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QUALITY ASSESSMENT OF COMMERCIAL BATING ENZYMES IN SELECTED KENYAN TANNERIES FOR USE IN DIFFERENT PRE-TANNING PROCESSES

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ABSTRACT

The aim of the study was to assess the quality of commercial bating enzymes in Kenyan market for potential use in different pre-tanning processes to reduce the use of hazardous chemicals. Five tanneries were involved in the study and 150 grams of enzymes were collected from the tanneries. Enzyme activity, moisture content and solubility of enzymes were done according to the Bureau of Indian standards 1991. ANOVA was used to test the significant difference between the analyzed parameters. Assessment of the protease activity indicated Microbate- Ewaso Nyiro to have 21,321.33± 54.64 (U/g), Microbate-Yetu leather 24,137.4 ± 65.25 (U/g), Micro enzyme P-Sagana 24,717.6 ± 109.84 (U/g), Microbate elbate -AHITI 11,341.2 ± 68.05 (U/g) and Microenzyme elbate- LIK 23,883.6 ± 97.10 (U/g). Most of the parameters measured such as protein content, fat content, total solids and suspended solids indicated no significant difference (p> 0.05) in soaking and unhairing process. Assessment of the Organoleptic tests of bated pelt gave a rate of 4-5 (good- very good) which is a good indication that the enzymes are suitable for bating process. In conclusion, commercial bating enzymes are very effective in bating process only. Therefore, special formulations are needed to be used in soaking, unhairing and degreasing stages.

INTRODUCTION

Leather industry utilizes hides and skins which are a by-product of meat industry [1]. The process of converting these putrescible raw hides and skins into leather is called tanning [2]. During tanning processes, both liquid and solid waste are produced and their disposal becomes a huge challenge to tanners [3]. Due to this, the sector has been classified to be among the most polluting agro-based industries in the world. The type and amount of the pollutants produced by different tanneries vary depending on the type of rawhides and skins used and the required properties of the finished products [4].

The increase in pollution causing environmental and human health risks has greatly accelerated people's focus on developing more environmentally friendly leather chemicals and cleaner leather manufacturing technologies [5]. One of the options is the application of enzymes in the pre-tanning process to reduce or eliminate most of the hazardous chemicals. Enzymes are large biomolecules that accelerate all biological processes and can increase reaction rates by 100 million to 10 billion times faster than any normal chemical reaction [6]. There are different types of enzymes that can be used by the tanner which include: protease, keratinase, lipase amylase, elastase and others but their use depends on availability and cost. According to Mamun et al., (2015) [7], protease enzymes account for more than 65% of the total industrial enzymes in the market. The second largest group are various carbohydrases and mostly amylases and cellulases [8].

The hides and skins contain water, protein, fat, and mineral matters. According to Maxwell, (2007) [9], cattle hide has 29% collagen, 2% keratin, 0.3% elastin, 0.3% globulins and albumins, and 0.7% mucins and mucoids. Goat skins have also been reported to have different chemical compositions. According to Hakim et al., (2021) [10], goatskins have 60%- 70% water, 25%-32% protein, 2.2%-3.2% fiber protein, and 7%-7.3% crude fat. From these two examples, it is evidence that the chemical composition of hides and skins varies from one animal to another. Due to these variations in the chemical composition of hides and skins, the processing recipe is different for different animals. Collagen is the main leather-forming material consisting of all 20 standard amino acids in addition to hydroxyproline and hydroxylysine. There are three main processes involved in transforming hides and skins into leather namely: pre-tanning, tanning, and post-tanning [11]. In the pre-tanning process, the non-collagenous components of the skin such as proteoglycans, albumin, globulin, fats, reticulin, and keratin are removed [11]. The non-collagenous components are partly or completely removed through several sequential steps from hides or skins and the characteristics of the final leather depend on the extent of their removal [12]. These processing steps include soaking, Unhairing, liming, fleshing, deliming, bating, degreasing and pickling [2].

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Soaking is the first pre-tanning process and it is carried out to remove dirt, salts, and nonfibrous proteins such as albumin and globulins [13]. In the conventional method, the addition of sodium carbonate or sodium sulfide help to raise the pH values between 9 and 10, and this quickens the rehydration process of hides and skins [13] Unhairing and liming are mostly done as a compact process. In this process calcium hydroxide and sodium sulphide are used and are known to cause huge water pollution in the tannery [14].

