

Antibacterial and Antioxidant Activity of Protein Hydrolysate Extracted from Different Indonesian Avian Egg White

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ABSTRACT

White egg contains the most significant protein source that has been explored to give more benefit for health purposes. This study evaluated the potential of antibacterial and antioxidant activity of white egg protein hydrolysate extracted from Indonesian avian eggs. The avian egg materials used local Indonesian chicken eggs (kampung chicken), chicken breed eggs, duck eggs, and muscovy duck eggs. The white egg was extracted by organic solvent and produced protein extracted which was dominated by ovalbumin and ovotransferrin. The protein extracted was hydrolyzed by pepsin and then powdered using a freeze dryer. Antibacterial and antioxidant activity was evaluated by agar well diffusion technique and 2,2-Diphenyl-1-picryl hydroxyl (DPPH) radical scavenging method. All samples showed antimicrobial activity against *Salmonella sp.*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Bacillus subtilis* with a concentration of protein hydrolysate at 40 mg/mL. The antioxidant activity between avian species was different and dose-dependent. Protein hydrolysate extracted from duck white egg exhibited the highest radical scavenging activity (65%) at 10 mg/mL concentration. However, all white egg protein hydrolysate extracted from Indonesian avian eggs can potentially help reduce oxidation and microbial deterioration.

Keywords: antibacterial, antioxidant, eeg white, hydrolysis, protein extracted

INTRODUCTION

The white egg is commonly considered a great protein source but not a functional food. During digestion, the high nutritional value of white eggs due to their high protein content will be converted into protein hydrolysates, peptides, and amino acids. However, it exhibited multiple biological activities, including antibacterial, antioxidant, anticancer, and hypotensive properties (Abeyrathne et al. 2014). Therefore, white egg is not only potentially advantageous for nutritious ingredients in food applications but has also generated significant interest from the biofunctional and biopharmaceutical industries (Benedé and Molina, 2020).

Various avian egg species have unique biochemical characteristics and functional properties of egg white (Benedé and Molina, 2020). The protein nitrogen content of white eggs from chicken, pheasant, quail, and duck varied from 71 to 82%, with the highest protein concentration found in duck white eggs, which contained over 80% protein. Indonesia has several species of local avian eggs, such as local Indonesian chicken called *ayam kampung*, local Indonesian duck, and muscovy duck that, intensive farming by human consumption as the primary protein resources, mainly egg and meat. Furthermore, the biofunctional activities of local

Indonesian avian white eggs have been studied, such as antioxidant, antihypertensive and antibacterial activity (Nariah et al. 2015). However, studies on specific protein egg white and hydrolysate from Indonesian avian egg species have never been investigated.

Recently, egg white protein hydrolysate has demonstrated several functional properties as a bioactive peptide to enhance human health, including anticancer and angiotensin-converting enzyme inhibitory effects (Jahandideh et al. 2014; Lee and Paik 2019). Hydrolysis with animal, plant or bacterial proteolytic enzymes could produce bioactive peptides. Pepsin is one of the most generally utilized enzymes to obtain egg white hydrolysates. Hydrolysate peptide from chicken egg white by pepsin activity has provided an antioxidant effect measured *in vitro* and *in vivo* assay. Egg white hydrolysate by pepsin successfully enhanced the plasma's radical-scavenging ability, reducing plasma malondialdehyde levels and a biomarker of oxidative damage in an obese model utilizing Zucker fatty rats (Garcés-Rimón et al. 2016). Therefore, pepsin is a well-known proteolytic enzyme to produce hydrolysate biopeptide mainly because naturally present in the human digestive tract.

