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Gelatinase Microbial Morphology from Leather Defect

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ABSTRACT

Microbes attach in pickle leather, wet blue leather, and upper shoeleather have been identified to have positive profile to produce extracellular protease enzymes with pH values of acidic to basic on 15% gelatin and 2% commercial skimmed milk as substrates. Defective tanned leather was added to a microbial growth solution enriched with 2% gelatin substrate and incubated for 7 days at room temperature on an open rotary shaker at 120 rpm and then stored at room temperature while waiting for gelatinase testing. There were 5 morphological colonies of microbial gelatinase from defective tanned skin, all of which were white.

Key words: Defect tanned leather, Gelatinase, Protease

Introduction

Leather is an organic material that has high economic value after tanning. Leather tanning converts unstable organic matter (collagen protein) into stable protein through the chemical processes of tanning. There are three types of tanned leather produced during the tanning process, consisting of 1) pickle that is produced from the beam house process, 2) wet blue that is produced from the pre-tanning process; and 3) finished leather or article leather, produced from the post-tanning and finishing process (Hermawan, 2014; Purnomo, 2016; Rachmawati *et al.,* 2018). Biological factors like microbes easily damage those three types of leather during and after the tanning process. Defects in tanned leather affected by microbial biology activity causes pickle leather that should be wet white to blackish brown on the grain and flesh surface, wet blue leather become blackish with holes, while the article leather becomes greenish-white due to mold that can penetrate from flesh to grain (Kurniawati *et al.,* 2021a).

Physical and chemical factors determine the growth of microbes that cause tanned leather defects. These two factors change simultaneously during the shelf life of the leather (Kurniawati *et al.,* 2022b). During the shelf life, leather should be stored in a place that is not exposed to direct sunlight and stacked in a way that the grain meets the grain and the flesh meets the flesh so that the water content and leather's moisture do not change when covered with plastic (Rachmawati *et al.,* 2018).

Microbes that can grow on leather have been identified as protease-producing microbes, one of them is gelatinase (Oruko *et al.*, 2019; Kurniawati *et al.,* 2021a). Gelatinase microbial of leather produce bio film to attach on leather surface (Cadirci et al., 2010). Gelatinase is an extracellular enzyme. It means that the test can be carried out by using a 15% gelatin gel liquefaction method with some modifications according to Kohn's (1953) method. This study was conducted to determine the microbial profile of gelatinase that causes defects in pickle leather, wet blue leather, and upper shoe leather. The gelatinase microbial profile of defective tanned leather that cannot be used to produce article is expected to become one of the raw materials for the protease enzyme industry, especially those from pickle leather.

Materials and Methods

Gelatinase microbial growth test sample preparation

Samples of pickle, wet blue, and upper shoe leather defects with an area of 1 cm x 1 cm were incubated in medium A solution, K_2HPO_4 0.07%, KH_2PO_4 0.01%, $MgSO_4.7H_2O$ 0.02%, and gelatine 2%, which was a modification of the Gautam and Azmi (2017) (Kurniawati *et al.,* 2021a) without the addition of peptone. Incubation was carried out at room temperature on an open rotary shaker at a speed of 120 rpm for seven

days. After incubated the solution was stored at room temperature and then observed for changes in pH while waiting for the gelatinase test.

pH measurement

Merck Universal pH indicator strip was used to measure the pH of the medium A solution by applying methods as indicated on the package.

Identification of gelatinase microbial morphology

The initial microbial profile of tanned leather defects was carried out with a sterile cotton bud containing approximately 1 mL of incubation solution and then rubbed onto the surface of a Petri dish containing material with the same composition as medium A added with 1.5% of agar. Morphological identification was carried out by scratching the four-quadrant method and then incubating at 37°C for at least 48 hours.

Gelatinase test

The gelatinase test applied the modified Kohn (1953) method by dissolving 15 g of Sigma-Aldrich beef gelatin into 100 mL of distilled water and sterilizing at 121°C, 15psi for 15 min. The sterilized gelatin solution was put into a 5 mL test tube and then stored at 4°C until solidified. The test was carried out at room temperature by adding 100 µL of growth solution of Medium A which had been overgrown with microbes from leather defects for seven days. A positive reaction was indicated by the gelatin not solidifying but melting at room temperature and 4°C.

Results and Discussion

pH value profile and gelatinase test

The pH value of the tanned leather defects microbial solution changed during storage. The pH value changed from acid to alkaline, but it did not alter the ability to melt the gelatin in the gelatinase test. The extracellular enzyme gelatinase from tanned leather defects has a wide range of pH values. It is due to the tanning ingredients and leather defect's collagen protein is dissolved, so that the pH value increases in addition to 2% gelatin added to medium A (Figure 1). The addition of carbon and nitrogen, tanning letaher defective dissolved, except for gelatin in the growth solution increases the number of OH^- ions and the pH of the solution becomes neutral or alkali. Leathers are lost water during storage added free ion OH^- into solution and incresed pH value (Kusmaryanti *et al.,* 2016). It induces the gelatinase production by microbes present in tanned leather defects used as a microbial source sample. The addition of carbon and nitrogen sources in the solution, changes in pH values, and test temperatures affect the ability of enzyme performance (Juwon and Emmanuel, 2012).

