

Effect of pH and Incubation Time on Dissolved Nitrogen During Autolytic Degradation of Chicken Intestine

Eldiani Aulia^[a], Achmad Sjaifullah^{*[a]}, Wuryanti Handayani^[a], Busroni^[a], Ika Oktavianawati^[a], Muhammad Reza^[a]

Abstract: Chicken intestine is a part of internal organs, which are rich in protein and protease enzymes. The protease enzyme could self-degrade (autolytic degradation process) proteins in the chicken intestine at an appropriate pH and incubation time. This process produces a shorter chain polypeptide having a higher solubility protein called protein hydrolysates. Protein hydrolysates have shown a good impact in foods and health applications. In this study, the autolytic degradation of chicken intestine was carried out to obtain protein hydrolysates. The effect of pH and incubation time on the dissolved nitrogen (%N) and protein content ([protein]) in hydrolysate from the

autolytic degradation of chicken intestine explained in this paper. The incubation pH used in this study was 2.5, 3.5, 5.5., and 6.3 while the the incubation time was 0, 6, 12, and 18 h. Chicken intestine was incubated for 18 h at several different pHs, and the % N and protein content were determined by using Formol titration and Bradford methods, respectively, within 6 h intervals. It was obtained the % N and [protein] content increase at pH 2.5 and 3.5 during 18 h of incubation time and they were decreased at a higher pH. The optimum % N and [protein] content were 5.98 ± 0.51 % and 25.3 ± 0.04 mg mL⁻¹, respectively, obtained at pH of 2.5 during 18 h incubation time.

Keywords: Chicken intestine, protein hydrolysate, autolytic degradation, incubation, protein content.

INTRODUCTION

Chicken is one of meat and egg-producing poultry that is widely used by the people in Indonesia. Chicken production, especially broiler, always increases every year (Badan Pusat Statistik, 2016). Chicken meat can generally be used as a whole, but internal organs, including intestines, are generally known as side products which are often not consumed in large quantities because of health reasons (Korver, 2022). Chicken intestine has a relatively high protein content, which is 57-60 % of its dry weight (Borda-Molina et al, 2019). This organ is also contain many protease enzymes (Lourenco et al, 2020).

Up to now, the use or processing of chicken intestines in Indonesia has only limited in food products such as satay and chips. These simple processed products have side effects that are not good for health if consumed in large quantities because chicken intestine contains 260 mg of cholesterol in every 100 g of wet sample (Saidin 1999), chicken intestine also has a fat content of 5.60 %. Consumption of cholesterol and fat in large quantities can have negative impacts on health, including hypercholesterolemia which can cause atherosclerosis, coronary heart disease, and high blood pressure (Listiyana 2013), therefore it is necessary to diversify processed chicken intestine products into protein hydrolysate products that have high added value of local commodities. Protein hydrolysates have an important role in the food industry applications as emulsifying, foaming and gelling ingredients. More recently, the interest in protein hydrolysates has shifted towards their biological activities with benefits to human health (Tang et al, 2023).

Hydrolysate is a product of protein hydrolysis. This product can be obtained through chemical or enzymatic processing (Tang et al, 2023; Hidayat et al, 2019). Enzymatic processing requires presence of proteolytic enzymes (Nasri, 2017). This enzyme can be obtained from several parts of the organs of living things, including chicken intestines. Chicken intestine contains proteolytic enzymes such as Cathepsin B, Cathepsin D, Cathepsin H, Cathepsin L and alkaline proteases (Harikumar &

Jamdar 2005).

The high content of protein and protease enzymes in chicken intestinal tissue allows autolytic degradation of protein, the process of degradation of a tissue because of enzymes contained in the tissue itself. This process is affected by pH and incubation time. Harikumar & Jamdar (2005) found that the crude extract of protease enzymes from chicken intestines had an optimum pH of 2.5. Therefore, this article reports the process of autolytic degradation of chicken intestine which was carried out by incubating chicken intestine porridge with varying times and pH and observing the effect of pH and incubation time on dissolved nitrogen levels using the Formol method and the Bradford method.

EXPERIMENTAL

Materials

The equipment used in this study were a common laboratory glassware, analytical balance, blender, pH meter, centrifuge speed of 4000 rpm, Rayleigh UV-9200 type UV-Vis spectrophotometer, and refrigerator.

The materials used in this study included small intestine of chicken, distilled water, phosphoric acid (8.5%), Bradford reagent, Na-oxalate 1:3 (w/v), PP indicator (1%), formaldehyde (37%), and 0.01 N NaOH. All reagents were analytical grade and were obtained from Merck.