Several studies reported the antibacterial and antioxidant activity focusing on the single protein from egg white protein purification and the hydrolysate of each type of protein such as ovotransferrin and ovomucoid (Abeyrathne et al. 2014; Nasution et al. 2018). However, not yet in a mixture of protein hydrolysate from white egg extraction or combining two or more protein types has been studied. Bioactivity assay using a mix or combining types of protein is likely to increase in activities because it will be rich in a specific protein, providing multiple mechanisms from each protein. Therefore, the objective of the study was to investigate the antibacterial and antioxidant activity of crude hydrolysate egg white extract dominated by ovotransferrin and ovalbumin from four avian egg species, namely: local Indonesian duck (*Anas platyrhynchos*), chicken breed (*Gallus gallus*), local Indonesian chicken (Ayam kampung) (*Gallus domesticus*), and muscovy duck (*Cairina moschata*).

MATERIALS AND METHODS

Extraction of egg white protein

Eggs were collected from four avian egg species, including local Indonesian duck (*Anas platyrhynchos*), chicken breeds (*Gallus gallus*), local Indonesian chicken (*Gallus domesticus*), and muscovy duck (*Cairina moschata*). By modification, protein extraction from egg white was carried out (Ko et al. 2009). Egg white was manually separated from the yolk and diluted with distilled water 1:1. Sample was added Fe^{3+} (0.8 mL/L of 500 mM FeCl_3) and then homogenized using a hand mixer for 2 min at high speed. The egg white proteins were precipitated by slowly adding 100% ethanol to the final concentration of 43% and centrifuged at 3,400 rpm for 20 min at 4°C. Supernatant protein was collected, increased the ethanol concentration to 61% adding slowly 100% ethanol and kept overnight at 4°C. The diluted protein was centrifuged at 3,400 rpm for 20 min at 4°C. Precipitate protein was collected and powdered by freeze dryer to obtain extracted protein from egg white (Abeyrathne et al. 2013).

Protein visualisation by SDS-PAGE

Protein visualisation employed vertical electrophoresis using polyacrylamide gel electrophoresis. Extract white albumen in powder was dissolved with water to make a 10 mg/mL. The sample was added loading buffer at a ratio of 1:1 and heated for 2 min. Polyacrylamide gel for gradient and stacking was prepared by concentration at 10% and 4 %, respectively.

Electrophoresis was carried out on a vertical chamber using tris-glycine buffer (pH 8.3) containing 0.1% of SDS and protein marker (ExcelBand 9-180kDa, SMOBIO Technology, Inc) at a constant electric current of 100V for 2.5h. Gels were stained with Coomassie Instant Blue (InstantBlue protein Stain, Merck) to visualize the protein.

Obtaining the hydrolysates

A homogenous freeze-dried egg white protein extracted was prepared by dissolving in distilled water, and then pH was adjusted to 2.5 with 1 M HCl aqueous solution. The hydrolysis reaction was performed by adding 0.1g/L pepsin (Merck) and incubating at 37°C in a water bath for six hours. Inactivation of pepsin was conducted by increasing the pH to 7.0 with 1 M NaOH, and the sample was heated in a boiling water bath for 10 min. The hydrolysates were powdered by freeze-dryer and stored at -20°C (Otte and Osman 2015).

Antibacterial assay

Antibacterial activity was evaluated using a modified version of the agar diffusion bioassay technique developed by (Moon et al. 2012). Each strain's inoculum was produced in nutrient broth (NB) and incubated at 37°C for one night. *Salmonella sp.*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Bacillus subtilis* were acquired from cultures at the Laboratory of Milk and Egg Technology, Faculty of Animal Science, University of Gadjah Mada, Indonesia. Those cultures were inoculated separately into 20 mL of nutrient agar (NA), mixed, and placed into a sterile petri dish. After the agar had solidified, four holes were punched in it, and 200 µl of hydrolysate samples with concentrations of 20 and 40 mg/mL were put into each hole. After incubating the agar at 37°C for 24 hours, the inhibitory zone was observed.

