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Research Article

Isolation and Protein Profile of Chicken, Pork and Processed Products Nugget with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Method

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ABSTRACT

Chicken nuggets are known as a nutritious processed meat food ingredient and are widely available in supermarkets and are very popular with consumers. Nugget is made by mixing it with various other additives, so that it raises a bit of concern about the ingredients used in terms of halal. The purpose of this study was to determine and analyze the protein profile of chicken, pork, nugget references, and nugget commercials. The method used in this study is an experimental laboratory analysis of variables using SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis). The data were obtained from observing the description of protein bands, calculations were carried out using linear regression, and descriptive analysis was carried out. The results of this study showed 4 protein bands in pork that were not found in chicken meat with a molecular weight of 62.95 kDa, 41.86 kDa, 31.28 kDa and 17.46 kDa. Based on the protein fraction, the protein referred to as BM 31.28 kDa is Tropomyosin and BM 17.46 kDa is Troponin C. Nugget reference shows similarities to the protein bands found in pork, whereas nugget commercially did not show specific similarity.

INTRODUCTION

In this era of advanced globalization, with the increase in all sectors including food, the demand for meat for public consumption is increasing, one of these food ingredients is chicken meat. Chicken meat can quickly spoil due to exposure to spoilage or storage factors. One way to prevent the rapid deterioration of chicken meat is to process it into processed products.

One of the processed products that can be made from chicken meat is nugget. In product manufacture nugget, some ingredients are mixed so that it raises a little concern about the ingredients used in terms of halal (Ratulangi and Rimbing, 2021). Halal is a mandatory aspect that every consumer pays attention to, especially Muslim consumers.

In Indonesia, the number of Muslim consumers can reach 204.8 million people. Halal product assurance is an important parameter in the food industry because food begins to be processed using various materials and techniques. This can raise concerns about contamination with haram elements (Charity, 2017).

One of the concepts of halal in Islam is that it does not contain dietary fat derived from pork, and consuming everything that contains elements of pork is forbidden in Islam in Q.S. Al-Baqarah (2): 173. Several meat-based food products were found mixed with pork. Mixing

using pork can lower the price of production to be cheaper or accidentally contaminated. Therefore, identification is needed to detect pork in food products in order to protect consumers, especially Muslim consumers. Food products can be identified using existing technology (Puspitasari *et al*., 2019).

In previous research conducted by Zilhadia and Betha (2014), stated that food products, namely sausages, can be identified using the method Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Identification was carried out by characterizing protein profiles by separating proteins and measuring retention time (Rf). Then, the difference in protein bands was obtained, in beef there were 3 protein bands which were not found in pork. Meanwhile, specific protein was also not found in 10 beef sausage samples (Ghozali and Murani, 2023).

Based on the above background and referring to previous research, this study aims to identify differences in protein profiles of chicken, pork, and processed products nugget, by method Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). So, it can provide information and increase consumer confidence and safety in consuming various processed food products made from meat, as well as as halal authentication of food products, especially for Muslims (Ghozali *et al*., 2023).

METHODS

Tools and materials

The tools used are beaker glass, mortar and stamper, knife, *Centrifuge Hettich centrifuges EBA-20*, analytical balance,*Vortex Super Mixer Gemmy Industrial, White tip; Blue tip; Yellow tip, Micropipet,* tubes conical, test tube, *Hellma quartz glass cuvette*, autoclave, *Refrigerated Centrifuge*. Then for the separation and characterization of proteins using electrophoresis apparatus *HOWEVER San Francisco*, and for the determination of protein content using *Spectrophotometer UV-Vis*.

The ingredients used include fresh chicken and pork, salt, starch or tapioca flour, paneer flour, distilled water, protein markers (PM 5100), normal saline solution, phenyl methane sulfonyl fluoride (PMSF); ammonium persulfate (APS) 10%; sodium dodecyl sulfate (SDS) 10% in distilled water; Tris-HCl 1.5 M pH 8.8; Tris-HCl 1.5 M pH 6.8; isobutane; N,N,N N'-Tetramethylenediamine (TEMED) 100%; electrophoretic buffer pH 8.7; reagent biuret. Running buffer SDS-PAGE: 3 gr Tris, 14,4 gr Glycine, and 1 gr SDS (10 ml SDS 10%). Ingredients for distaining: 50% Methanol, 10% Acetic Acid Glacial, 40% Aquabidest. Ingredients for staining (dye): 0.2% Coomassie Blue in destaining solution. Ingredients for 5x Sample Buffer: 2.5 ml Tris 1.5M pH 6.8; 2 gr Tris; 5 ml mercaptoethanol; 10 mg *Bromphenol blue*; 10 ml *Glycerin* added to a volume of 20 ml using sterile Aquabides.

