



# Characterization of the Allergen from Egg White with SDS-PAGE

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## General Note

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## ABSTRACT

Food allergies are one of the biggest problems in the world of health. Egg is the type of food that most often causes allergies. The study aims to determine in different in the characteristics of egg white allergic from locally chicken, layer chicken and duck with analysis of SDS-PAGE. The results of the analysis there are six types of the proteins that can be identified in which four suspected protein is a allergens such as ovalbumin, ovomucoid, ovotransferrin and lysozyme, and from protein band that emerged showed that the allergens more guess contains in chicken layer egg white. The conclusion from the analysis that the chicken-layer egg-white is more likely to causes allergies.

**Key words:** Allergens, Egg White and SDS-PAGE

## 1. INTRODUCTION

Allergy is one of the biggest problems in the health world. Allergy or hypersensitivity reaction is a condition caused by a reaction that occurs in the immune system with antigens from the environment. One of the most common allergic factors is food.

Food allergies are caused by glycoprotein characterized by having a light molecular weight ranging from 18-80 kDa which is resistant to heat and resistant to several enzymes such as proteolytic enzymes. Food allergic reactions to the human body can vary depending on the body's immune condition when consuming these foods. One type of food that most often causes allergies is eggs.

Allergy to egg white is caused by four major proteins present in the egg white; ovomucoid, ovalbumin, ovotransferrin and lysozyme. Egg yolk allergy is caused by the currently identified allergens  $\alpha$ -livetin dan YPG-42 (Sophioglu, 2015). The protein contained in egg whites that can cause can be distinguished by characteristics, such as protein ovalbumin molecular weight of 45 kDa composed of 385 amino acids and is located at the isoelectric point of 4.5, ovalbumin is phospho-lipoprotein that are resistant to heat.

Ovomucoid is a type of protein that is the main cause of allergies in egg whites. Ovomucoid is at a molecular weight of 28 kDa consisting of 186 amino acid groups and at an isoelectric point of 4.82, ovomucoid is usually called a protease inhibitor and is heat-resistant. The third allergen is ovotransferrin at a molecular weight of 76-80 kDa with an isoelectric point of 6.1 and composed of 686 amino acids (Kaewmanee, 2011: 706).

In addition to the features already mentioned protein these allergens also have the properties and characteristics even more so this study aims to characterize differences in protein allergen egg white chicken eggs locally, layer chickens and ducks by a molecular weight with electrophoresis SDS-PAGE.

## 2. MATERIALS & METHODS

### Preparation of egg white

Samples were extracted by 2 methods, with dilution and without dilution. Egg white samples were separated from egg yolks, the first method with dilution, egg white was added with ethanol in a ratio of 1: 1. The second method without dilution is egg white dissolved using 30% ethanol in a ratio of 1:10. Then each sample was centrifuged at 4000 rpm for 30 minutes at 4. The resulting supernatant is separated from the precipitate. The resulting precipitate was an egg white protein isolate to be analyzed using SDS-PAGE.

### SDS- Polyacrilamide gel electrophoresis

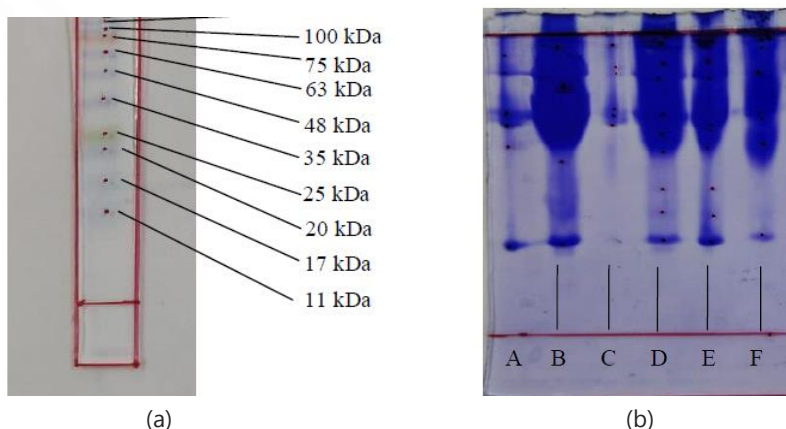
Separation using SDS-PAGE was carried out based on the Lemmli (1970) method. This analysis uses acrylamide gel with a concentration of *separating gel* 12% and *stacking gel* 5%, the samples used are protein extracts of local chicken egg white, layer chicken and ducks.

