Production of poultry feather hydrolysate using HCl and NaOH as a growth medium substrate for indigenous strains

N A Fitriyanto*, Y Ramadhanti, Rismiyati, I Rusyadi, A Pertiwiningrum, R A Prasetyo, Y Erwanto

Faculty of Animal Science, Universitas Gadjah Mada, Jl. Fauna No.3 Bulaksumur, Yogyakarta, 55281, Indonesia

*Email: nanungagusfitriyanto@ugm.ac.id

Abstract. The poultry feathers have a very high protein content due to it consists of 90% of crude protein, and it is an ideal material to obtain keratin protein. Due to Keratin's difficulties and time-consuming decomposition, further processing is needed to degrade Keratin into simpler proteins that can be used as an alternative N-source. This study was aimed to evaluate the keratin hydrolysate from poultry feathers prepared by acidic (HCl) and alkaline (NaOH) compound utilization and its potency as the substrate medium for growth keratinolytic bacteria at a laboratory scale. Poultry feathers, including kampung (local breed) chicken feathers, layer chicken feathers, and local goose treat with HCL 12% and NaOH 20%. The results of the hydrolysate of poultry feathers using 12% HCl showed no significant changes. Visually, the feathers of birds that have been treated with 12% HCl show a colour change to brownish-yellow. The results of hydrolysis using NaOH showed better results than HCl for producing feather meals. The highest yield has occurred at local goose feathers at 95.7%, followed by Kampung and Layer chicken feathers at 93.17% and 78.75%. Based on the viability test, three indigenous strains (Bacillus cereus TD5B, B. cereus LS2B and Pseudomonas sp. PK4) grew in a medium with a substrate of kampung chicken feathers, layer chickens, and local goose feathers. It can be concluded that the hydrolysed poultry feathers made by NaOH 20% preparation had a potency as N-source in the bacterial growth medium.

1. Introduction

Poultry farming produces meat and eggs, one of the businesses with high demand by the Indonesian community. However, the increasing development of poultry farming has increased feather waste production. At every chicken slaughter processing, feather waste will be produced at about 5-7% of its total body weight [1]. Thus, it will cause problems if this feather waste is not handled properly. Feathers are one of the largest wastes in the livestock industry due to the high demand for poultry meat in Indonesia. The total production of poultry meat in 2018 was 2,313,518 tons [2]. The production of broiler feathers per head is about 9.6%, and it can be projected the volume of chicken feather waste produced in one year [3]. Although the poultry feathers have a very high protein content since it consists of 90% crude protein, it might be causing an environmental problem due to Keratin's difficulties and time-consuming decomposition. The protein content in chicken feathers reaches 80 to 90% [4]. The protein that makes up chicken feathers is dominated by keratin substrate, which is difficult to digest, so further processing is needed to degrade Keratin into simpler proteins.
Keratin is a protein that is not easily dissolved and is the main component that makes up feathers. The process of making poultry feather hydrolysate aims to change the nature of the protein in the feather, which is not easily dissolved into soluble protein. The process of protein hydrolysis is breaking the peptide bonds of proteins into simpler molecules with the help of water molecules. The hydrolysis process can also occur due to the influence of chemical components such as acids and bases, heating, and enzymatic treatment [5]. Keratin in poultry feathers can be converted into soluble protein using an acid or alkaline solution and converted into digestible protein using trypsin and pepsin enzymes [6]. Therefore, the present study was aimed to evaluate the keratin hydrolysate from poultry feathers prepared by acidic (HCl) and alkaline (NaOH) compound utilization and its potential as the substrate medium for growth keratinolytic bacteria at laboratory scale.

2. Materials and methods

2.1. Material preparation
Several poultry feathers including kampung (local breed) chicken feathers, layer chicken feathers, and local goose feathers, were collected from the poultry slaughterhouse at Terban Market, Special Region of Yogyakarta. The feathers were washed under running water and soapy water. After cleaning, the feathers were dried in an oven at 105°C for 48 hours. The feathers were then ground to reduce the particle size and sterilized using an autoclave (121°C, 15 psi for 15 minutes). Feathers are then stored in storage media [7].