Processing *Nugget* (Ratulangi and Rimbing, 2021)

Each fresh meat is cut into small pieces and mashed using a mortar and stamper. Then mixed with other ingredients such as starch or tapioca and salt. Made with different concentrations of meat, nugget 100% chicken meat, nugget 100% pork, and nugget reference with 1%; 5%; 10%; 15%; 30%; 50%; and 75% pork. The additional ingredients and finely ground meat are mixed homogeneously, the homogeneous dough is formed into rounds and steamed at 75OC for 45 minutes. Furthermore, nugget was removed and prepared for further treatment.

Protein Isolation

Samples of fresh chicken meat and fresh pork and their processed products viz nugget, weighed 30 mg and washed using normal saline solution and then mashed. Then, 300 µl of PBS pH 7.2 extraction reagent was added and homogenized using a vortex (Hermanto and Meutia, 2016). Furthermore, after being mixed homogeneously, 4 µl of PMSF was added and homogenized (Baehaki *et al*., 2008). Then, cold centrifugation was carried out at 4OC using a speed of 12,000 rpm for 5 minutes. Then the supernatant was taken slowly and put into a new Eppendorf tube and then stored at -18OC (Hermanto *et al*., 2022).

Measurement of Protein Levels

Creation of Standard Standard Curves

Used 100 mg *Bovine Serum Albumin* as a standard solution put in 10 ml of distilled water, dissolved. BSA concentration is made at 0; 0.1; 0.2; 0.4; 0.6; and 0.8 mg/ml. 4 ml of reagent was added biuret, allowed to stand for 30 minutes and the absorbance was measured with a UV-Vis spectrophotometer with a wavelength (λ) of 540 nm. The measurement results are entered into the graph and the linear line aquation is determined (Amir *et al*., 2013).

Sample Preparation and Absorbance Measurement

The sample was weighed 0.5 g and pulverized, then dissolved with *Aquades.* Then, it

was centrifuged at 250 rpm for 10 minutes until it was divided, and 0.5 ml of the supernatant was taken into a test tube. Reagent added 4 ml and left for 30 minutes. The absorbance was measured using a UV- Vis spectrophotometer with a wavelength (λ) of 540 nm. The measurement results are entered into the graph and the linear line equation is determined (Amir *et al*., 2013).

Separation and Characterization of Protein Profiles with SDS PAGE

Protein isolation samples were taken as much as 13 µl and added 5 µl *loading buffer*. Protein marker used 10 µl. Each sample and protein marker was boiled in water at 100OC for 1 minute. The protein profile of the samples was characterized by SDS-PAGE (*Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis*). The sample is slowly introduced into the well and electrophoresis is carried out at 80- volts until the sample reaches the tip separating *gel.* Then at the end of the process, the power source is removed and the gel is taken from the plate (Hermanto and Meutia, 2016). Gel staining is carried out using *Coomassie Blue.*

Molecular Weight Analysis of Protein Profile SDS-PAGE Results

Analysis of the molecular weight (MW) of each protein produced by electrophoresis of fresh chicken and pork, nugget reference and nugget commercially, performed using protein markers as standard. Results Scanning The protein bands were then entered into the linear regression equation curve, and the MW protein values were obtained for each sample (Hermanto, 2009).

RESULTS AND DISCUSSION

Protein Band Profile Analysis

In this study used the *Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis* (SDS- PAGE) method to analyze the differences in protein band profiles of each sample. The concept of the gel used in electrophoresis, the pores in the gel are formed the bigger the concentration used in the gel is smaller, the protein molecules will run fast (Susanto, 2010). Another study by (Ginting, 2009), suggested that when processing meat and heating nugget, it can cause the protein in the meat to be damaged or lost.

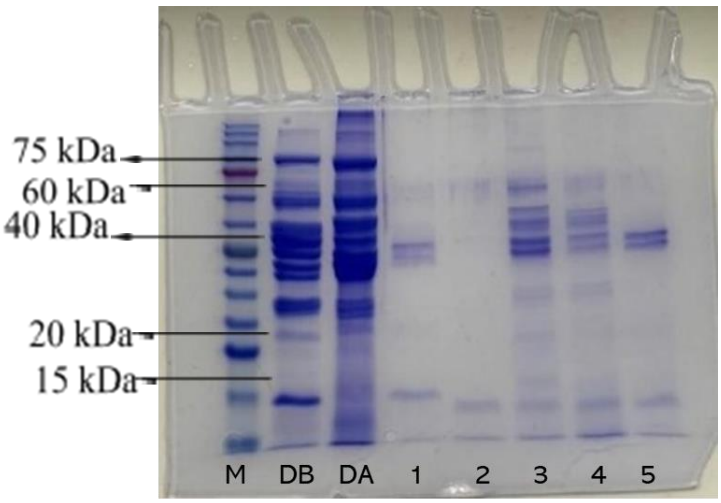

Figure 1. Gel 1 SDS-PAGE Electrophoresis Results of Protein Markers and Samples *Nugget* References (M: Protein Marker; DB: Pork; DA: Chicken; 1:*Nugget* Pork Ref 100%; 2: *Nugget* Chicken Ref 100%; 3:*Nugget* Reference with 1% pork; 4:*Nugget* Reference with 5% pork; 5:*Nugget* Reference with 10% pork.