Methods

Chicken intestines were mixed with distilled water with a ratio of 1:2 (w/v) and were homogenized and produce a slurry, the slurry was analyzed the %N and [protein] content at 0 h of incubation.

[a] E. Aulia, A. Sjaifullah, W. Handayani, B. Busroni, I. Oktavianawati, M. Reza.
Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Jember
*e-mail: sjaiful.fmipa@unej.ac.id

The chicken intestine slurry was then placed in an incubation glass and the pH was adjusted by adding 8.5 % phosphoric acid to obtain a sample with an incubation pH of 2.5; 3.5; 5.5 and incubation time of 0, 6, 12, and 18h, respectively. In addition, a control was also prepared by incubating the intestinal slurry without phosphoric addition, i.e a pH = 6.8. During the incubation, every 6 h the levels of dissolved nitrogen and protein were measured using the formol titration method (Sudarmadji 1997) and the Bradford method (Bradford 1976).

Table 1. %N value (in %) obtained from autolytic degradation of chicken intestine

pH	Incubation time (h)			
	0	6	12	18
Control		3.46±0.36	Not detected	Not detected
2.5	1.79±0.18	4.22±0.24	5.75±0.00	5.98±0.51
3.5		3.77±0.65	4.60±0.18	5.33±0.18
5.5		3.02±0.20	Not detected	Not detected

During the incubation time from 0 to 18 h, the % N value in samples with a pH of 2.5 and 3.5 always increased. Meanwhile, in samples with a pH of 5.5 and pH 6.3 there was an increase in the %N value only at an incubation time of 0 - 6 h and becomes undetectable after more than 6 h of incubation. The undetectable dissolved nitrogen resulting from autolysis degradation at pH 5.5 and pH 6.3 during 12 h incubation and so on is thought to be due to the fact that the short peptides resulting from autolysis may have been used by bacteria that had developed in the sample after 12 h of incubation. Axelsson, 2004 states that bacteria need short peptides and amino acids as a source of nitrogen for their growth process. Bacteria will more easily consume short chain proteins than long chain proteins. There is bacterial degradation in the sample by spoilage microbes, such as *Salmonella Typhimurium* which lives at pH 4.5-6 (Guiter 2016); and *Enterococcus* bacteria at pH 4.4-9.4 (USDA 2012) causes the autolysis process to stop. Bacterial degradation was indicated by the appearance of a foul odor on the sample after the 6 h incubation time. The odor that arises comes from ammonia gas or H₂S, which is formed from the results of protein degradation by microbes. Nurwantoro and Djarijah, (2001), stated that spoilage microbes that become contaminants in food protein will metabolize low molecular weight organic compounds, such as amino acids, dipeptides, lactic acid and sugar into foul-smelling metabolites such as cadaverine, putrescine, H₂S, and NH₃.

The results in table 1 show that the highest % N value was obtained at pH of 2.5. The results obtained are in accordance with the research conducted by Harikumar & Jamdar, (2005). It is possible that the protease enzyme that plays a role in the autolytic degradation process is the Cathepsin D enzyme. This enzyme is an acidic protease enzyme which has optimum activity at pH 2-2.5 (Conner 1998). The % N value of the sample at pH 6.3 was higher than that of the sample at pH 5.5. This makes it possible for the active role of aminopeptidase enzymes to be active at pH 5.5-9 and work optimally at pH 6-7.5 (Harikumar, 2003). The results of this study indicate that there are various roles of enzymes during the autolytic degradation process. Each enzyme has optimum activity at different pH conditions. Harikumar & Jamdar, (2005) stated that chicken intestine contains various

RESULT AND DISCUSSION

Dissolved nitrogen content in hydrolysate

Table 1 shows the %N value during the autolysis degradation process in the chicken intestine were affected by pH and incubation time. The %N value of all samples at 0 h incubation time (start point of autolysis) had the same value of 1.79 ±0.18%.

kinds of protease enzymes, such as Cathepsins, Aminopeptidases, and Alkaline proteases.

The process of autolysis degradation is not only influenced by pH, but also by incubation time. This is shown by the research results which can be seen in Figure 1. The % N value of samples at pH 2.5 and 3.5 continued to increase during the incubation time from 0 to 18 h. These results show conformity with Prasetyo's statement, (2012) which states that hydrolysis time affects the amount of hydrolyzed connective tissue, the longer the time given, the more hydrolyzed tissue. The % N value at pH 5.5 and control (pH 6.3) only increased from 0-6 h of incubation. This is due to bacterial degradation, which causes decay in the sample.

