NOTE:

Enhancing the Amino Acid and Reducing the Metal Ions Contents in the Hydrolysate Resulting from Hydrothermal Carbonization of Chicken Feather Waste by Chemical Phosphorylation

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Abstract: *Chemical phosphorylation of hydrolysate resulting from hydrothermal carbonization of chicken feather waste was performed to enhance the amino acids and reduce the metal ions content. The aim of this research is to improve the functional properties of chicken feathers hydrolysate without impairing the nutritional availability thereof with the cheapest chemical method by phosphorylation. Phosphorylated hydrolysate can function as animal feed and fertilizer. The hydrolysate of chicken feathers was obtained by hydrothermal carbonization in an alkaline condition using a CaO and KOH catalyst, by the ratio of water:dry matter of chicken feathers is 5:1, at 9–10 atm pressure, and in a temperature of 190–200 °C during 3 h. Phosphorylation has been carried out by reacting the hydrolysate with H_3PO_4 85% in pH of 5, 6, 7 and using the original hydrolysate as control. The sample that has been prepared was characterized and semi-quantitative analyzed by HPLC and AAS. The phosphorylation results showed that the total maximum protein of soluble protein, their minimum metal ions, and anion in soluble protein was obtained at pH 7, while the higher the pH, the lower the liquid protein that was obtained.*

Keywords: *hydrothermal carbonization; phosphorylation; liquid protein*

■ INTRODUCTION

The non-composting method was proposed for the treatment of chicken feather pathogenic waste [1]. Chicken feathers are the main by-product waste in the poultry industry that constitute about 5–7% of the total chicken weight [2-3]. Based on Central Statistics Agency (BPS) data, broiler chicken production in Indonesia reached 3,495,090.5 tons in 2019, with 6% of the live weight produced being feathers. So, it is assumed that the production of chicken feathers in 2019 reached 236,705.4 tons [4]. It can be estimated about several million tons of chicken feathers were discarded for years. Therefore, chicken feathers storage is quite important instead of collecting them in an abundant area without treatment to avoid the risk of microbial growth and infections that may become hazardous to the environment and affect human health due to their keratin content [5-7]. Chicken feathers contain 91% keratin, 8% water, and 1% lipid [8]. Keratin has a high protein and amino acids content that can be a low-cost source of nitrogen (up to 15% total N) [9-10], but keratin is difficult to digest by the organism and it needs a long time to be degraded in the environment [3,11]. Keratin is an insoluble protein in polar solutions such as water, weak acids and weak bases, and even in some organic solvents, which makes them resistant to microbial degradation and proteolytic enzymes such as pepsin and trypsin [12-14]. The

presence of cross bonds from disulfide bonds caused by the contains of sulfur amino or cysteine, hydrogen bonds, and hydrophobic interactions are driving the stability of keratin [15].

Keratin in chicken feather waste can potentially be converted into raw material for animal feed and fertilizer through chemical treatment for application in agricultural and food fields [16-18]. To be converted as raw material, the cystine bond of keratin in chicken feathers must be broken down [19]. Hydrothermal carbonization (HTC) is the thermochemical method that involves the application of high temperature and high pressure to convert biomass into a solid product called hydrochar, liquid product and gaseous product that is rich in organic and inorganic compounds in the sub-critical water environment [20-21]. The liquid product must be separated from the hydrochar using phosphorylation. KOH catalyst can be used as an activation agent in the HTC process, which has an effect on the change of morphology and chemical functional group in the product, and also can enhance the degree of carbonization [22-23]. Production of organic fertilizer from poultry feather wastes excluding the composting process was investigated by several researchers [24-28]. This research reports the combination of HTC and phosphorylation to convert the chicken feather pathogenic waste into valuable liquid protein, simultaneously to get digestible feed animals, and organic fertilizer by non-composting method.

■ EXPERIMENTAL SECTION

Materials

The materials used were hydrolysate, H_3PO_4 85% (Brataco Chemical), KOH (Brataco Chemical), CaO (Brataco Chemical), distilled water, and filter paper.

Instrumentation

The equipment used in this research were laboratory glassware, Buchner filter, Atomic Absorption Spectroscopy (AAS, ContrA A 300 Analytik Jena), High-Performance Liquid Chromatography (HPLC, LiChrospher 100 RP-18 column with Thermo Ultimate 3000 RS Fluorescence detector), UV-Vis spectrophotometer (Shimadzu UV-1800) and Hydrothermal carbonization (HTC reactor of 1145 L capacity).