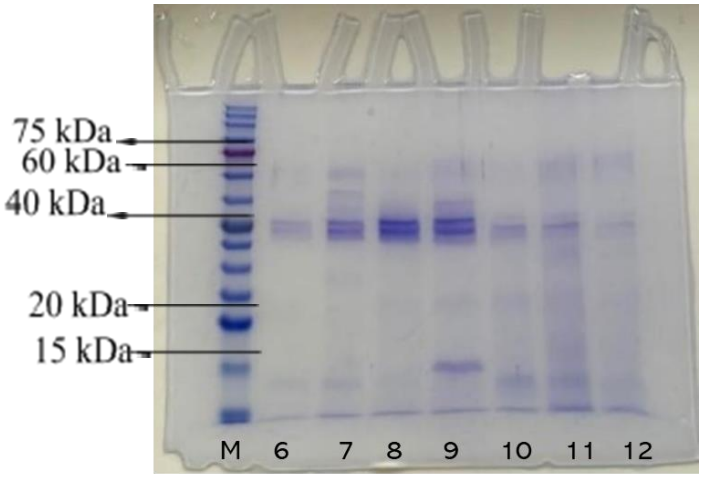

Figure 2. Gel 2 SDS-PAGE Electrophoresis Results Marker Protein, Sample*Nugget* References, and Samples*Nugget* Commercial (M: Marker Protein; 6: *Nugget* Reference with 15% pork; 7:*Nugget* Reference with 30% pork; 8:*Nugget* Reference with 50% pork; 9:*Nugget* Reference with pork 75%, 10;*Nugget* Commercial A; 11:*Nugget* Commercial B; 12:*Nugget* Commercial C.

The protein bands formed in the gel shown in Figures 1 and 2 appear blue due to use *Coomassie blue* as *staining solution* reacts to bind proteins with covalent bonds (Emami Bistgani *et al*., 2017; Lee *et al*., 2020). Based on Figures 1 and 2, it can be seen that there are thick and thin protein bands. This was explained by Hermanto *et al* (2022), thick or thin the formation of protein bands is the large amount of protein in the protein profile. The lower the protein concentration of the sample, the thinner the protein bands are formed.

In the gel electrophoresis results, 10 protein bands appeared in fresh chicken meat and 12 protein bands in fresh pork. On the reference sample nugget 100% pork, there are 4 protein bands, whereas in the reference sample nuggets 100% chicken there are 3 protein bands. On the sample nugget another reference, namely with a composition of 1% pork, 7 protein bands were obtained, nugget pork reference 5% obtained 6 protein bands, nugget pork reference 10% obtained 4 protein bands, nugget pork reference 15% obtained 2 protein bands, nugget pork reference 30% obtained 4 protein bands, nugget 50% pork reference obtained 3 protein bands, and nugget pork reference 75% obtained 4 protein bands. Nugget commercial, respectively on samples 10, 11, and 12, there were 3, 4, and 3 protein bands.

Sample Molecular Weight Measurement

In this study, the Retention factor (Rf) was calculated and the relationship with the logarithm of the molecular weight (BM) of the protein marker was entered into a linear regression (y = -1.4176x + 5.3053) to obtain an equation in order to calculate the molecular weight of the sample (Ginting, 2009).

Sample Molecular Weight and Protein Characteristics

Based on the electrophoresis results in Figures 1 and 2, as well as the calculation of the Rf value with the linear regression equation that has been obtained, it is possible to calculate the molecular weight of the sample and analyze the characteristics of the protein.

Based on Table 1, there are several protein bands in fresh pork that are not found in fresh chicken meat, namely at molecular weights of 62.95 kDa, 41.86 kDa, 31.28 kDa and 17.46 kDa. on sample nugget references 1 and 5, found the similarity of bands formed on fresh pork and not formed on fresh chicken meat, namely with a molecular weight of 62.95 kDa. On sample nugget references 3 and 4, found unknown protein with a molecular weight of 66.73 kDa which is not found in fresh pork or fresh chicken.

Meanwhile, on the sample nugget references 7 and 9, it was found that the bands formed on fresh pork and not on fresh chicken meat were found, with molecular weights of 56.03 kDa and 17.46 kDa. on sample nugget references 6 and 8, found unknown protein with a molecular weight of 59.39 kDa and 47.04 which is not found in fresh pork or fresh chicken meat. On all samples nugget commercially did not show a specific protein band similarity with pork. This shows that all nugget commercial qualitatively there is no pig.