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In addition, the treatment of unhairing and liming liquors is very difficult and expensive [14]. Deliming is another pre-tanning process carried out after the liming process to solubilize residual lime and deflate the structure by lowering the pH down to 8.5-9.0 [13]. The most commonly used chemicals in this process include ammonium sulfate or ammonium chloride because they penetrate quickly into the hide and have good buffering action in the suitable pH range required for deliming. The bating process is the only process in the tannery that cannot be substituted by chemicals [13]. During bating, the skins are treated with proteolytic enzymes in order to open the fibrous structures of the skins to increase their softness. Pickling and degreasing are also carried out but may differ depending on the type of tannage needed and the amount of fat in hides and skins. Tanning is the process that transforms putrescible raw hides and skins into non-putrescible and stable products [11]. Post tanning operations include operations such as coloring and lubrication of the leather. Due to pollution associated with the leather sector, a lot of research is being carried out to assess the effectiveness of enzymes in different pre-tanning stages. They have been reported to play an important role in soaking, dehairing, bating, and degreasing processes.

The use of clean technology has not been adopted by most tanneries in Kenya and they use enzymes mostly in the bating process. There have been a lot of complaints from the tanners that the enzymes in the Kenyan market are not effective. Therefore, the current study was conducted to assess the quality of commercial bating enzymes for use in different pre-tanning stages.

MATERIALS AND METHODS

Sample collection

Five tanneries were involved in the study and 150 grams of enzymes were collected from the tanneries.

Determination of enzyme activity

Determination of enzyme activity was done according to Bureau of Indian standards (1991) [15]. One gram of enzyme was weighed accurately in a beaker and 100 ml of distilled water was added. The beaker with its contents was kept at 37°C for 2 hours with occasional stirring and the enzyme extract was collected after filtration. Two point five milliliters each of the enzyme extracts and distilled water were added to 10 ml of casein solution prepared earlier. The mixture was kept in a conical flask and incubated at 45°C for 30 minutes. After the period, the reaction was stopped by adding 30 ml of 5 percent trichloroacetic acid solution and the mixture heated on a boiling water bath for 2-3 minutes. It was then cooled to room temperature and filtered. Two milliliters of distilled water, 5.0 ml of sodium hydroxide and 1.5 ml of diluted Folin phenol reagent were added to 0.5 ml of the filtrate.

The intensity of the blue color developed by a spectrophotometer at 660nm was measured after shaking. A control was also used in the same manner except that trichloroacetic acid was added before the addition of the enzyme solution and before putting them in the incubator. The Control values were subtracted from experimental values for the calculation of the enzyme unit.

Determination of moisture content

The moisture content was determined according to Bureau of Indian standards (1991) [15]. Five grams of the bate was weighed into a porcelain basin and dried in an oven at 105°C for six hours. The basin was cooled with its content in a desiccator and weighed. The process was repeated till a constant mass was obtained.

$$\text{Moisture, percent by mass} = \frac{(M - m)}{M} \times 100$$

M= Mass, in g of the sample taken for the test
m= mass, in g of the residue

Determination of insoluble matter

The insoluble matter was determined according to Bureau of Indian standards (1991) [15]. Ten grams of bate was weighed accurately into a 500-ml beaker then dissolved in a 400 ml of distilled water. The solution was stirred until apparently complete. The solution was filtered using Whatman filter paper (No 4) and the filter paper was washed with distilled water for several times. The paper was dried in a hot oven at 105°C to constant mass. It was cooled in a desiccator and weighed.

$$\text{In soluble matter, percent by mass} = \frac{m}{M} \times 100$$

m=mass, in g of the residue after deducting the mass of the filter paper
M= mass, in g of the sample taken for the test

Effectiveness of enzymes in different pre-tanning processes

To assess the effectiveness of the commercial bating enzymes in different pre-tanning processes, 8 goat skins were used for the study. Samples weighing around Ten grams were sampled from the butt area as recommended by Society of leather technologist and chemists (2001) [16]. For soaking and unhairing process, 5 % of the enzymes were used for a duration of 5 and 24 hours respectively. Several parameters were assessed such as percentage weight gain and fat content of the residual samples. Total solids, suspended solids and protein content were also assessed on the liquor according to APHA, AWWA, WPCF (1989) [17]. For bating process, the sampled skins were processed by conventional sodium sulphide unhairing method and the pelts bated by application of 2% enzymes. Parameters such as thumb imprint, softness, appearance, flexibility and grain firmness were assessed by rating them from a rate of 1-5 (very poor – very good).

