Fibrous Keratin Powder as by-Product of Keratinase Fermentation under Solid State Fermentation using Chicken Feathers as Substrate

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Abstract. Billion tons of chicken feathers are being generated every year from the poultry industry. Ironically, although egg white is one important source of proteins for a human, it only contains 57% of protein, while chicken feathers contain about 98% of protein. However, due to its complex structure, chicken feathers are not consumable by the human. This makes chicken feathers are classified as biowaste material which causes environmental problems. The huge amount of this biomaterial offers a potential raw material for polypeptide and amino acids source.

Chicken feathers contain a highly fibrous protein called keratin. X-ray diffraction model of keratin architecture showed supercoiled of helical proteins. The structure is also strengthened by disulphide bonds which make keratin insoluble in water and an organic solvent. Many attempts have been tried to break down the chemical bonds and convert chicken feathers into simple structural materials for sustainable uses. Currently, hydrothermal degradation is a preferable technique to concert chicken feathers into keratin powder because it is cheap for industrial purposes. Enzymatic degradation offers a greener process. We have successfully isolated a Gram-positive bacterium named Bacillus sp. MD24 which is capable of producing keratinase, an enzyme that degrades chicken feathers. In this paper, we described fibrous keratin powder as a by-product of keratinase fermentation by Bacillus sp. MD24 under solid-state fermentation (SSF). Scanning electron micrograph showed a tremendous reduction in the size of keratin after fermentation.

Keywords: chicken feather, solid-state fermentation, by-product, microfiber

1. Introduction

Increasing environmental awareness drives significant efforts in exploring and exploiting natural resources as raw materials to develop new green materials. Protein-based natural biopolymer such as wool and silk has been applied since the dawn of human civilization. Wool and silk contain a specific fibrous protein called keratin. Keratin is also found as major component of a chicken feather. Proximate analysis of chicken feathers revealed crude protein content of 82% [1]. Chicken feathers are abundant biowaste from the poultry industry. Feathers constitute between 8 and 10 percent of chicken weight. According to the Ministry of Agriculture of Indonesia, the chicken production in Indonesia in 2018 is predicted 3.56 million tons. It would generate about 0.35 million tons of chicken feather wastes [2]. In Indonesia, at present, most of the chicken feathers from small-scale industry are disposed of the landfill.
Due to its availability and renewability, the chicken feathers are considered as raw material to be converted into high-value biomaterial product such as biofertilizer [3,4], animal feed [5,6], fibrous material for textile development [7], and biodegradable plastics [8,9].

None of the natural and synthetic fibers which are commercially available today has a density as low as chicken feathers. The low density of chicken feathers is caused by specific honeycomb structure of chickens [10]. Study on chicken feathers durability showed that the material is though [1]. Chicken feathers did not dissolve in cold and hot water, weak acids and alkalis, strong alkalis, strong acids except concentrated sulphuric acid. The superior properties of chicken feathers make chicken feathers suitable as raw material for developing lightweight materials. However, the processing of the properties of chicken feathers into valuable materials faces some problems.

Many efforts have been addressed to convert chicken feathers into valuable materials. Cellulose-keratin biocomposites have been prepared using chicken feather keratin through physical and chemical modification [11,12]. Chitosan-cellulose-keratin biocomposites have also been prepared for medical purposes [13–15]. Chicken feathers have been studied to reinforce thermoplastic such as high-density polyethylene (HDPE), polypropylene (PP), and polylactic acid (PLA) [8,9,16]. Preparation of keratin from chicken feathers in previous researches was done by chemical and physical degradation. Chemical degradation is high cost and non-environmentally friendly, while physical degradation produced a big size of molecules. Keratinase is the only enzyme which is capable of degrading keratin. We have studied keratinase fermentation by Bacillus sp. MD24 using chicken feathers as a sole nitrogen and carbon source under submerge fermentation (SmF). The fermentation process yielded the degraded chicken feathers as by-product [17]. However, SMF was not effective to produce a big amount of degraded chicken feathers. Solid State Fermentation offers a fermentation process with low cost and high productivity [18]. SSF is a fermentation technique which has been established in the Eastern country for food industry [19]. This research evaluated chicken feathers degradation under SSF using Bacillus sp. MD24 as enzyme producing keratinase.