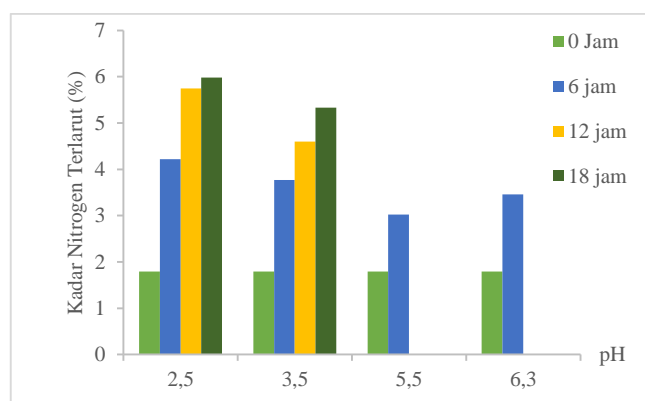


Figure 1. The obtained % N profile as a function of pH and incubation time.

Dissolved protein content in hydrolysate

Analysis of dissolved protein levels ([protein]) begins with the creation of a calibration curve. The maximum wavelength used in this study is 570 nm. The wavelength is in accordance with the theory put forward by Bradford, 1976 namely, Bradford's reagent can form complexes with proteins and has a maximum absorbance absorption in the range of 465-595 nm. The resulting calibration curve linear equation gives a value of $y = 1.6264x + 0.0761$ with an R² of 0.9917. The results of the analysis of [protein] in the sample can be seen in Table 2.

Table 2. [protein] value (in mg mL⁻¹) obtained from autolytic degradation of chicken intestine

pH	Incubation time (h)			
	0	6	12	18
Control		5.5±0.016	Not detected	Not detected
2.5	2.70±0.10	7.5±0.009	17.1±0.026	25.3±0.037
3.5		3.77±0.65	10.8±0.009	18.5±0.005
5.5		3.6±0.003	Not detected	Not detected

Based on Figure 2, it can be seen that the trend of [protein] produced has the same trend as % N value which were analyzed using the formol titration method. Dissolved protein levels at pH 2.5 and 3.5 always increased during the incubation time, whereas for pH 5.5 and control (pH 6.3) the dissolved protein levels could only be identified up to 6 h of incubation. When the incubation time was extended from 12 to 18 h at pH 5.5 dan 6.8 the presence of dissolved nitrogen cannot be detected, it was due to the dissolved peptides we consumer and degraded by bacteria which was indicated by the presence of gas bubble during incubation.

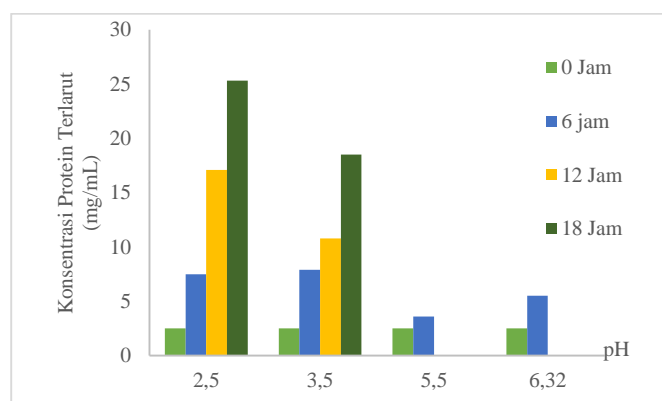


Figure 2. The obtained [protein] profile as a function of pH and incubation time.

CONCLUSION

It can be concluded that the levels of dissolved nitrogen and protein resulting from the autolyzed proteases in the intestine of chickens are affected by pH and incubation time. During 18 h of incubation at pH 2.5 and 3.5 always produce an increase in dissolved nitrogen and dissolved peptides. On the other hand, the levels of dissolved nitrogen and protein in samples at pH 5.5 and 6.3 was increase at incubation time of 0-6 h The best pH that can be used for the autolysis degradation process of chicken intestine in this study is pH 2.5.