Data analysis

The data were analyzed using the statistical package for social science (SPSS) version 21. The results were presented using descriptive statistics such as means, standard deviation and graphs. ANOVA was used to test the level of significance and also post hock test was performed using Duncan multiple comparison tests to identify the means that were significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

The quality and effectiveness of enzymes was assessed in different pre-tanning. Microenzyme p, microbate elbate and micro enzyme elbate were the commercial enzymes used in Kenyan market. These enzymes gave different moisture, solubility and protease activity as indicated on [Table 1].

Table 1: Enzyme activity, percentage moisture and solubility of commercial enzymes.

Enzyme	% Moisture	Solubility (%)	Enzyme activity (U/g)
Microbate- Ewaso Nyiro	3.08 ± 0.88	60.53 ±2.42	21,321.33± 54.64
Microbate-Yetu leather	0.28 ± 0.08	65.65 ± 2.16	24,137.4 ± 65.25
Microenzyme P-Sagana	0.91 ± 0.07	64.06 ± 1.95	24,717.6 ± 109.84
Microbate elbate -AHITI	0.20 ± 0.07	53.81 ± 7.28	11,341.2 ± 68.05
Microenzyme elbate- LIK	1.86 ± 0.23	67.74 ± 1.9	23,883.6 ± 97.10
p- Values	p< 0.05	P=0.140	p< 0.05

Micro enzyme-p had the highest activity of 24,717.6 ± 109.84 U/g while Microbate elbate enzyme from AHITI tannery had the lowest activity of 11,341.2 ± 68.05 U/g. Dancan multiple comparison tests indicated a significant difference ($p < 0.05$) in enzyme activity among all the enzymes. All the enzymes had more than 10, 000 U/g which is the required minimum activity for bating enzymes [15]
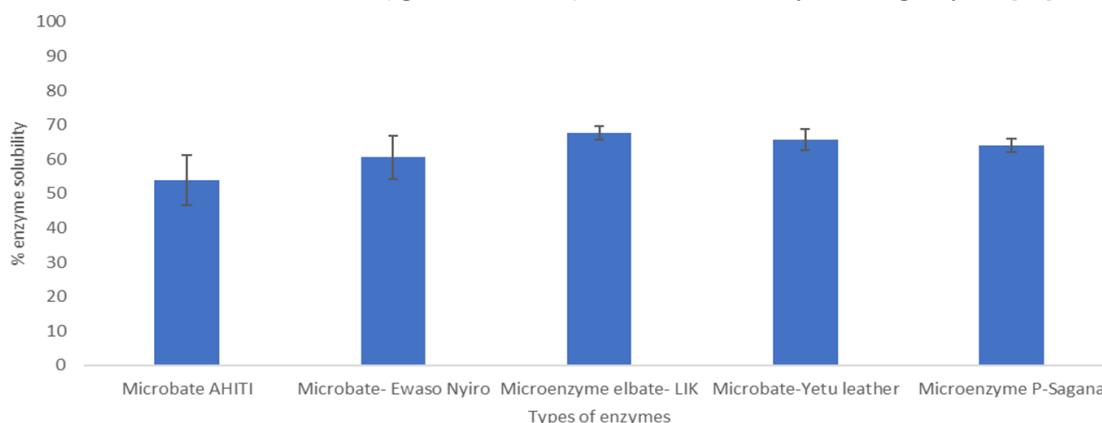

Fig. 1: Solubility of commercial enzyme

Most of the leading enzyme manufacturers does not include detailed information about the enzymes and therefore their activities are not known [18]. Different researchers have reported the use of enzymes with different activities: Novo Nordisk 174U/g, UAB Biosinteze: 23U/g, TM enzyme (activity: 100, 000 units/g, AprA and AprA-PPC specific activity of 42567.1 U mg-1 and 99511.9 U mg-1 [19, 20, 21]. The moisture content was within the required range (<5 %) while all the enzymes failed the solubility test which should be above 90% as described by bureau of Indian standards [15]. Micro enzyme elbate- LIK had the highest

solubility of 67.74 ± 1.9 while Elbate -AHITI had the lowest solubility of 53.81 ± 7.28 as indicated on [Fig 1].