Despite having a wide range of pH values, tanned leather defect's gelatinase showed a significantly positive result on the liquefaction test of 15% gelatin gel at room temperature (Table 1). This occurrence indicated that the induction of 2% gelatin in the growth solution, coupled with important defects of leather pieces, can create optimal growth conditions for gelatinase microbes from leather defects. The tanned leather defect has become a suitable substrate for the growth of gelatinase microbes. It triggers changes in the pH value of the growth solution during the shelf life before the gelatinase test. Extracellular synthesis of the enzyme gelatinase and other enzymes is formed slowly, adjusting to the formation of the appropriate pH of the solution. The optimum performance of the enzyme system in a flask fermenter is strongly influenced by external factors, consisting of carbon and nitrogen substrates that change simultaneously, pH values, and temperature (Negi and Banerjee, 2010).

Gelatinase microbial morphology

The identification of the morphology of the colonies produced from the growth solution containing the tanned leather defect sample that was the origin of the gelatinase microbial and the nitrogen and carbon sources other than 2% gelatin showed the same color, namely white (Table 2). There were five different colonies which were positive for the gelatinase test and also positive for the protease test with 2% commercial skimmed milk as a substrate (Kurniawati *et al.,* 2021a). It showed that extracellular protease enzymes, except for gelatinase were also

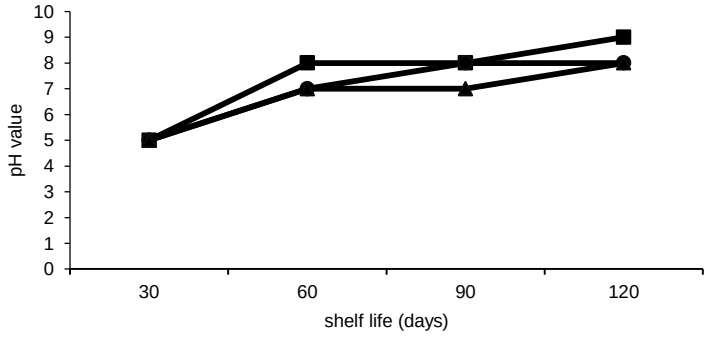

Figure 1. The results of the pH value test of the defect tanned leather solution during the shelflife. The defect tanned leather pickle (square). The defect tanned leather wet blue (round), The defective upper shoe leather (Triangle).

Table 1. Gelatinase test results during storage of defective tanned leather solution

Solution	Shelf life	pH values	Gelatinase test
Pickle	August	5	+
	September	8	+
	October	8	+
	November	9	+
Wet Blue	August	5	+
	September	7	+
	October	8	+
	November	8	+
Upper shoe	August	5	+
	September	7	+
	October	7	+
	November	8	+

Table 2. Microbial morphology of gelatinase defective tanned leather

Defective leather sample	Colony number	Colony shape	Colony color
Pickle	P1	Regular flat round	White
	P2	Irregular round	White
Wet blue	WB1	convex round	White
	WB2	Irregular flat round	White
Upper shoe	A1	Irregular flat round	White

produced by other protease enzymes. The protease enzyme also has a broad pH value as the results of the gelatinase test for defective tanned leather.

In this research results showed that the gelatinase enzyme from microbes that grew on the defective leather had a wide range pH value acidic, neutral, and basic. The longer the solution is stored, the increase in pH value occurs which is caused by the dissolution of the defective leather sample which causes an increase in the amount of carbon and nitrogen substrates. The addition of a substrate other than 2% gelatin is what causes the gelatinase test at acidic, neutral, and alkaline pH to be detected. This is in line with the results of research by Gioia *et al.* (2010) which successfully tested gelatinase in the wide range pH value of 6 and 9.2. The results of the microbial morphology test for defective tanned leather also showed that bacteria growth dominated the defective pickle leather and defective wet blue leather, than fungal growth on defective artickles leather (Table 3). These results have similarities with the results of research by Orlita, 2004.

Tabel. 3. The microbial morphological test of defective pickle leather, defective wet blue leather, and defective artickle leather

Defective leather sample	Colony number	Microbial test
Pickle	P1	Bacterial
	P2	Fungal
Wet Blue	WB1	Bacterial
	WB2	Fungal
Upper shoe	A1	Fungal

Conclusions

Gelatinase microbes from the pickle, wet blue, and article defects had characteristic pH values from acid to alkaline. All treatment showed positive results on gelatinase testing of 15% gelatin at room temperature and 4°C. The shelf life changes the pH due to the addition of carbon and nitrogen source solutions from tanned leather defects. There were five positive extracellular protease microbial colonies on gelatin and skim milk substrates with the same morphology in color, namely white.

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