Antioxidant assay

The DPPH (2,2-Diphenyl-1-picryl hydroxyl) (Merck) radical scavenging activity was evaluated by modifying the method of (Jeevithan et al. 2015). Briefly, various concentrations of extract protein albumen were combined with 500 µl of methanol and 125 µl of methanol containing 0.02% (w/v) DPPH. The combination was kept in the dark for 60 minutes. The absorbance of a solution was measured at 517 nm using a spectrophotometer. Ascorbic acid served as a positive control, and every finding was based on

the average of three measurements. The calculation of DPPH radical scavenging activity was calculated as follows:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sampel}}{\text{Absorbance of control}} \times 100$$

Data analysis

The protein extraction yield result was subjected to variance analysis (ANOVA) with a 5% tolerance using SPSS 16.0.

RESULT AND DISCUSSION

Yield and purity of protein extracted

Figure 1 depicts the visualization of protein extraction from egg white by SDS-PAGE. Two primary bands at 40 kDa and 75 kDa have been identified as ovotransferrin and ovalbumin (Figure 1), with some differences that appear to be shared across species. The white egg extraction pattern by electrophoresis from local Indonesian chickens and chicken breeds was comparable; however, local Indonesian ducks and Muscovy ducks exhibited slight variations in intensity at 60 kDa. This electrophoretic profile indicated that

chicken groups (*Galliformes order*) and duck groups (*Aseriformes order*) varied in their egg white protein extracted results.

Ovotransferrin was found in all species with a similar electrophoretic profile and a molecular mass of around 70-80 kDa. However, the molecular weight of ovotransferrin varies between various extraction methods. Using cellulose dialyzed, egg white extracted from chicken, turkey, duck, and goose yields ovotransferrin with a molecular weight of 78 kDa (Wellman-Labadie et al. 2008; Abeyrathne et al. 2013) (Miguel et al. 2005) demonstrated that white eggs from hens, pheasants, and quail contained ovotransferrin with a molecular weight of 70 kDa, but ostrich eggs had a molecular weight of approximately 75 kDa. In addition, ovalbumin, the major protein in egg white, had a molecular weight of 45 kDa (Miguel et al. 2005).

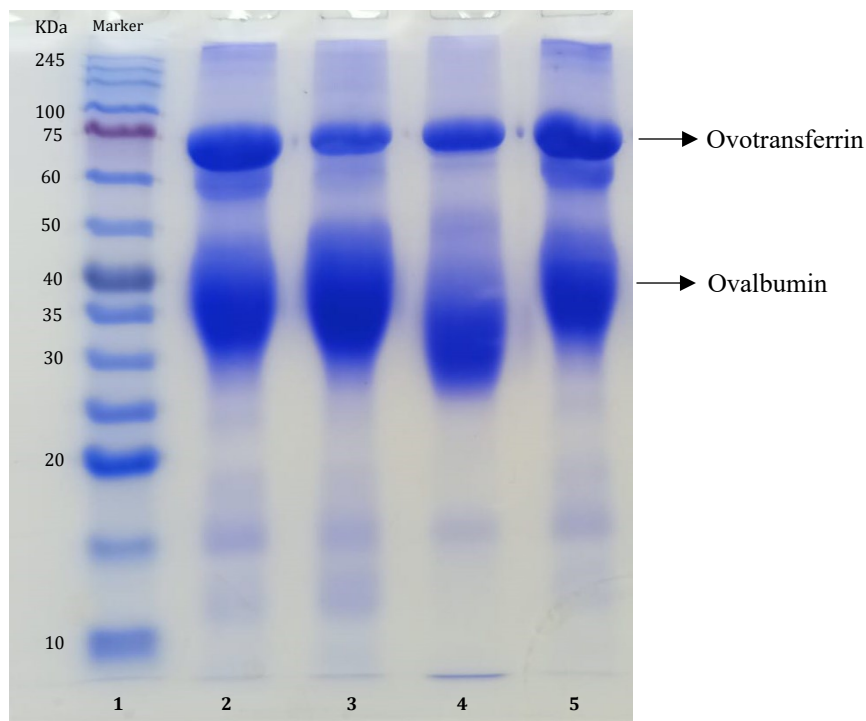


Figure 1. The SDS-PAGE of egg white protein was extracted from four different egg sources. Lane 1: marker; lane 2: protein egg white extracted from local Indonesian duck; lane 3: protein egg white extracted from chicken breed; lane 4: protein egg white extracted from egg white Indonesian local chicken; lane 5: protein egg white extracted from muscovy duck.