2.2. Feather hydrolysate production
The principle of making poultry feather hydrolysate is to weaken or break the bonds in keratin through hydrolysis used acidic and alkaline compounds. In this study, the hydrolysate of poultry feathers was made using chloric acid (HCl) and sodium hydroxide (NaOH). Making hydrolysate using HCl solution was done by mixing 20 mL HCl 12% solution with 40 grams of feathers substrate (kampung chicken, layer chicken, and local goose) at the ratio of 2:1 (w/v). After that the mixture was stored in a closed container and allowed to stand for 4 days. The feather was then washed to neutralize and dry in an oven. The dried feathers were then ground to obtain hydrolysate of poultry feathers [8].

The hydrolysate of poultry feathers made using NaOH solution was carried out by adding 1 M NaOH solution to the feathers with the ratio 5:1. A total of 200 mL of 1 M NaOH was mixed with 40 grams of substrate for each feather (kampung chicken, layer chicken, and local goose). The solution was then closed using a cotton plug and allowed to stand for two days. The crude hydrolysate that has been formed for two days is then washed and filtered using a cloth. The results of the hydrolysate filter were then oven-dried at 70°C to dry (to remove the water content) and then the dried hydrolysate was crushed [9].

2.3. The medium preparation of indigenous keratinolytic bacterial culture
Stock solution (100 mL) was made from 1 grams of meat extract, 1 grams of biological peptone, and 0.5 grams of NaCl were put in a 100 mL beaker glass which had previously been filled with 70 mL of distilled water. The medium was set at pH 7.2, and then distilled water was added to reach a volume of 100 mL. Pre-culture medium (50 mL) was made from 5 mL of stock solution and then put into an erlenmeyer flask which already contained 45 mL of distilled water. The medium was sterilized by autoclaving (121°C, 15 psi for 15 minutes). Before application, the medium has been cooled in laminar air flow. One use of indigenous strains (B. cereus TDSB, B. cereus LS2B, and Pseudomonas sp. PK4) were inserted into the pre-culture medium prepared previously. Indigenous bacteria including B. cereus TDSB, B. cereus LS2B dan Pseudomonas sp. PK4 were maintained at Laboratory of Leather, Waste, and By-Products Technology, Faculty of Animal Science, UGM, Yogyakarta.

The erlenmeyer flasks containing bacterial strains were incubated on a 120 rpm at rotary shaker for 18 hours. Nutrient agar medium was made from 100 mL of stock solution, and 1.5 grams of agar powder was put into a 250 mL erlenmeyer flask. The agar medium homogenized by stirred and heated to boiling.
using a magnetic stirrer. After that, the medium was sterilized using an autoclave (121ºC, 15 psi for 15 minutes). The medium that has been sterilized was then poured into a sterile Petri dish, cooled, and placed in laminar air flow (LAF) [10].

2.4. Viability cell growth assay
Minimum Growth Medium (basal phosphate solution) was made by 0.05 grams NaCl, 0.07 grams KH$_2$PO$_4$, 0.14 grams K$_2$HPO$_4$, and 0.01 grams MgSO$_4$ were added in a 100 ml beaker filled with 70 mL of distilled water. The medium was adjusted to pH 7.5 then added with distilled water until it reached a volume of 100 mL [11]. A total of 5 ml of minimum growth medium was added with 1% (w/v) hydrolysate of poultry feathers. Then the medium was sterilized using an autoclave (121ºC, 15 psi for 15 minutes). Cultures of indigenous strains (B. cereus TD5B, B. cereus LS2B and Pseudomonas sp. PK4) which have been shaken overnight were then inoculated into the medium and incubated at 120 rpm shaker for 72 hours. Every 24 hours, 1 µL sample was taken and grown on the nutrient agar medium.

2.5. Data analysis
The data obtained in this study was analysed using descriptive analysis supported by figure and table.

3. Results and discussions
3.1. The production of poultry feather keratin hydrolysate substrate
Keratin is a protein that is not easily dissolved and the main component of feathers. The process in making poultry feather hydrolysate aims to change the characters of the protein in the feather which is not easily dissolved into soluble protein. The manufacture of the hydrolysate of feathers using acid compounds have done using HCl solution. The results of the hydrolysate of poultry feathers using a 12% HCl solution are presented in Figure 1.