The next step is preparation and sample injection and running SDS-PAGE. The sample is put into a tube and a sample *buffer* is added and then heated for 5 minutes in boiling water. The sample is then injected into the well. After the sample is injected, running is done by means of a valve electrode mounted with current flowing to the anode. The power source is turned on and kept constant. Running carried out for 180 minutes until the migration of *dye* remaining sekitar 0.5 cm from the bottom. After completion, the electricity is turned off and the electrode valve is removed and the plate is removed from the electrode.

After running, the gel is removed for the gel coloring stage. Staining is carried out in a closed container that contains a solution of *commasie brilliant blue R-250*, then allowed to stand for a few minutes. After it is done destaining gel. The colored gel is removed and washed using distilled water several times. Then wash it with a color solution to lose color and shake it for several minutes until the protein band is visible on the gel. After clearing the gel, was analyzed by calculating the Rf value of each band formed.

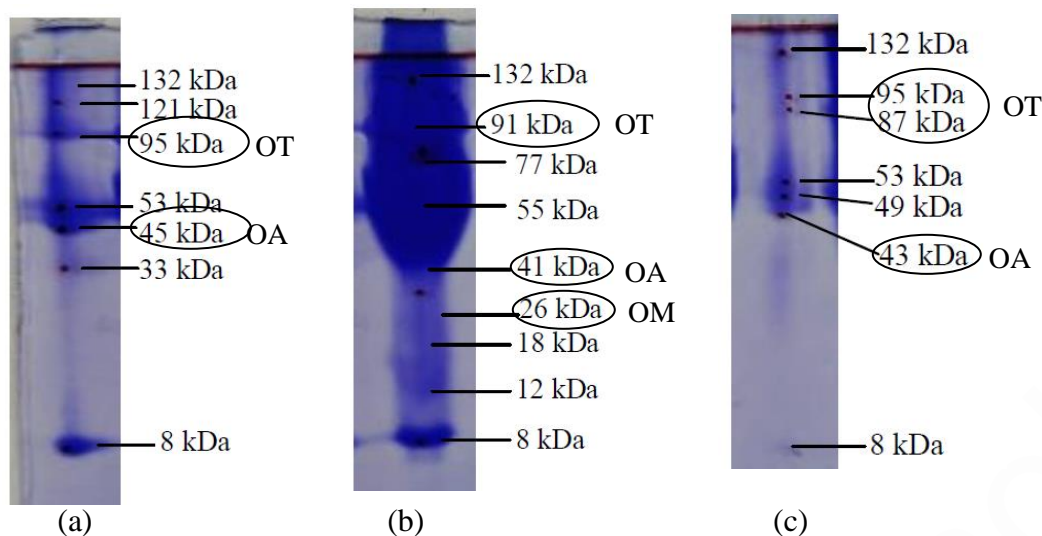
## 3. RESULTS AND DISCUSSION

### Analysis using SDS PAGE method



**Figure 1:** (a) Protein Marker; (b) SDS PAGE Analysis of egg white samples by 2 methods. The first method with dilution before adding 30% ethanol is code (A) with a sample of local chicken eggs, (B) layer chicken eggs and (C) duck eggs. The second method is without code dilution (D) with local chicken egg samples, (E) layer chicken eggs and (F) Duck eggs

