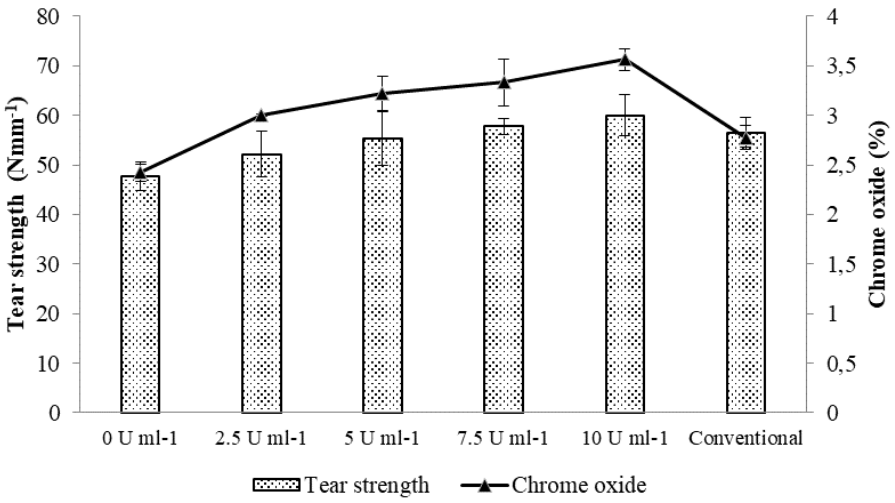

Figure 1. Correlation between concentration of chromium oxide in the leather and the resulting tear strength of the leather (wet blue)

Tensile Strength

Tensile strength is the force required to pull the leather to break per unit length times the thickness of the leather. The results showed that the levels of enzyme activity applied in leather peeling affected tensile strength of the leather. Table 2 shows that increasing enzyme activity up to 7.5 U mL^{-1} tended to increase tensile strength of the leather. However, at enzyme activity of 10 U mL^{-1}, there was a decrease in tensile strength of the leather produced. The highest tensile strength was 27.85 ± 2.41 N mm^{-2} found at 7.5 U mL^{-1} treatment. On the other hand, the lowest tensile strength (23.31 ± 2.22 N mm^{-2}) was observed in the leather treated with 0 U mL^{-1} enzyme activity. However, statistical analysis showed that the differences in the tensile strength values as influenced by the different levels of enzyme activity as well as the conventional treatment were insignificant (Table 2). Based on the Indonesian National Standard (SNI) issued by [13], the tensile strength values of the leather produced by the all treatments met the standard value determined in SNI 4593:2011, i.e., above 14 N mm^{-2}.

The results from this study were in agreement with those reported by [16] indicating that tensile strength of the leather decreases at a certain concentration of bating agent. This phenomenon could be due to decreased levels of elastin in the leather. According to [17], when an enzyme used for pulverizing the skin works more effectively, the collagen fibers are largely exposed to tanning materials. This facilitates strong interactions, and thus increases tensile strength. Previous studies showed that degradation of elastin in leather influences the quality/properties of the final leather product [18, 19]. However, excessive elastin degradation may cause a decrease in the quality of the skin itself [20]. This is also supported by [21] reporting that the use of proteases results in skin damage due to excessive elastin degradation. Furthermore, [22] found that proteases exhibit a hydrolytic activity against elastin, and the affecting factors are pH and temperature.

Elongation at Break

Elongation is the increase in length of the leather pulled up to break divided by the initial length of the leather. Table 2 shows that the levels of enzyme activity influenced elongation of the leather produced significantly. The higher the enzyme activity was, the lower the elongation of the leather was. It is shown that the highest elongation

of the leather (51.65 ± 4.89 %) was obtained when the enzyme activity applied was 0 U mL^{-1}. Conversely, the lowest elongation of the leather (34.42 ± 2.59 %) was found at the enzyme activity of 10 U mL^{-1}.

Further analysis shows that the elongation at 0 U mL^{-1} was not significantly different from that at 2.5 U mL^{-1}. The elongation started decreasing significantly when enzyme activity was applied at 5 U mL^{-1}, and it was not significantly different from those of 2.5 U mL^{-1}, 7.5 U mL^{-1}, and the conventional treatment. Further significant decrease in elongation of the leather occurred at enzyme activity of 10 U mL^{-1}, which was significantly lower than that of the conventional treatment (Table 2). The elongation values of the leather produced by the all treatments met the standard elongation value for goat/sheep leather jacket as specified in SNI 4593:2011, which is below 60 % [13].

The decrease in the elongation value of the tanned leather was in line with the increased level of enzyme activity used during leather pulverization. The presence of elastinolytic activity in enzymes causes a decrease in elastin levels in the skin, so that skin elasticity is lower. This is also supported by previous findings from [23]. However, the decrease in elastin levels does not only occur in skin crushing, but also liming, which is the stage before removing lime and peeling the skin. According to [22], elastin plays an important role in skin elasticity and affects the quality of the final skin product.

CONCLUSIONS

Crude enzyme extract from *R. oligosporus* applied in the bating process influenced positively mechanical properties of the leather. In this study, the best treatment was application of the synthesized enzyme with an activity level of 5 U mL^{-1}. The best treatment produced tanned leather with good tear strength and elongation at break values, which were significantly different from those of 0 U mL^{-1}, but not significantly different from those of 10 U mL^{-1} and the conventional treatment. No significant differences were observed in the tensile strength values of all treatments. All mechanical properties of the leather produced in this study met the standard values for goat/sheep leather jacket as specified in Indonesian National Standard (SNI) 4593:2011. The results suggest that the crude enzyme extract from *R. oligosporus* can potentially be used to replace the conventional enzymes in the bating process.

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