However, the electrophoretic profile of local Indonesian chicken was less concentrated. This ovalbumin exhibited a distinct migratory pattern and reduced molecular mass at around 35 kDa, which indicated a lower degree of glycosylation. The greater degree of glycosylation in ostrich ovalbumin has a larger molecular mass of 58 kDa. Most egg white protein consists of glycoproteins, which must be considered when analyzing them using electrophoretic techniques in the presence of SDS, as the carbohydrate moieties result in an overestimated molecular weight. According to these variables, there are variances in the ovotransferrin and ovalbumin of avian species (Miguel et al. 2005; Wellman-Labadie et al. 2008).

The yield of protein extracted from four avian egg white can be seen in Table 1. The protein yield of white eggs extracted from local Indonesian ducks, chicken breeds, local Indonesian chicken, and muscovy ducks was identical. The composition of the egg white's protein component is comparable to that of avian eggs. Meanwhile, the production of crude extraction is mostly impacted by the variance in egg size and white egg size (Miguel et al. 2005).

Antibacterial activity

Antibacterial activity derived from protein egg white extracted hydrolysate exhibited bacterial strain-dependent variability. Protein hydrolysate among four avian egg species at a concentration of 20 mg/mL exhibited no antibacterial activity, except for muscovy duck egg, which slightly inhibited *Salmonella sp*, *Bacillus subtilis*, and *Bacillus cereus* (Table 2). The potential of antibacterial activity from protein egg white extracted hydrolysate was categorized following Moon et al. (2012). Increasing hydrolysate concentrations up to 40 mg/mL showed antibacterial effectivity against all five strain microorganisms. This study indicated that the hydrolysate of the protein extracted, which was dominated by ovotransferrin and ovalbumin, possessed antibacterial properties.

Several studies have been conducted to evaluate the antibacterial activity of the

antibacterial from the white egg in various forms. Al-Mohammadi et al. (2020) found the antibacterial activity of whole white egg chicken hydrolyzed by pepsin depended on the protein concentrations. The minimum concentration of egg albumen hydrolysate was achieved at 100 µl/mg against *L. monocytogenes*, *B. cereus*, *S. aureus*, *S. typhimurium*, *S. pyogenes*, and *K. oxytoca*. Furthermore, the antibacterial activity of native form ovotransferrin from a commercial chicken egg was able to against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, and *Helicobacter pylori* at a concentration of 60 mg/mL (Moon et al. 2012). Furthermore, Kim et al. (2012) found no inhibition zone at 20 mg/mL ovotransferrin against *E. coli* O157:H7, while antibacterial activity was demonstrated at 80 mg/mL. Ovotransferrin is antibacterial by binding iron (Fe³⁺) and preventing bacterial development (Lee et al. 2017).

Moon et al. (2012) reported that the antibacterial effectivity of hydrolysate ovotransferrin was achieved by concentration at 80 mg/ml against *S. aureus* KCCM 32395, *B. cereus* KCCM 40935, *L. monocytogenes* ATCC 15313, *E. coli* O157:H7, and *H. pylori* HpKCTC 26695. Rathnapala et al. (2021b) studied ovotransferrin hydrolyzed by papain, trypsin, and -chymotrypsin. By the concentration of 20 mg/mL, there was no bactericidal activity, and it did not produce an inhibitory zone on a whole plate. (Kim et al. 2012) also found no inhibition zone at 20 mg/mL ovotransferrin against *E. coli* O157:H7, while antibacterial activity was demonstrated at 80 mg/mL.