2. Methods
Chicken feathers were collected from a local slaughterhouse and washed three times with tap water to remove blood and other water-soluble materials. The washed chicken feathers were dried under sunlight. The dried chicken feathers were degraded under Solid State Fermentation by Bacillus sp. M24. The solid state fermentation was done at pH 8 (pH optimum of keratinase from Bacillus sp. M24) at 37 °C. The experiment was carried out at the water to chicken feathers ratio between 2 to 7 (v/w). Keratinase activity was measured accordingly [17]. The solid by-product which contained the degraded chicken feathers was separated from fermentation batch by filtration and washed three times with demineralized water. The residue was dried and mashed using a kitchen blender.

3. Results and Discussion
3.1. Fermentation of keratinase under semi-SSF
Many filamentous fungi generally are more desirable for SSF compared to unicellular microorganism [19]. However, due to substrate solubility and microorganism capability, SSF was chosen to ferment chicken feathers by Bacillus sp. MD 24. Keratinase was produced under SSF using chicken feathers as sole carbon and nitrogen source and producing degraded chicken feathers as by-product. Keratinase fermentation was done at the water to chicken feathers ratio of 2, 3, 4, 5, 6, and 7 (v/w) (Figure 1A). The optimum keratinase activity was achieved at the water to chicken feathers ratio of 5. The optimum production of keratinase under SSF was observed on the third day (Figure 1B), while the incubation of chicken feathers without the addition of Bacillus sp. MD 24 did not yield any chicken feather degradation or keratinase. Drastic reduction of water content did not change the optimum fermentation time. As we reported previously the optimum fermentation time of keratinase production was also observed on the third day when the fermentation was undertaken under submerge fermentation (SmF) [17].
In the absence of soluble carbon and nitrogen source, *Bacillus* sp. MD 24 produced keratinase to degrade the chicken feathers and use the amino acids as its carbon and nitrogen source. The enzyme activity increased during the first three days due to the demand for the nutrient for bacterial growth. After the third day, the enzyme activity decreased and on the fifth day and the remaining enzyme activity was about 78.5%. This could happen due to the limitation of other nutrients, such as minerals or the amino acids supply which were enough for the cells. Keratinase production is a good indicator to know that the chicken feather degradation occurs enzymatically.

![Figure 1](image)

**Figure 1.** (A) Profile of keratinase production at different water/chicken feathers ratios. (B) Profile of keratinase production at water/chicken feathers ratio of 7 (v/w) within five days incubation time

3.2. Fibrous keratin powder from chicken feathers as by-product of keratinase fermentation

Keratinase activity changed the morphology of chicken feathers. Figure 2A shows the fermented chicken feathers at the water to the chicken feathers ratio of 2, 3, 4, 5, 6, and 7, respectively. Chicken feather pulp was formed after the fifth day. Although the highest activity was observed at the water to chicken feathers ratio of 5, the best pulp was achieved at the water to chicken feathers ratio of 7. Water content might help the swelling process of the chicken feathers.

After filtration and washing, the chicken feathers were mashed mechanically to form keratin powder. Figure 2B shows the fibrous keratin powder which was obtained as by-product of keratinase fermentation at the water to the chicken feathers ratio of 2, 3, 4, 5, 6, and 7, respectively. The keratin powder showed apparent fibrous powder with different macrostructures, however, scanning electron microscopy photograph at 4000x magnification showed similar microstructure of fibrous micro keratin. The difference in macrostructure most likely was caused by water content in the powder as we normally observed in wet cotton. Keratin powder obtained from keratinase fermentation at the water to chicken feathers ratio of 2 has longest apparent fibrous macrostructure. This could be explained that water content might not be enough to form a shorter fiber.