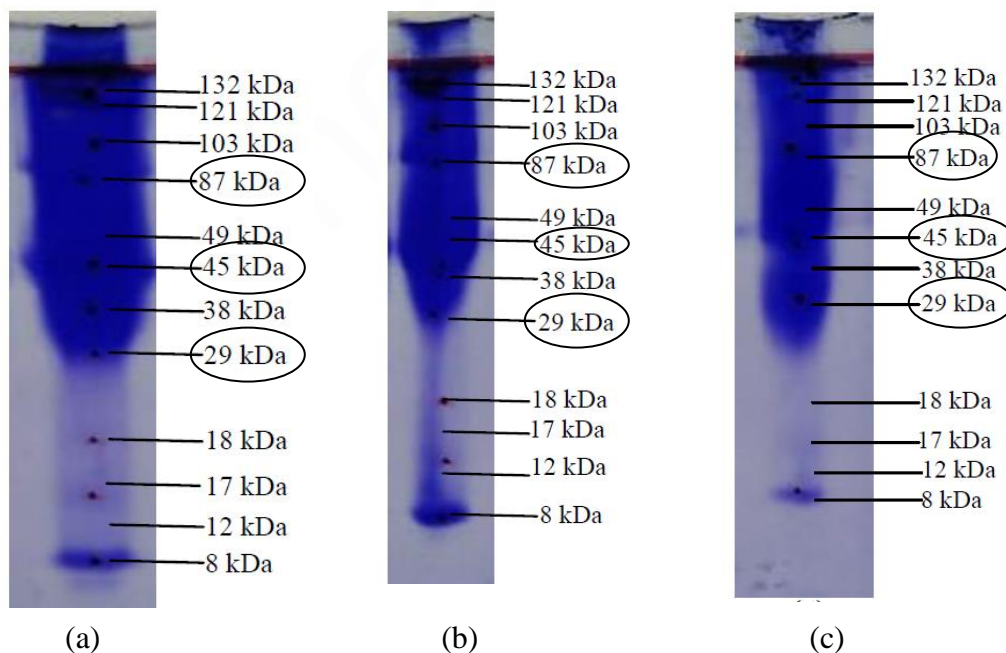
## Results of protein analysis method I



**Figure 2:** Results of protein molecular weight analysis using a dilution method before adding 30% ethanol. (a) local chicken egg whites; (b) layer chicken egg white ; (c) duck egg whites.

Figure 2.a with a layer chicken egg white from samples was obtained molecular weight in a row of 8, 63 kDa; 33,80 kDa (Ovoflavoprotein); 45,14 (ovalbumin); 53,26 kDa; 95,00 kDa (ovotransferrin); 121,75 kDa and 132,2 kDa. Figure 2.b with a locally chicken egg white samples was obtained 9 protein bands with molecular weight are 8,63 kDa; 12,53 kDa(lysozyme); 18,94 kDa; 26,37 kDa(ovomucoid); 41,56 Kda (ovalbumin); 55,51 kDa; 77,26 kDa; 91,16 kDa (ovotransferrin) and 132,24 kDa. Figure 2.c with duck egg white samples obtained 7 protein bands with molecular weight are 8,63 kDa; 43,31 kDa (ovalbumin); 55,51 kDa; 77,26 kDa; 91,16 kDa (ovotranferrin) and 132,2 kDa. Based on the resulting protein bands chicken egg white layer more containing globulin type of protein than chicken egg locally and duck egg which globular protein is a protein which is not soluble in water. Then based on the thickness of the bands formed layer chicken egg produce thicker bands than locally chicken egg and duck. So that the chicken egg layer contains more allergens than the locally chicken egg whites and duck because in the protein band that is thought to be an allergen, the resulting tape is thicker

## Results of protein analysis method II



**Figure 3 :** Results of protein molecular weight analysis with the addition of 30% ethanol. (a) local chicken egg whites; (b) layer chicken egg white; (c) duck egg whites.

Figure 3 (a) locally chicken egg white method 2, the results of the study were carried out showed that the band is produced in all three samples together which resulted in 12 bands of protein by weight molecule are the same. The molecular weight obtained are 8,63 kDa; 12,53 kDa; 17,44 kDa; 18,18 kDa; 29,85 kDa; 38,26 kDa; 49,03 kDa; 87,46 kDa; 103,19 kDa; 121,75 kDa and 132,24 kDa. From the data obtained on results of molecular weight, there are several types of proteins that can be identified including 12,53 kDa suspected to be a types of lysozyme protein, 17,44 kDa suspected avid in protein type; 29,85 kDa suspected ovomucoid protein type; 38,26 kDa suspected ovoflavoprotein protein type; 45,14 kDa suspected ovalbumin protein type and 87,46 kDa suspected to be type of ovotransferrin protein. From the results there are 3 types of protein that are suspected to be a type of allergens including ovalbumin, ovomucoid and ovotransferrin.

Ovalbumin is a type of protein that is most contained in egg whites, which is about 54% with a molecular weight ranging from 45 kDa and is a type of globulin protein. In this study, ovalbumin protein was suspected to be in the molecular weight range of 41-45 kDa. Yoo, et al (2013) by isolating and characterizing chicken egg whites, it was found that ovalbumin was identified in the 45 kDa molecular weight range. Kaewmanee, et al (2011) by identifying the protein present in duck egg whites obtained that ovalbumin is in the molecular weight range of 45-54 kDa, Aberathney (2015) study, by separating the protein in the egg white, also obtained the result that ovalbumin protein in the range of 45 kDa molecular weight. Ovalbumin protein is also found in some samples other than egg whites such as research Nugroho (2013), obtained the result that ovalbumin protein was found in cork fish with a molecular weight of 45 kDa.

Ovalbumin is also thought to be one type of protein that can cause allergic reactions, this is evidenced from the research of Leasa (2010), by giving ovalbumin as a cause of allergies to guinea pigs, the results obtained indicate that administration of ovalbumin and Al (OH)<sub>3</sub> cause allergies in Guinea pigs, which are allergy formed in guinea pigs, are observed from a systematic effect that shows the whole body of the guinea pig is blue due to allergies.

