EVALUATION OF PHYSICAL PROPERTIES OF LEATHER ON THE BATING PROCESS BY COMBINATION OF PAPAIN ENZYME WITH SURFACTANT

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ABSTRACT. Bating is one of the important stages in the tanning process that has a role as a determinant of leather properties. It is commonly carried out with the assistance of protease enzymes such as papain. By using surfactants, sorbitol and Sodium Dodecyl Sulfate (SDS), it can help the penetration of enzymes into the skin or hides. Thereby, the study aimed to evaluate the use of papain on the bating process of *kacang* goat skin by assisted surfactants. Accordingly, three different concentrations (1, 1.5, & 2%) of enzymes were added with two different types of surfactants (SDS and Sorbitol) for the bating process. For the result, the leather was evaluated based on physical properties and histology studies. The presence of surfactant in the bating process improves the absorption of papain towards the hydrolysis of non-collagenous proteins. Based on the physical properties and histology studies, they perform better than leather without surfactant agents. Cleary, the surfactant agents can be used as bating auxiliary.

KEY WORDS: bating, papain, surfactant, *kacang* goat skin, physical properties

EVALUAREA PROPRIETĂȚILOR FIZICE ALE PIELII ÎN URMA PROCESULUI DE SĂMĂLUIRE PRIN COMBINAREA ENZIMEI PAPAINĂ CU UN SURFACTANT

REZUMAT. Sămăluirea este una dintre etapele importante ale procesului de tăbăcire, cu un rol determinant în obținerea anumitor proprietăți ale pielii. Se efectuează în mod obișnuit cu ajutorul enzimelor proteolitice, cum ar fi papaina. Folosirea unor surfactanți, sorbitol și dodecil sulfat de sodiu (SDS) poate facilita pătrunderea enzimelor în piele. Prin urmare, studiul și-a propus să evalueze utilizarea papainei în procesul de sămăluire a pielii de capră din specia *kacang* utilizând surfactanți. În consecință, s-au utilizat trei concentrații diferite (1, 1,5 și 2%) de enzime și două tipuri diferite de agenți tensioactivi (SDS și sorbitol) în procesul de sămăluire. S-au efectuat caracterizarea proprietăților fizice și studii histologice pentru a evalua pielea obținută. Prezența surfactantului în procesul de sămăluire îmbunătățește absorbția papainei până la hidroliza proteinelor non-colagenice. Proprietățile fizice și studiile histologice arată că pielea obținută are o performanță mai bună decât pielea fără agenți tensioactivi. În mod evident, agenții tensioactivi pot fi utilizați ca auxiliari pentru sămăluire.

CUVINTE CHEIE: sămăluire, papaină, surfactant, piele de capră *kacang*, proprietăți fizice

L'ÉVALUATION DES PROPRIÉTÉS PHYSIQUES DE LA PEAU APRÈS LE PROCESSUS DE CONFITAGE EN COMBINANT L'ENZYME PAPAÏNE AVEC UN SURFACTANT

RÉSUMÉ. Le confitage est l'une des étapes importantes du processus de tannage, avec un rôle décisif dans l'obtention de certaines propriétés de la peau. Il est généralement réalisé à l'aide d'enzymes protéolytiques, telles que la papaïne. L'utilisation de tensioactifs, de sorbitol et de dodécylsulfate de sodium (SDS) peut faciliter la pénétration des enzymes dans la peau. Par conséquent, l'étude a eu le but d'évaluer l'utilisation de la papaïne dans le processus de confitage de la peau de chèvre de l'espèce *kacang* à l'aide de tensioactifs. Par conséquent, trois concentrations différentes (1, 1,5 et 2%) d'enzymes et deux types différents de tensioactifs (SDS et sorbitol) ont été utilisés dans le processus de confitage. On a réalisé la caractérisation des propriétés physiques et des études histologiques pour évaluer la peau obtenue. La présence de l'agent tensioactif dans le processus de confitage améliore l'absorption de la papaïne jusqu'à l'hydrolyse des protéines non-collagéniques. Les propriétés physiques et les études histologiques montrent que la peau obtenue a de meilleures performances que la peau sans tensioactifs. De toute évidence, les tensioactifs peuvent être utilisés comme auxiliaires pour le confitage.