Most of the parameters measured to assess the effectiveness of enzymes in soaking process showed no significant difference (p> 0.05) as indicated in [Table 2]. Duncan multiple comparison tests indicated that time had a significant effect on percentage weight gain (P= 0.02) while the type of enzymes had no significant effect (P= 0.75). A similar study by Stockman et al., 2008 [22] reported an increase in percentage weight gain on cured hide by 45% when a mixture of enzyme, nonionic surfactant and dry powdered soda ash were used in a soaking process. The study also indicated that the hide gained more weight within the first one hour when soaked with the soaking aids but the weight declined after four hours except on the samples soaked by use of enzymes alone [22]. The protein content of the effluent which is an indication of the nonfibrous protein removed from the skin was also analyzed. The samples processed by use of water (Blank) had the least crude protein of 0.41 ± 0.30% but the difference was not statistically significant (P= 0.68). The fat content from the samples processed by use of Microbate-Yetu leather had the lowest amount of fat content (3.98 ±1.22%) but the difference was not significant from the others (P= 0.42). Afsar and Cetinkaya (2008) [23], reported that application of enzymes (alkaline protease/ lipase at liming and unhairing stage and acidic lipase) can reduce the fat content of the pelt to below 4%. Among the studied enzymes only microbate-Yetu leather gave a fat content below 4% (3.98 ±1.22). Total solids represent the dirt and soluble component of the skin while suspended solids represent insoluble dirt's as indicated in [Table 2]. There was no significant difference (P= 0.18) for the mean total solids and suspended solids among all enzymes and the blank.

Table 2: Assessment of enzymes effectiveness in a soaking process

	Total solids (g/l)	Suspended (g/l)	Protein (%)	% Weight gain (g)- (5 Hrs)	Fat content (%)
Microbate- Ewaso nyiro	99.22 ± 42.28	10.35 ± 2.19	0.49 ± 0.18	57.34 ± 4.94	5.96 ±2.28
Microbate-Yetu leather	115.58 ± 6.26	10.25 ± 8.10	0.48 ± 0.16	66.29 ± 5.06	3.98 ±1.22
Microenzyme P-Sagana	89.07 ± 28.76	11.38 ± 0.90	0.47 ± 0.14	57.17 ± 0.82	4.80 ±2.4
Micro enzyme elbate-LIK	93.68 ± 39.25	8.14 ± 3.76	0.56 ± 0.18	75.01 ± 5.56	7.47 ±1.28
Elbate AHITI	71.79 ± 1.51	11.73 ± 0.12	0.42 ± 0.11	63.69 ± 8.77	7.88 ±3.39
Blank	73.57 ± 5.44	7.67 ± 1.80	0.41 ±0.30	56.86 ± 3.13	7.82 ±1.483
p-values	0.183	0.4229	0.682	0.02	0.423

Table 3: Assessment of the effectiveness of enzymes in unhairing process

	Proteins (%)	Suspended solids (g/l)	Total solids (g/l)	% Weight gain (g)- (24Hrs)	Fat content (%)	Unhairing
Microbate- Ewaso Nyiro	0.79 ± 0.28	10.39 ± 2.48	79.81 ± 14.82	57.38 ± 0.79	8.15 ± 1.42	No
Microbate- Yetu leather	0.70 ± 0.13	12.44 ± 3.21	81.37 ± 0.79	66.29 ± 5.06	7.04 ± 0.01	No
Micro enzyme P-Sagana	0.75 ± 0.19	11.32 ± 0.95	79.29 ± 11.41	57.17 ± 0.82	8.65 ± 1.43	No
Micro enzyme elbate -LIK	0.67 ± 0.08	15.69 ± 2.12	85.96 ± 18.72	72.88 ± 4.58	7.42 ± 1.16	No
Microbate elbate-AHITI	0.66 ± 0.09	14.88 ± 3.44	57.35 ± 23.35	54.86 ± 2.57	7.97 ± 0.65	No
Blank	0.61 ± 0.14	11.10 ± 3.43	64.95 ± 4.39	58.21±0.749	9.21 ±0.62	No
P – values	0.967	0.273	0.684	p< 0.05	0.971	