Ovalbumin contains carbohydrates and amino acids in the order Glu-Ala-Gly-Val-Asp-Ala-Ala. Nisbet (1980), ovalbumin contains thiol and disulfide bonds and there are 4 amino acids cysteine, 1 amino acid cystine and peptide binding residues. Purified ovalbumin produces amino acid sequences with N-terminals inhibited by the acetyl group and C-terminals containing the amino acid Cys-Val-Ser-Pro-COOH. Although included in one of the allergen proteins, ovalbumin does not really give an allergic effect due to its relatively stable heat which causes changes in conformation to become more stable.

Ovomucoid is identified at a molecular weight of 26-29 kDa while ovotransferrin is identified at a molecular weight of 87 kDa-95 kDa, Abyrathne (2015) by separating egg white protein, the results obtained are 3 types of proteins analyzed including ovalbumin, ovomucoid in the molecular weight range 26-39 kDa and ovotransferrin range from 79 kDa-95 kDa. Then according to Kaewmanee (2011), the molecular weight range of ovomucoid ranges from 29 kDa-37 kDa and ovotransferrin ranges between 66-97 kDa. According to Hiidenhovi (2015), which isolated one protein protein in egg white, namely ovomucin with a molecular weight of 120-150 kDa, also obtained ovotransferrin, ovalbumin and ovomucoid protein bands in the range of molecular weight, 66 kDa-97 kDa respectively; 45 kDa and 29 kDa-37 kDa.

Ovomucoid is the most dominant type of protein that causes allergies, this is because according to Lin (2016), ovomucoid has been identified as a major allergen, due to ovomucoid characteristics that are resistant to heat and stable in acidic solutions. Ovomucoid is a major glycoprotein which is a protease inhibitor and is found in egg whites, in ovomucoid there are 3 disulfide bonds, 2 tyrosine residues and 1 active group. Ovomucoid is composed of globular molecules with 2 double bonds  $\beta$ -sheet containing 22-23 residues and  $\alpha$ -helix of the amino acid Asn 33-Ser 44, the active group on the peptide bond is an opening with the composition of Lys 18-Asp 19 and closed by Asn 33. The primary structure of ovomucoid-forming amino acids consists of Asp, Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, His, Lys and Arg with the most dominant aspartic amino acids.

As with ovalbumin, ovotransferrin and lysozyme have little effect on causing allergies, because they are stable to heat. Ovotransferrin is the only dissolved glycoprotein that consists of 2 bonds, which bind iron with different solubility, in the first bond serum ovotransferrin is involved in iron transport and cell delivery and the second with lyoferrin which is involved antibodies to cause immunity in the body. Ovotransferrin is formed from the merging and cloning of genes, consisting of 15 disulfide bridges with a stable structure, 6 of which bind to Lobus N and Lobus C, while 3 disulfide bridges only bind to lobus C.

Lysozyme on the egg white very small amounts, the activity of lysozyme is determined by the presence of several types of amino acids, hydroxyl group and carboxyl group. Lysozyme molecules consist of a single polypeptide chain that is connected by a disulfide bridge. At the end of the N-terminal consists of 4 residues which are arranged including Lys-Val-Phe-Gly. The fifth residue is a type of amino acid serine and arginine is also present (Tomson, 1954). Lysozyme has a major role in egg white as an antibacterial such as *Streptococcus auerus* and *E.coli*.

Based on the result shows that are proteins that can cause allergies such as ovomucoid, ovalbumin, ovotransferrin and lysozyme. The bands that was produced also showed a layer chicken egg white over potentially cause allergies compared to the type of egg to

another, respect is due to the first method only in the layer chicken egg that shows the presence of protein ovomucoid which is a protein the main cause of allergies. Sophioglu (2015), there are 4 types of proteins causes allergies in egg white are ovalbumin, ovomucoid, ovotransferrin and lysozyme. Ovomucoid is a major allergies because one of the characteristic of allergens is resistance to heat. In addition, the factors other causes of allergies depends on the state of the body when consumed types of proteins such as well as the materials chemistry that may bind to the types of proteins are this potentially major cause of allergies.

#### 4. CONCLUSION

The conclusion of this study is the difference in protein characteristics between local chicken egg whites, layer chickens and ducks analyzed using SDS-PAGE obtained results of layers of egg white chicken containing a higher quantity of allergen protein than local chicken eggs and ducks. Identified proteins include ovalbumin, ovomucoid, ovotransferrin, lysozyme, avidin and ovoflavoprotein.

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