In addition, purified biopeptide from ovalbumin hydrolysate that was digested by trypsin and chymotrypsin exhibited a high activity level against *Bacillus subtilis* (Pellegrini et al. 2004). Besides, antibacterial activity by a purified peptide from ovotransferrin hydrolysate (Leu109-Asp200) inhibited Gram-positive and damaging bacteria at 32 and 128 µg/mL, depending on the resistance of each strain of bacteria. Biopeptide have an antibacterial activity through a membrane damage mechanism.

Table 1. The yield of crude protein white egg extract from several Indonesia avian egg species

Result	Local Duck	Chicken breed	Local chicken	Muscovy duck
White egg (g)	128.05±0.35	166.73±7.01	79.56±5.35	91.47±3.00
Crude protein extract (g)	1.16±0.25	2.07±0.20	0.93±0.06	1.23±0.22
Yield (%) ^{ns}	1.02±0.20	1.27±0.43	1.17±0.11	1.34±0.20

Note: ^{ns} is not significantly different at P>0.05

Table 2. The potential inhibition hydrolysate protein of egg white extracted against pathogen bacteria

Bacterial strain	Hydrolysate of egg white extracted from different avian egg species							
	20 mg/mL				40 mg/mL			
	Local Duck	Chicken Breed	Local chicken	Muscovy Duck	Local Duck	Chicken breed	Local chicken	Muscovy Duck
<i>Salmonella sp</i>	-	-	-	+	+	+	+	+
<i>E. coli</i>	-	-	-	-	+	+	++	+
<i>S. aureus</i>	-	-	-	-	+	+	+	+
<i>B. subtilis</i>	-	-	-	+	+	-	+	+
<i>B. cereus</i>	-	-	-	+	+	+	+	++

Notes:

No inhibition (<2.0 mm) : -
 Slight inhibition (2.1- 4.0 mm) : +
 Moderate inhibition (4.1 - 6.0 mm) : ++
 Strong inhibition (6.1 - 8.0 mm) : +++

Peptides can attach to and penetrate membrane bilayers due to their amino acid composition, amphiphilicity, cationic charge, and side. Natural peptides can disrupt cytoplasmic membrane permeability, inhibit cell wall production, restrict nucleic-acid synthesis, and decrease protein synthesis, causing bacterial cell death (Cruz et al. 2021).

Antioxidant activity

The DPPH (2,2-Diphenyl-1-picryl hydroxyl), a stable and commercially accessible organic nitrogen radical, was generally deployed to examine the radical scavenging activity. The intensity of the yellow colour increases with increasing DPPH radical scavenging activity. Figure 2 shows the variance of antioxidant activity from protein hydrolysate egg white extracted between four avian species during this study.

In this study, antioxidant activity was optimized by different concentrations of protein hydrolysate of egg white extracted to provide radical scavenging activity. Protein hydrolysate of egg white extracted from local Indonesian duck started having radical scavenging activity for approximately 36.63% with a concentration of 2 mg/mL. In the chicken egg breed, radical scavenging activity was detected by protein hydrolysate at 20 mg/mL at about 11.99%. Moreover, protein hydrolysate from local Indonesian chicken and muscovy duck egg white extract by concentration at 62.5 mg/mL presented radical scavenging at 2.24% and 2.06%, respectively, which was to be the lowest radical scavenging activity compared to the other sources.