![Figure 1. Poultry feathers hydrolysate visualization. Sequentially from left to right are the feathers of kampung chickens, local goose, and layer chickens without processing (a) and feather hydrolysate with HCl 12% (b).](image)

The results of the hydrolysate of poultry feathers using 12% HCl showed no significant changes in physical characteristics. The poultry feathers that have been treated with 12% HCl show a change in
colour into brownish-yellow. The hydrolysate damage of feathers could be indicated by a colour change from white to brown due to the browning reaction and chemically indicated by the high levels of ammonia produced (> 30 mM) [8]. In addition, the different types of feathers in this study were thought due to the effect of the process and duration of hydrolysis occurred. In a previous study, the addition of 12% HCl for 4 days was maximally hydrolyses the chicken feathers [8].

The results hydrolyzation of poultry feathers using 1 M NaOH solution gave the higher results. In less than 2 days, the feathers were completely hydrolysed. The hydrolysis of feathers using 1 M NaOH solution resulted in a relatively fast time. Less than 2 days, the feather samples were hydrolysed entirely. The hydrolysis process of the peptide bond would cause changes in the protein [12]. The alkaline NaOH solution functions to remodel the bonds in the protein components of the feathers. The changes in the protein structure of feathers caused an increase in solubility due to an increase in the number of NH₃⁺ and COO⁻ compounds and, including a reduction in the size of the protein or polypeptide molecule [9]. The hydrolysate of feather keratin using NaOH and the yield value can be seen in Figure 2 and Table 1.

![Figure 2](image)

**Figure 2.** Hydrolysate of keratin with NaOH: (a) Kampung chicken feathers, (b) Layer chicken feathers, and (c) Local goose feathers.

| Feather Substrate          | Initial dry weight (g) | Final dry weight (g) | Yield (%) |
|----------------------------|------------------------|----------------------|-----------|
| Kampung chicken feathers   | 40                     | 37.27                | 93.17     |
| Layer chicken feathers     | 40                     | 31.5                 | 78.75     |
| Local goose feathers       | 40                     | 38.28                | 95.7      |

The results of hydrolysis using NaOH showed better characters, compare to HCl treatment. The highest yield has occurred at local goose feathers which observed at 95.7%, followed by kampung and layer chicken feathers at 93.17% and 78.75%. This results was in line with another research which conducted by [13] which states that NaOH with enzymes has an excellent ability to dissolve N feathers. The combination dissolved 50.34% N feathers, with 30% hydrolysis by NaOH for 2 hours and the remaining 20% by enzymes with incubation for 24 hours. The application of the HCl combination with enzymes showed less effect on the solubility. Usage of alkaline solutions could loose protein bonds in feathers [9]. These changes have increased the solubility, due to an increase of NH₃⁺ and COO⁻, followed by decreasing in the molecular weight of proteins and polypeptides. Hydrolyzation of poultry feathers keratin using NaOH was chosen to be applied as a keratin substrate in future studies.

### 3.2. Viability cell bacterial growth

Indigenous strains (*Bacillus cereus* TD5B, *B. cereus* LS2B and *Pseudomonas* sp. PK4) were observed for their viability on poultry feather hydrolysate medium, which had previously been made using 1 M NaOH solution and presented by figure in Table 2. The strains were conditioned in a liquid medium containing hydrolysate of poultry feathers and shaken for 72 hours on the rotary shaker 120 rpm. At every 24 hours the strains were inoculated into the nutrient medium in order to see their viability. The
results showed that all strains could grow in all hydrolysate medium for kampung chicken feathers, layer chickens and local goose

Table 2. Viability test of indigenous keratinolytic strains (Bacillus cereus TD5B, B. cereus LS2B and Pseudomonas sp. PK4).

| Bacterial Strain         | Visual Observation |
|--------------------------|-------------------|
|                          | 24 hours          | 48 hours          | 60 hours          |
| *Note: N source in culture medium: (a) Kampung chicken feather hydrolysate, (b) Layer chicken feather hydrolysate and (c) Local goose feather hydrolysate |

Based on the picture of bacterial growth in Table 2, it can be seen that the three strains (Bacillus cereus TD5B, B. cereus LS2B and Pseudomonas sp. PK4) were able to grow in a medium that was given the addition of hydrolysate of kampung chicken feathers, layer chickens, and Local goose feathers. The incubation time of three days showed that all strains could grow on the medium with the addition of different feather hydrolysates. This can be seen from the size of the colonies that grow on each plate. The medium colonies that were added with the hydrolysate of layer chicken feathers had a smaller size than the medium with the addition of hydrolysate of kampung chicken feathers and goose feathers. On the other hand, the medium colonies that were added with the hydrolysate of kampung chicken feathers and local goose feathers had almost the same colony size.