Procedure

HTC of chicken feathers

The hydrolysate of chicken feathers was obtained using hydrothermal carbonization 1145 L HTC reactor. First, as much as 200 kg of wet chicken feathers containing 25% of dry matter of chicken feathers was put in the HTC reactor and then added to 100 L of water. The ratio of water:dry matter of chicken feathers is 5:1. After that, as much as 7.50 kg of CaO and 2.25 kg of KOH were added. The HTC reactor was heated up to a temperature of 190–200 °C for 3 h, and the pressure was controlled at 9–10 atm. The hydrolysate obtained from the HTC process was then phosphorylated by H_3PO_4 85% in various pH of 5, 6, and 7.

Sample preparation of phosphorylated hydrolysate for AAS analysis

The emulsion of hydrolysate was moved into a 100 mL beaker glass; it was reacted with 0.5 mL of H_3PO_4 85% and then repeated by adding 1 and 2 mL of H_3PO_4 85%. Each result of phosphorylation was vacuumed using a Buchner funnel that was covered using Whatman 40 filter paper. After the liquid fraction was filtered, the liquid fraction and the residue were analyzed using the AAS spectrophotometer to prove the content of Cu, Fe, Mn, Mg, Zn, Ca, and K. The content of P was analyzed using a UV-Vis spectrophotometer, and the profile of amino acids in liquid protein was analyzed using HPLC.

Characterization of profile amino acids

The characterization by HPLC was done for all samples. Each sample was weighed for 60 mg and then added with 4 mL of HCl 6 M. The sample was heated for 24 h at a temperature of 110 °C, then cooled down to room temperature and neutralized (pH 7) using NaOH 6 M. After that, the sample was added with distilled water until the volume reached 10 mL and then filtered with 0.2 mm Whatman filter paper. Characterization of profile amino acids in the sample was carried out by HPLC. The HPLC instrument that was used was LiChrospher 100 RP-18 (5 µm) column with Thermo Ultimate 3000 RS Fluorescence detector. The sample

was taken around 50 µL, and as much as 300 µL of OPA was added. The sample was stirred for 5 min before 10 µL of the sample was injected into the HPLC injector.

■ RESULTS AND DISCUSSION

This research aimed to conduct the chemical phosphorylation of hydrolysate resulting from HTC of chicken feather waste, to enhance the proteins and reduce the metal ions content. The product obtained was liquid hydrolysate and its solid phase that separated using phosphorylation. Phosphorylation was conducted to enhance the quality of protein without impairing the soluble protein that was available. The results of HPLC analysis of phosphorylated hydrolysate in various pH solutions are given in Table 1. It is worthy of being noted here that there is no free amino acid detected in the hydrolysate resulting from the HTC of chicken feather waste. Analysis of HPLC conducted the profile of amino acids of protein hydrolysate.

Table 1 shows that the optimum pH for the highest concentration of dissolved protein was obtained at pH 7. These phenomena conduct that phosphorylation enhances the reaction between dihydrogen phosphate ion and a group of amino acids in proteins of hydrolysate. It is also generally conducts protein in essential amino acids with maximum concentration at pH 7 except threonine. The results indicate that the lower the pH, the higher the liquid protein that is obtained. The variation of solubility of protein reveals that the interaction between dihydrogen phosphate ion and amino acids group facilitated by hydrogen bonding force to form an ion protein phosphate that is more soluble in water at pH 7 [29-31]. The product of the reaction between the ion dihydrogen phosphate and amino acid is shown in Fig. 1 [32].

The content of metals (Fe, Mn, Zn, and Mg) in all pH can be seen in Fig. 2. These results show that the protein dihydrogen phosphate anion precipitates Fe, Mn, Zn, and Mg ions in their protein biphosphate neutral salt [13]. Except for the Cu ion, with a pH of 7, which formed a complex of copper ions in the form of ions copper protein dihydrogen phosphate. The concentration of Cu in liquid protein at different pH showed in Fig. 3.