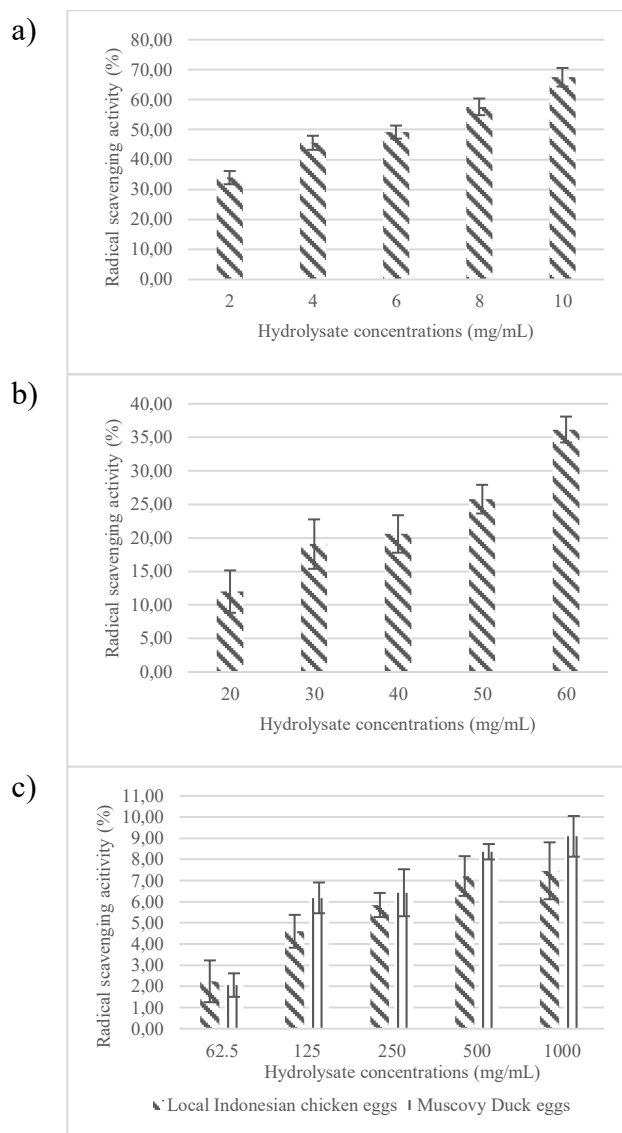


Figure 2. Antioxidant activity from crude hydrolysate egg white extract. a) local Indonesian duck; b) Chicken breed; c) local Indonesian chicken and Muscovy duck.

Ovotransferrin hydrolysate was thought to have minimal antioxidant properties. According to Moon et al. (2012), ovotransferrin hydrolyzed by papain, trypsin, and α -chymotrypsin at a 20 mg/mL concentration did not present antioxidant activity. However, the antioxidant activity of chicken ovotransferrin in its original form and its hydrolyzed form was contrasted by Kim et al. (2012). The hydrolyzed ovotransferrin had approximately 3.2 to 13.5 times higher antioxidant properties than undigested ovotransferrin, which depends on acid and enzymes. However, Lee et al. (2017) reported that 2 mg/mL ovotransferrin showed 40.30% DPPH radical scavenging, which had similar radical scavenging activities with ovotransferrin hydrolysate by thermolysin and promod278. Moreover, according to Abeyrathne et al. (2014), the antioxidant activities of ovalbumin hydrolysate were identified as one-fourth higher than those without hydrolysis.

Sun et al. (2014) demonstrated that DPPH radical scavenging and superoxide anion radical-scavenging activities assay from egg white hydrolysates, digested by pepsin, depended on molecular weight (MW) of protein fractions. The egg white hydrolysates with concentration at 50 mg/mL, the fraction 2-5 kDa peptides, have the most potent radical scavenging activity at $99.93 \pm 2.54\%$, while the lowest was the fraction 1-2 kDa with radical scavenging activity at $34.98 \pm 0.96\%$. In addition, Lin et al. (2013) reported that antioxidant activity from egg white hydrolysate digested by alcalde was higher than that digested by pepsin and trypsin. However, the optimum condition by pepsin hydrolysis with the most potent antioxidant capacity was determined by 4.56% of egg white protein as a starting material with an enzyme-to-substrate ratio of 1.58% at pH 1.99 and incubation at 37°C for one hour. Therefore, the variation of enzyme and hydrolysate protein resources also provides the differences in the capability in antioxidant activity. A comprehensive condition screening is entirely required to obtain optimal antioxidant activity.