MOTS CLÉS : confitage, papaïne, tensioactif, peau de chèvre *kacang*, propriétés physiques

INTRODUCTION

Hides and skins are an end product of animal production, which are turned into a value-added product through a series of chemical, enzymatic, and mechanical procedures [1, 2]. To obtain the high quality of leather products is needed adequate preparatory treatment of hides or skins during the wet end phase [3]. The main features of desired customers, i.e., softness, grain smoothness and fullness, can be improved in the beam house operation [4]. Bating is one of the important stages of the beam house operation to produce the soft leather products such as gloves, upholstery, and garments [3]. Therefore, it is of high interest to promote novel biocatalysts in the bating process.

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Bating is one stage of the beam house operation, which employs protease enzyme as biocatalyst at neutral pH [5, 6]. This process is essentially performed to remove non-collagenous proteins from pelt, open up collagen fiber network and make the pelt soft and smooth for the purpose of tanning [6]. Non-structural proteins can be degraded by general proteases such as nagarse, trypsin [6, 7], chymotrypsin [8], papain [5], and pronse [9]. Previous studies revealed that trypsin enzyme is regarded as the best bating enzyme due to its moderate proteolytic activity and safety [8], and a mixture of trypsin and ammonium salts is commonly used in bating to provide better performance [6].

Currently, novel approaches are being carried out to identify other enzymes and examine the enzymes that have been used for bating of leather processing. For example, papain is very heat-stable [10], easy to produce, and has very broad specificities to break off specific peptide bond in pelt protein [5, 7]. However, the activation of enzyme is affected by other factors such as surfactants, cofactor, chelator, and reducing agents [5]. Surfactants have suitable properties to facilitate the penetration and adsorption of the enzyme in addition to fiber swelling [5, 11]. Accordingly, there are few studies on surfactans and other agents for enzyme processing were examined in the application of tanning process (soaking and fatliquoring) [5, 6, 11]. Here, this study evaluates the application of surfactants [Sodium Dodecyl Sulfate (SDS) and Sorbitol] in papain treatment of bating process of *kacang* goat skin. The existence of surfactants is expected to improve the papain solubility, and is predicted to help the penetration and adsorption of enzyme and fiber structure of pelt.

EXPERIMENTAL

Materials and Methods

Twenty-one *kacang* goat skins were used as the leather material. Chemicals used for leather processing were of commercial/ industrial grade. Papain was purchased from local company, Yogyakarta, Indonesia. The chemical analysis and physical testing of leathers were conducted at the Center for Leather, Rubber and Plastics-Yogyakarta, Indonesia. Microscopic studies were performed at Department of Anatomy, Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia.

Tanning process was carried out according to recipe in Table 1. For control, chromed leather was processed without surfactant of bating step, while experimental leather was processed by combination level of enzyme (1-2%) with surfactant (SDS and Sorbitol).

Table 1: The recipe for leather processing

Process	Product	(%)	Duration (min)	Remarks
Soaking	Water	300		
	Water	0.15	30	
	Calcium hypochlorite	0.5		
Unhairing	100% Water, 1% Sodium sulphide, 0.3% Degreasing agent		30	
	Sodium sulphide	1	30	
	1% Sodium sulphide, 0.5% Lime		20	Stop 40 min
Liming	Water	300	30	
	Sodium sulphide	2	60	18 h (run 5 min/h)
	Calcium hydroxide	6	30	
Fleshing, Trimming, Weighing				
Deliming	Water	100		
	Ammonium chloride	1	10	
	Deliming agent	1	25	

Process	Product	(%)	Duration (min)	Remarks
Bating	Water	100		Temp. 37°C
	Surfactant	X		Control = no surfactant X = SDS (0.2%) or Sorbitol (0.15%)
	Enzyme	Y	45	For control was used 1% of Basozym T 1000-BASF Y = 1, 1.5, and 2% of papain
Pickling	Water	100		
	Sodium chloride	7	10	
	Formic acid	0.8	30	pH = 2.9 – 3.0
	Sulfuric acid	0.7	120	
Tanning	33% Basic chromium sulfate	6	60	
	Sodium formate	1	60	pH = 3.8 – 4.2
	Sodium bicarbonate	1	70	

Determination of Tensile Strength and Percent Elongation at Break

Samples for various physical tests from experimental and control crust leathers were obtained as per IUP methods [13]. Specimens were conditioned at 20±2°C and 65±2% RH over a period of 48 hrs. Tensile strength and elongation at break was measured as per standard procedures. Each value reported is an average of three samples.