A comparison of Ca, P, and K concentrations in liquid protein at different pH can be seen in Fig. 4. The

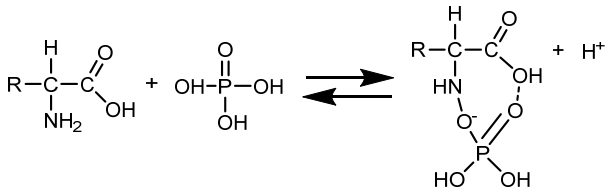

Fig 1. Phosphorylation reaction of a hydrolysate

Table 1. Amino acid profile of hydrolysate protein before and after phosphorylation

Amino acid	Before phosphorylation		After phosphorylation					
	pH 8–9		pH 7		pH 6		pH 5	
	weight	% AA	weight	% AA	weight	% AA	weight	% AA
Aspartic acid	0.51	0.85	0.48	0.80	0.46	0.76	0.48	0.80
Glutamic acid	0.90	1.51	0.94	1.56	0.87	1.45	0.89	1.49
Serin	0.48	0.80	0.51	0.85	0.48	0.79	0.48	0.80
Glycine	0.63	1.05	0.72	1.20	0.68	1.14	0.73	1.22
Threonine*	0.22	0.37	0.20	0.34	0.20	0.33	0.18	0.30
Arginine*	0.61	1.01	0.68	1.13	0.65	1.09	0.67	1.12
Alanine	0.55	0.91	0.65	1.08	0.61	1.02	0.65	1.08
Tyrosine	0.50	0.83	0.53	0.89	0.48	0.81	0.48	0.81
Valine	0.73	1.21	0.84	1.40	0.79	1.32	0.78	1.31
Phenylalanine*	0.61	1.01	0.66	1.10	0.62	1.04	0.61	1.02
L-Leucine*	0.55	0.92	0.63	1.05	0.60	0.99	0.60	1.00
Leucine*	0.77	1.28	0.89	1.48	0.82	1.37	0.84	1.40
Total	7.12	11.86	7.80	13.00	7.33	12.22	7.46	12.47

*essential amino acid

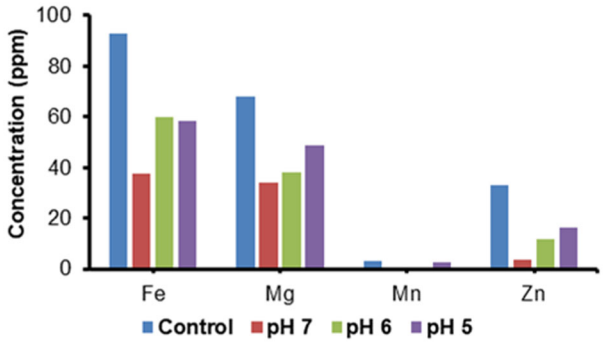

Fig 2. Comparison of Fe, Mg, Mn, and Zn concentrations in liquid protein at different pH

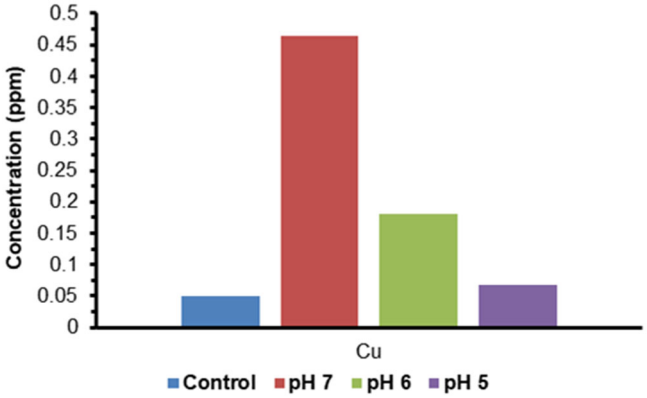

Fig 3. Comparison of Cu concentration in liquid protein at different pH

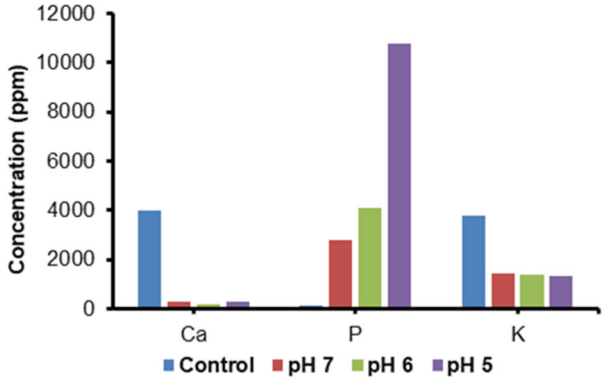

Fig 4. Comparison of Ca, P, and K concentrations in liquid protein at different pH

maximum Ca and K ions concentration was conducted at its origin hydrolysate. This concentration was obtained due to their solubilization of catalysis Ca and K utilized during HTC. The decreasing of pH conducts the diminution of concentration Ca and K ions in the liquid phase to form their neutral protein phosphate, except for the concentration of P ion. It indicates that the higher of H^+ conducted, the higher the phosphate concentration can be obtained in the liquid phase.