The antioxidant activity between protein and hydrolysate has a different mechanism. The antioxidant activity of biopeptides is hypothesized by improving the accessibility of the functional side chain (R-group) to reactive species and electron-dense peptide bonds that may exhibit antioxidant function more efficiently. Hence, peptide length is correlated with the antioxidant capacity of peptides, and biofunctional peptides are typically identified as fragments containing

between 2 to 20 amino acid residues. An alternative mechanism for antioxidants is attributed to tyrosine at the N terminus as a hydroxyl group in the aromatic structure that allows it to break the antioxidant chain by a hydrogen atom transfer mechanism. Moreover, the amino acid composition appears to affect antioxidant activity significantly. Acidic amino acids (glutamine and asparagine) and hydrophobic amino acids (proline, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and tryptophan) have a considerable favourable influence on antioxidant activity. The sulfur-containing amino acids are also susceptible to oxidation because their S-groups interact with reactive species to create stable oxidation products. The presence of methionine in the peptide structure could also provide cyclic oxidation and reduction of methionine residues (Giansanti et al. 2015).

CONCLUSIONS

To conclude, hydrolysate of white egg extracted dominated by ovotransferrin and ovalbumin has the potential for antibacterial and antioxidant activity. The protein hydrolysate extracted from four Indonesian avian eggs has a different degree of antioxidant activity. The most potent antioxidant activity was protein hydrolysate from local Indonesian duck white egg extract, followed by the chicken breed, Indonesian chicken, and muscovy duck.

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REFERENCES

- Abeyrathne, E. D. N. S., H. Y. Lee, J. S. Ham, and D. U. Ahn. 2013. Separation of ovotransferrin from chicken egg white without using organic solvents. *Poult Sci.* 92(4):1091–1097.
- Abeyrathne, E. D. N. S., H. Y. Lee, C. Jo, K. C. Nam, and D. U. Ahn. 2014. Enzymatic hydrolysis of ovalbumin and the functional properties of the hydrolysates. *Poult Sci.* 93(10):2678–2686.
- Al-Mohammadi, A. R., A. Osman, G. Enan, S. Abdel-Shafi, M. El-Nemer, M. SitoHy, and M. A. Taha. 2020. Powerful antibacterial