REFERENCES

- [1] L. Axelsson. Lactic acid bacteria: classification and physiology. In Salminen, S., Wright, A.V., Ouwehand, A., editors. Lactic Acid Bacteria: Microbiological and Functional Aspects. 3rd edition, revised and expanded. New York:Marcel Dekker, Inc, 2004.
- [2] Badan Pusat Statistik. 2016. Produksi Daging Ayam Ras Pedaging menurut Provinsi, 2009-2016. <https://www.bps.go.id>. [18 September 2017].
- [3] A.J. Barret, and H. Kirschke. "Cathepsin B, Cathepsin H, and Cathepsin L," *Method in Enzimology*. Vol. 80, pp. 535-561, 1981.
- [4] D. Borda-Molina, T. Zuber, W. Siegert, A. Camarinha-Silva, D. Feuerstein, and M. Rodehutsord. "Effects of protease and phytase supplements on small intestinal microbiota and amino acid digestibility in broiler chickens," *Poultry Science*, vol. 98, no. 7, pp. 2906-2918, 2019
- [5] M.M. Bradford, M.M. "A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding," *Anal Biochem*, vol. 72, p. 248, 1976.
- [6] G.E. Conner. Cathepsin D. In: Barret, A. J., Rawling, N., Woessner, J.F., (Eds). Handbook of Proteolytic enzymes. New York: Academic Press, 1998.
- [7] P.M. Davidson and F.M. Critzer. Interventions to inhibit or inactivate bacterial pathogens in foods. In: Oyarzabal, O.A., S, Backert. (Eds), Microbial Food Safety. New York: An Introduction Springer, 2012.
- [8] D.R. Korver. "Intestinal nutrition: role of vitamins and biofactors and gaps of knowledge," *Poultry Science*, vol. 101, vol. 4, p. 101665, 2022.
- [9] M. Gauiter and F. Baron. Microbial spoilage. In: Jeantet, R., Croguennec, T., Schuck, P., Brule, G. (Eds.), Handbook of Food Science and Technology 1: Food Alteration and Food Quality. New York: Willey NY, 2016.
- [10] P. Harikumar, and S.N. Jamdar. "Autolytic degradation of chicken intestinal proteins," *Bioresource Technology*, vol. 96, no. 11, pp. 1276-1284, 2005.
- [11] M. Hidayat, S. Prahastuti, A.A. Soemardji, M. Yusuf, and K. Hasan. "Protein hydrolysate as a functional food for chronic kidney disease," *JKKI: Jurnal Kedokteran Dan Kesehatan Indonesia*, vol. 10, no. 3, pp. 211-212, 2019.
- [12] A.D. Listiyana, Mardiana, and N.P. Galuh. "Obesitas sentral dan kadar kolesterol darah total," *KEMAS*, vol. 9, no. 1, pp. 37-43, 2013
- [13] J.M. Lourenco, S.C. Nunn, E.J Lee, C.R. Dove, T.R. Callaway, and M.J. Azain. "Effect of supplemental protease on growth performance and excreta microbiome of broiler chicks," *Microorganisms*, 8, 475, 2020.
- [14] M. Nasri. "Protein hydrolysates and biopeptides: production, biological activities, and applications in foods and health benefits. A review," *Adv Food Nutr Res*, vol. 81, pp. 109-159, 2017.
- [15] Nurwantoro, and Djarijah. "Pengaruh kontaminasi mikroba. in: P.S. Maulana, C. Nirmala, S. Budiayati. Pembuatan kecap ikan gabus secara enzimatis menggunakan sari nanas," *Jurnal Teknologi Pangan*, vol. 1, no. 1, pp. 270-276, 2001.
- [14] M.N. Prasetyo, N. Sari, and S. Budiayati, S. "Pembuatan Kecap dari Ikan Gabus Secara Hidrolisis Enzimatis menggunakan Sari Nanas," *Jurnal Teknologi Kimia dan Industri*, vol. 1, no. 1, 2012

- [15] S.N. Jamdar, and P. Harikumar. "Autolytic degradation of chicken intestinal proteins," *Bioresource Technology*, vol. 96, no. 11, pp. 1276-1284, 2005
- [16] M. Saidin. "Kandungan kolesterol dalam berbagai bahan makanan hewani," *Buletin Penelitian Kesehatan*, vol. 27, no. 2, 1999.
- [17] S. Sudarmadji, B. Haryono, and Suhardi. *Prosedur Analisa Bahan Makanan dan Pertanian*. Yogyakarta: Liberty Yogyakarta, 1997.
- [18] T. Tang, N. Wu, S. Tang, N. Xiao, Y. Jiang, Y. Tu, and M. Xu. "Industrial application of protein hydrolysates in food," *J. Agric. Food Chem*, vol. 71, no. 4, pp. 1788-1801, 2023.
- [19] USDA. *Introduction to the Microbiology of Food Processing*. Washington: U.S Department of Agriculture, 2012.