Figure 3 shows photographs of whole chicken feather and degraded chicken feathers under SSF at the water to chicken feathers ratio of 7 at different magnifications. Figure 3A shows the whole chicken feather which has a specific structure. The typical feathers consist of a central shaft called rachis and branches called barbs. Barbs possess further branches called barbules [20]. The structure of chicken feathers was broken into smaller fiber upon enzymatic degradation. Figure 3B shows the degraded chicken feathers at 250x magnification. The chicken fathers were degraded into smaller fiber size with diameter about 5-10 µm with various lengths and differences between rachis and barbs that could not be distinguished any more.
Figure 2. The degradation of chicken feathers into fibrous micro keratin: A is laboratory scale of keratinase fermentation under solid state fermentation using chicken feathers as a sole carbon and nitrogen source at the water to chicken feathers ratio of 2:1, 3:1, 4:1, 5:1, 6:1, and 7:1; B is fibrous keratin powder as by-product of keratinase fermentation after mechanical treatment at respective water to chicken feathers ratio, and C is SEM photograph of keratin microfiber water to chicken feathers ratio.

Figure 3. Chicken feather degradation under SSF at the water to chicken feathers ratio of 7 at different magnifications: (A) the whole chicken feather; (B) the degraded chicken feathers at 250X magnification; (C) the degraded chicken feathers at 250X magnification; (D) zoom out of the selected area of the degraded chicken feathers at 250X magnification.
3.3. The effect of sugar bagasse on fibrous keratin powder from chicken feathers by-product

Optimizing keratinase production might be done by optimizing fermentation substrate. Sugarcane bagasse was tested as an additional carbon source. Simple carbohydrates such as glucose and sucrose lower the enzyme production [17]. The addition of complex carbohydrate might balance the production of keratinase and carbohydrate degrading enzyme. Cell biomass would increase due to carbon source availability. The addition of 1% sugarcane bagasse seems to slow down the chicken feather degradation (data are not shown).

Surprisingly, unusual microstructure was observed as a result of fermentation at the water to the chicken feathers ratio of 7. Figure 4A shows keratin powder produced which was less fibrous compared to Figure 2C. Despite fibrous keratin microfiber, the material in a sheet form was observed (Figure 4B) which had a different structure compared to the degraded chicken feathers at Figure 4B. Figure 4C and 4Dand show closer look into the sheets. The sheet contains fibrous material. Two possibilities might explain the phenomena. Firstly, the sheets are undegraded chicken feathers or sugarcane bagasse. However, sugarcane bagasse was only 1% of total chicken feathers total weight; therefore, sugarcane bagasse would not be dominant structure. Secondly, the degraded chicken feathers were blended with sugarcane bagasse to form a new material. This idea needs to be further studied. This might lead to a finding of a new biocomposite with a novel character.

![Figure 4](image_url)

**Figure 4.** The degradation of chicken feathers under SSF at the water to chicken feathers ratio of 7 in the presence of 1% sugarcane bagasse: (A) keratin powder; (B), (C), and (D) keratin powder at the magnifications of 250X, 2500X and 4000X, respectively.

4. Conclusion

Fibrous keratin powder was produced as by-product of keratinase fermentation under SSF fermentation. The powder has diameter size between 5–10 µm at experimental conditions. Higher water content might promote further degradation to keratin nanofiber and soluble keratin, therefore, further experiments need to be done by increasing water content or combining enzymatic degradation and other methods such as chemical and thermal degradation. The combination of method would provide a greener process compared to chemical or thermal degradation alone. Interestingly, the addition of 1% sugarcane bagasse led to a different type of powder; its natural fibrous character was lost; a sheet consisted of fibers was formed. This phenomenon should be studied further.
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