4. Conclusions
Based on the results in this study, it can be implied that the preparation feathers meals from poultry feathers gave better result in the alkaline hidrolysis bases compared to acidic hidrolysis bases. The preparation feather meals from poultry feather hidrolysate using NaOH 20% showed better results than HCl 12%. The highest yield has occurred at local goose feathers at 95.7% followed by kampung and layer chicken feathers at 93.17% and 78.75%. Based on the viability test, it can be seen that the three strains of strains (Bacillus cereus TD5B, B. cereus LS2B and Pseudomonas sp. PK4) were able to grow
in a medium that was given the addition of hydrolysate of kampung chicken feathers, layer chickens, and Local goose feathers. It shown that poultry feather meals after NaOH 20% preparation had a potency as N-source in the bacterial growth medium.

Acknowledgement
This research was financially supported by grant aid from the Directorate General of Higher Education (DIKTI), Ministry of Education and Culture, the Republic of Indonesia through The Program Rekognisi Tugas Akhir 2021 under Directorate of Research, Gadjah Mada University management with grant number: 3143/UN1.P.III/DIT-LIT/PT/2021

References
[1] Godbole S, Pattan J, Gaikwad S and Jha T 2017 Isolation, Identification and Characterization of Keratin degrading microorganisms from Poultry soil and their Feather degradation Potential Int. J. Environ. Agric. Biotechnol. 2 2060–8
[2] DJPKH 2018 Statistik Peternakan dan Kesehatan Hewan 2018 (Jakarta: Kementerian Pertanian)
[3] Murtidjo B A 2003 Pedoman Beternak Ayam Broiler (Yogyakarta: Kanisius)
[4] Sari E P, Putri I S , Putri R A, Imanda S, Elfidasari D and Puspitasari R L 2015 Pemanfaatan limbah bulu ayam sebagai pakan ternak ruminansia Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia vol 1 pp 136–8
[5] Riffel A, Lucas F, Heeb P and Brandelli A 2003 Characterization of a new keratinolytic bacterium that completely degrades native feather keratin Arch. Microbiol. 179 258–65
[6] Gupta A, Perumal R, Yunus R B M and Kamarudin N B 2012 Extraction of keratin protein from chicken feather J. Chem. Chem. Eng. 6 732–7
[7] Wandita T G, Triatmojo S, Gumilar J and Fitriyanto N A 2016 Production and application of keratinase enzyme from 4 strains of Bacillus spp. isolated from Yogyakarta and Garut city, Indonesia Asian J. Microbiol. Biotechnol. Environ. Sci. 18 351–7
[8] Puastuti W, Yulistiani D and Mathius I-W 2004 Nilai biologis (in vitro dan in sacco) bulu ayam yang diolah secara kimiai sebagai sumber protein by-pass rumen Jitv 9 73–81
[9] Said M I, Yuliati F N and Sukma M 2019 The effects of acidic and alkaline hydrolysis process on some physical and chemical properties of broiler chicken feathers Iran. J. Appl. Anim. Sci. 9 529–40
[10] Fitriyanto N A, Winarti A, Imara F A, Erwanto Y, Hayakawa T and Nakagawa T 2017 Identification and growth characters of nitrifying pseudomonas sp., LS3K isolated from odorous region of poultry farm J. Biol. Sci. 17 1–10
[11] Shabaan M T, Attia M, El-Sabagh S M and Ahmed A M 2014 Isolation, Screening and Selection of Efficient Feather Degrading Bacteria Curr. Sci. Int. 3 488–98
[12] Kim W K and Patterson P H 2000 Nutritional value of enzyme-or sodium hydroxide-treated feathers from dead hens Poult. Sci. 79 528–34
[13] Kim W K, Lorenz E S and Patterson P H 2002 Effect of enzymatic and chemical treatments on feather solubility and digestibility Poult. Sci. 81 95–8