A multiple comparison test was performed to identify the means that were significantly different. The type of enzymes and time of unhairing process seemed to have a significant difference on the percentage weight gain of the pelt (P< 0.05). Microbate-Ewasonyiro, Microbate- yetu leather, microenzyme p-sagana, microbate elbate-AHITI and the blank had no significant difference (p> 0.05) while microenzyme elbate-lik seemed to be different from all the other treatments. The percentage weight gain also varied with time. The skin gained more weight within the first three hours and a multiple comparison test indicated a significant difference in percentage weight gain (p< 0.05) in first hour, 4th, 5th, and 24 hours. The total suspended solids ranged from 10.39- 14.88 g/L which was comparable to a study by Ranjithkumar et al., 2016 [24], which found the suspended solid to be 10.1 g/L.
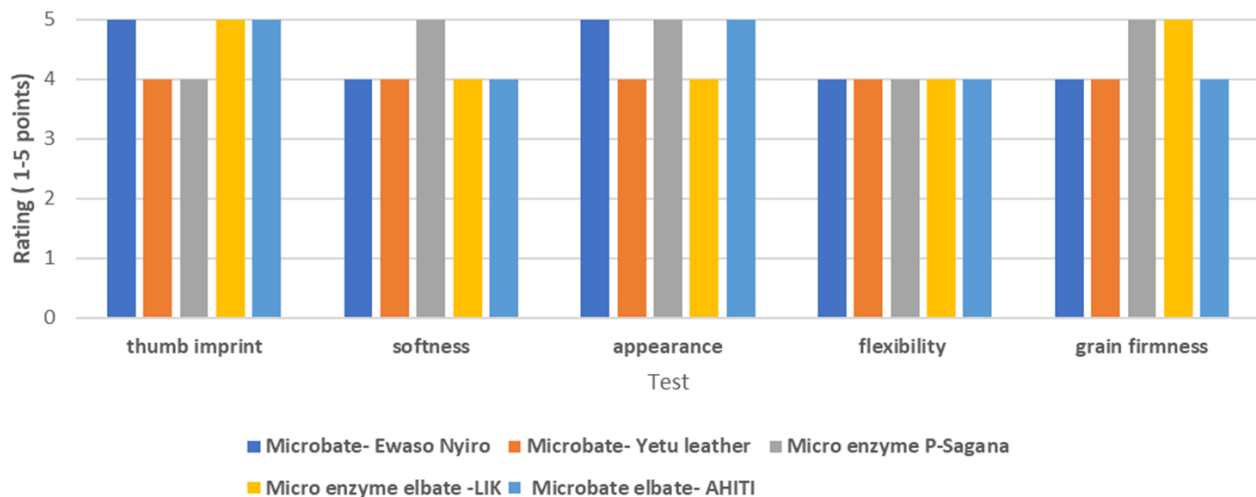
Fig. 2: Solubility of commercial enzyme

For all the other parameters tested, there was no significant difference (p> 0.05) among all the treatments: percentage protein, total solids (g/l), % weight gain (g)- (24Hrs) and fat content [Table 3]. A related study by Ângela et al., (2018) [25], found that wet-salted pelt soaked for a period of 24 hours had 41.1% weight gain which was lower compared to the findings of the current study where the percentage weight gained ranged from 72.88-54.86%.

Assessment of the Organoleptic tests of bated pelt (thumb imprint, softness, appearance, flexibility and grain firmness) was also carried out by rating them from 1-5 (Very poor - very good). For all the properties tested all of them were given a rate of 4-5 (good- very good) and this is a good indication that the enzymes are suitable for this processing steps [Fig 2].

CONCLUSION

For most of the parameters measured: percentage fat content, protein content, total solids and suspended solids indicated no significant difference p> 0.5 in the soaking and unhairing process. The application of enzymes to unhair the skin was not effective even after twenty-four hours but all the enzymes were very effective in the bating process. Although all the enzymes failed the solubility test, they can be applied in a bating process successively. In conclusion, commercial bating enzymes are not effective in soaking, unhairing and degreasing process and therefore, new enzymes formulations are needed to improve, soaking, unhairing, and degreasing processes.

AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration with all authors.: "'Author ANK' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author PBG, LWK, ASO, KC' and 'Author PGM' managed the analyses of the study. 'Author ANK' managed the literature searches. All authors read and approved the final manuscript."

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