Determination of Shrinkage Temperature

The shrinkage temperature of both control and experimental tanned leathers were determined using the Theis shrinkage tester [14]. A 2 cm sample, cut out from the leather was clamped between the haws of the clamp, which in turn was immersed in a solution of glycerol: water mixture (3:1). The solution was stirred using a mechanical stirrer attached with the shrinkage tester. The temperature of the solution was gradually increased and the temperature at which the sample shrinks was noted. Each value reported is an average of three experiments.

Histological Studies

Histological examination was carried out on the bated *kacang* goat skins. Samples were taken and preserved in 10% formalin for 48 hours. The fixed samples were dehydrated in an aqueous alcohol series (50 to 100%) and then cleared in xylene. Samples were finally embedded in paraffin wax and 10 µm sections were cut on a microtome, mounted and stained with Van Gieson staining method [15].

Statistical Analysis

All the physical parameters tests performed in triplicate were compared for the different types group of surfactants with papain concentration level using variance of ANOVA. The Turkey test for post hoc analysis was chosen because of its robustness. Statistical Package for Graphpad prism 8 program was used for all statistical analyses. All tests were considered statistically significant when they had a *p*-alpha value of less than 0.05.

RESULTS AND DISCUSSIONS

Bating is very important process in leather making, which facilitates achieving the softness feature of leather. This process connects to the proteolytic enzyme system and cannot be substituted by any mechanical and chemical procedure. However, the acceleration of enzymes needs to be improved by using surfactants. Surfactants have been widely used in many industrial products such as detergents, medicines, and anti-corrosive treatments due to their unique structures consisted of two different molecular parts and the broad range of selection [16]. Applying a surfactant can help the enzymatic process of bating stages. Hence, this work evaluated the use of surfactants in papain treatment of bating process of *kacang* goat skin.

Table 2: Physical properties of tanned leather (control) and combination of papain concentration (1%) with surfactant (SDS or Sorbitol)

Physical properties	Control	Type of surfactant		BIS standards [17]
		SDS	Sorbitol	
Tensile strength (Kg/cm^2)	261.76±0.34	352.11±0.53	346.60±0.76	200
Elongation at break (%)	75.17±0.53	64.02±0.96	66.37±0.67	40-65
Shrinkage temperature (°C)	90.67±0.58	94.67±0.58	94.33±0.58	-

Note: The values are means ± S.D. of three values

Physical properties are essential to examine the addition of surfactant on bating process. The physical strength measurements and shrinkage temperature of experimental and control leathers are given in Table 2. The tensile strength measurement was found to be better for experimental leathers, while the elongation at break was slightly lower than control. However, all the physical properties for both control and experimental leathers are found to meet the requirement of BIS standards for upper leathers. While the shrinkage temperature data show that the addition of surfactants in the bating process gives a good shrinkage temperature around 94°C compared to control 90°C. Despite the meet requirements, the tensile strength value of the control is relatively close to the BIS standards [17]. Clearly, the collaboration of enzyme and surfactant resulted in an improvement in tensile strength and shrinkage temperature of leather.