- peptides from egg albumin hydrolysates. *Antibiotics* 9(12):1–21.
- Benedé, S., and E. Molina. 2020. Chicken egg proteins and derived peptides with antioxidant properties. *Foods* 9(735).
- Cruz, V. L., J. Ramos, J. Martinez-Salazar, M. Montalban-Lopez, and M. Maqueda. 2021. The Role of Key Amino Acids in the Antimicrobial Mechanism of a Bacteriocin Model Revealed by Molecular Simulations. *J Chem Inf Model* 61(12):6066–6078.
- Garcés-Rimón, M., C. González, J. A. Uranga, V. López-Miranda, R. López-Fandiño, and M. Miguel. 2016. Pepsin egg white hydrolysate ameliorates obesity-related oxidative stress, inflammation and steatosis in Zucker fatty rats. *PLoS One*. 11(3): e0151193.
- Giansanti, F., L. Leboffe, F. Angelucci, and G. Antonini. 2015. The nutraceutical properties of ovotransferrin and its potential utilization as a functional food. *Nutrients*. 7(11): 9105–9115.
- Jahandideh, F., K. Majumder, S. Chakrabarti, J. S. Morton, S. Panahi, S. Kaufman, S. T. Davidge, and J. Wu. 2014. Beneficial Effects of Simulated Gastro-Intestinal Digests of Fried Egg and Its Fractions on Blood Pressure, Plasma Lipids and Oxidative Stress in Spontaneously Hypertensive Rats. *PLoS One* :1–21.
- Jeevithan, E., B. Bao, J. Zhang, S. Hong, and W. Wu. 2015. Purification, characterization and antioxidant properties of low molecular weight collagenous polypeptide (37 kDa) prepared from whale shark cartilage (*Rhincodon typus*). *J Food Sci Technol*. 52(10):6312–6322.
- Kim, J., S. H. Moon, D. U. Ahn, H. D. Paik, and E. Park. 2012. Antioxidant effects of ovotransferrin and its hydrolysates. *Poult Sci*. 91(11):2747–2754.
- Ko, K. Y., A. F. Mendoncam, H. Ismail, and D. U. Ahn. 2009. Ethylenediaminetetraacetate and lysozyme improves antimicrobial activities of ovotransferrin against *Escherichia coli* O157:H7. *Poult Sci*. 88(2):406–414.
- Lee, J. H., S. H. Moon, H. S. Kim, E. Park, D. U. Ahn, and H. D. Paik. 2017. Antioxidant and anticancer effects of functional peptides from ovotransferrin hydrolysates. *J. Sci. Food Agric*. 97(14):4857–4864.
- Lee, J. H., and H. D. Paik. 2019. Anticancer and immunomodulatory activity of egg proteins and peptides: a review. *Poult Sci*. 98(12):6505–6516.
- Lin, S., Y. Jin, M. Liu, Y. Yang, M. Zhang, Y. Guo, G. Jones, J. Liu, and Y. Yin. 2013. Research on the preparation of antioxidant peptides derived from egg white with assisting of high-intensity pulsed electric field. *Food Chem*. 139(1–4):300–306.
- Miguel, M., M. A. Manso, R. López-Fandiño, and M. Ramos. 2005. Comparative study of egg white proteins from different species by chromatographic and electrophoretic methods. *European Food Research and Technology* 221(3–4):542–546.
- Moon, S. H., J. H. Lee, Y. J. Lee, J. Y. Paik, D. U. Ahn, and H.-D. Paik. 2012. Antioxidant, Antimicrobial, and Cytotoxic Activities of Ovotransferrin from Egg White. *Korean J Food Sci Anim Resour*. 32(5):612–617.
- Legowo, A.M., E. Abustam, and A. Hintono. 2015. Antioxidant and Antihypertensive Activity Egg White Powder Produced by Pan Drying at Different Temperature and Drying Time. *Jurnal Ilmu dan Teknologi Peternakan* 4(1):28- 34.
- Nasution, S., E. Kusumaningtyas, D. N. Faridah, and H. Kusumaningrum. 2018. Lysozyme from Chicken Egg White as an Antibacterial Agent. *Wartazoa* 28(4):175–188.
- Otte, J., M. Abdel-Hamid, and A. Osman. 2015. Comparative assessment of peptide concentration in milk protein hydrolysates and fractions. *International Journal of Dairy Science* 10 (5):228–235.
- Pellegrini, A., A. J. Hülsmeier, P. Hunziker, and U. Thomas. 2004. Proteolytic fragments of ovalbumin display antimicrobial activity. *Biochimica et Biophysica Acta*. 1672 (2):76–85.
- Rathnapala, E. C. N., D. U. Ahn, and S. Abeyrathne. 2021. Functional properties of ovotransferrin from chicken egg white and its derived peptides: a review. *Food Sci Biotechnol*. 30 (5):619–630.
- Sun, S., H. Niu, T. Yang, Q. Lin, F. Luo, and M. Ma. 2014. Antioxidant and anti-fatigue activities of egg white peptides prepared by pepsin digestion. *J. Sci. Food Agric*. 94 (15):3195–3200.
- Wellman-Labadie, O., J. Picman, and M. T. Hincke. 2008. Comparative antibacterial activity of avian egg white protein extracts. *Br Poult Sci*. 49(2):125–132.