The profile concentration of metals contained in dry matter of solid precipitate can be shown in Fig. 5 and 6. This figure showed the diminution of concentration of metals observed by decreasing its pH value.

Fig. 7 shows that the concentration of K in the dry matter is relatively stable on various pH, while the

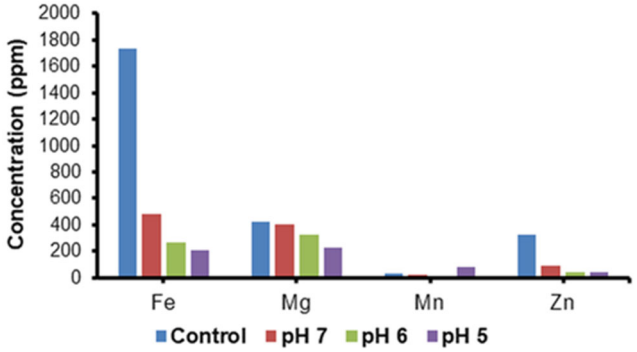

Fig 5. Comparison of Fe, Mg, Mn, and Zn concentration in solid humus at different pH

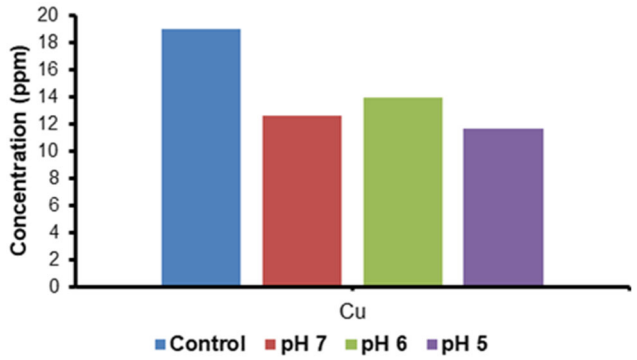

Fig 6. Comparison of Cu concentration in solid humus at different pH

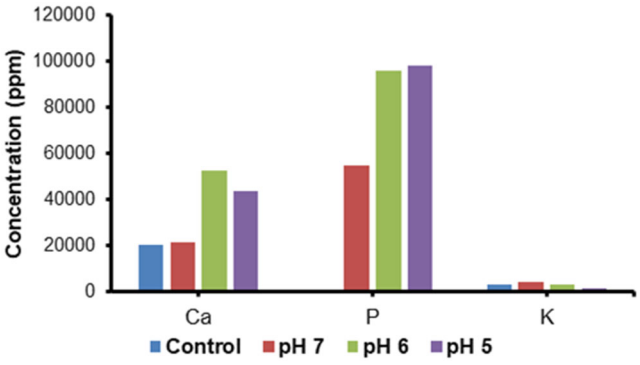

Fig 7. Comparison of Ca, P, and K concentrations in solid humus at different pH

concentration of Ca and P ions in the diminution of pH conduct the augmentation of their content in dry matter.

The results of this research show that chemical phosphorylation can enhance the protein content and reduce the metal ions content in the hydrolysate. The maximum soluble protein and minimum metal ions condition of hydrolysate was obtained at pH 7 and indicating the higher the pH, the lower the liquid protein that was obtained. The enhancement of soluble protein in hydrolysate had advantages as a source of nitrogen that can be functioned as a digestible animal feed and potential organic fertilizer, while the reduction of metal ions content in hydrolysate is suitable with minimum heavy metals content requirements of feed animals and organic fertilizer.

■ CONCLUSION

Based on the results of this research, the optimum phosphorylation condition to produce maximal soluble protein and minimum concentration of metal content in chicken feather hydrolysate has been obtained at pH 7. It can be concluded that chemical phosphorylation constitutes the simple and cheapest method to improve the functional properties of chicken feathers hydrolysate resulting from HTC without impairing the nutritional availability. This research opened the opportunity to easily get proteins with a smaller chain that can be used as digestible animal feed and organic fertilizer.

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