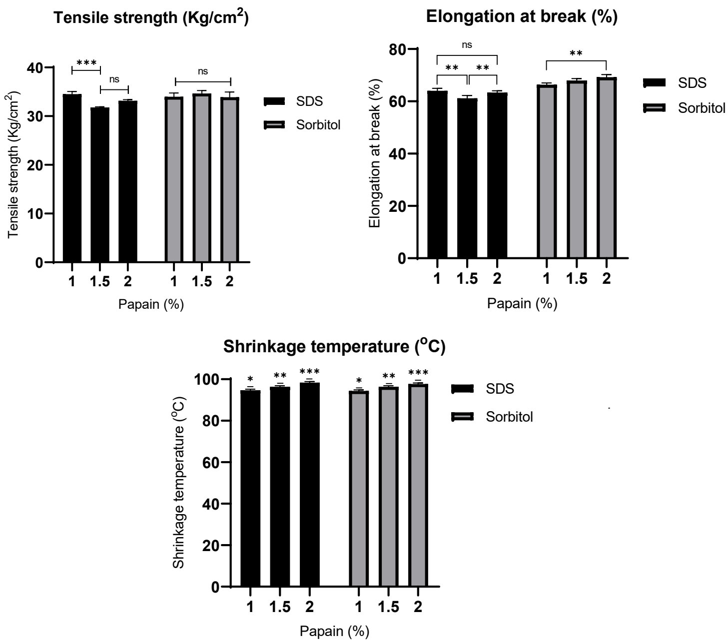
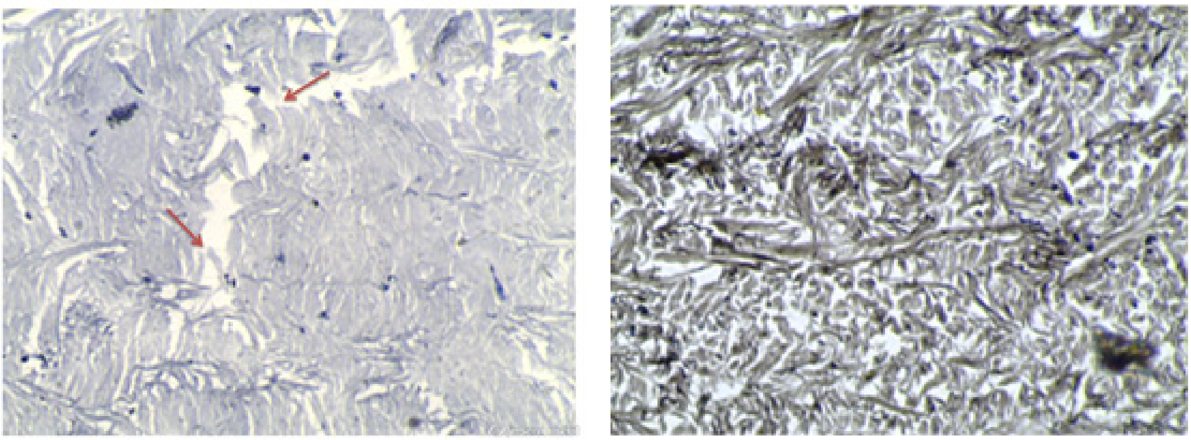


Figure 1. Physical properties of tanned leather from combination of papain concentration level with surfactant (SDS or Sorbitol)

Previous findings show that the use of enzymes and surfactants gives excellent physical properties of leather. This prompted us to investigate a papain concentration level (1%, 1.5%, and 2%) with two kinds of surfactant (anionic and nonionic) to gain information about the utilization of surfactant in bating process. Generally, the result of the examination gives an effect on the elongation at break and shrinkage temperatures of leather except for the tensile strength (Figure 1). For Sorbitol, elongation at break and shrinkage temperature of the samples are obviously improved linearly with the increase of papain in the range 1-2% whereas SDS is only on elongation at break measurement. Both tensile strength values have no effect by the increase of papain due to tanning enhancing the structural stability and the mechanical strength of the leather. While, the various types of surfactants in bating have an impact to the leather tensile strength, the elongation at break, and the shrinkage temperature [5]. The addition of surfactants can improve the removal of non-collagenous protein, and hence useful for the opening up of the fiber network [4, 18]. Concurrently, this causes the increase of the collagen reactivity group, thus the bond point between the chrome tanning agents and the skin was stronger [18].



A. Experimental leather **B. Control leather**

Figure 2. Residual of elastin fiber in grain layer (100 x hor.sec.)

The optical microphotographs taken for the bated skins are set out in Figures 2. The microphotographs show that the surfactant boost enzymes to open up more collagen fibers than the control, making the cavities among collagen as indicated by the red arrows. These results demonstrate that surfactants promote the acceleration of enzymatic reaction towards to the degradation of non-protein fibrous.

CONCLUSION

In conclusion, we here describe the application of surfactants in the bating process. The addition of surfactant improves the penetration of papain towards to proteolysis non-collagenous in satisfactory bating effect. Surfactant agents, such as SDS and sorbitol, perform better than control of leather non-surfactant according to the physical properties and histology studies. As a result, the surfactant agents can be considered as supplemental molecules for bating process.

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