At least the appearance of protein bands in processed product samples nugget this, showed significant differences in protein bands in fresh

meat. This is because reduced protein bands can be caused by denaturation or breakdown of most of the protein during processing (Susanto, 2010).

Friction that occurs with the smoothing tool can cause the separation of proteins which are inhibited, then protein coagulation will occur. Heating during the manufacturing process nugg*et al*so causes a low concentration of dissolved total protein, so that the protein will be denatured (Hermanto *et al*., 2022).

Protein Band Fraction

In this study, the estimation of the protein band fraction was carried out using Price and Schweigert's (1987) protein fraction standard.

There is a significant difference between fresh pork and fresh chicken. Fresh pork samples contained tropomyosin with a molecular weight of 31.28 kDa and troponin C with a molecular weight of 17.46 kDa, which were not found in fresh chicken meat. According to Listrat *et al*., (2016), tropomyosin and troponin act as myosin binders in muscle cells. Tropomyosin is a protein with a secondary structure that has an α- helix with two chains, namely alpha and beta tropomyosin, attached to the filament pathway of F-actin. Meanwhile, troponin C, one of the troponin complex proteins that is in the heart muscle (Rosana, 2019).

Table 1. Results of Measuring Protein Content

No	Sample	Protein (mg/mL)	Molecule Weight (kDA)
1	Fresh Pork	2.758	159.97 134.31 100.5 79.48 70.74 62.95 56.03 52.85 41.86 31.28 17.46 12.31
2	Fresh Chicken Meat	1.343	190.54 159.97 134,31 94.67 79.48 70.74 59.39 52.85 39.49 12.31
3	*Nugget* 100% Reference Pig	0.459	62.95 56.03 18.51 12.31
4	*Nugget* 100% Reference Chicken	0.310	112.76 16.47 12.31
5	*Nugget* Reference Pig 1%	0.367	100.35 84.25 74.98 66.73 59.39 16.47 12.31
6	*Nugget* Reference Pig 5%	0.550	106.38 84.25 66.73 59.39 16.47 12.31

7	*Nugget* Reference Pig 10%	0.819	70.74 62.95 16.47 12.31
8	*Nugget* Reference Pig 15%	0.462	59.39 12.31
9	*Nugget* Reference Pig 30%	1.088	94.67 56.03 52.85 12.31
10	*Nugget* Reference Pig 50%	1.289	52.85 47.04 12.31
11	*Nugget* Reference Pig 75%	1.353	56.03 49.86 17.46 12.31
12	*Nugget* Commercial Chicken A	1.729	52.85 15.54 12.31
13	*Nugget* Commercial Chicken B	0.981	100.35 52.85 15.54 12.31
14	*Nugget* Commercial Chicken C	1.934	100.35 52.85 12.31

Troponin C was not present in samples 6, 7, 8, 10, 11, and 12. This was stated by Dalilah (2006), that troponin C protein has properties that dissolve in salt solution. This can result from additional ingredients, namely salt in processing nugget reference. Therefore, it is very possible that Troponin C protein is not detected because it has dissolved in the added salt.

There is also actinin α protein in fresh meat and samples 3 and 7. Actinin α protein is unstable or labile and does not dissolve in the range of 50OC (Susanto, 2010). In addition, in the process of adding other materials for processing nugget references can also affect protein levels (Dalilah, 2006).

Measurement of the Protein Levels

In this study, protein levels were measured using a UV-Vis spectrophotometer with a wavelength (λ) of 540 nm and using the UV-Vis method Biuret. Measurement of protein content using samples of processed products, which during the processing of samples can also affect the content of protein content (Riyanto, 2010).

After all samples were read the absorbance, then entered into the linear regression equation obtained from the standard curve that has been made with several concentration series.

The results in table 1 show that the protein content in fresh pork is higher than the protein content in fresh chicken meat. This is because according to Vee rman and R usman (20 15) , protein in pork is as much as 20-28%, while protein in chicken according to Rukmini *et al* (2019), there is generally 18- 20%, which is less than pigs.

On sample nugget reference, the highest protein content is shown in the sample nugget pork reference 75% with a protein content of 1.353 mg/mL. Meanwhile, the lowest protein content was Shown in the sample nuggets 100% chicken reference with a protein content of 0.310 mg/mL. The less fresh pork added, the lower the protein content (Hetharia *et al*., 2013).

On sample nugget commercially, the highest protein content is in the sample nugget commercial chicken C with levels of 1.934 mg/mL. Meanwhile, the lowest protein content was in the sample nugget commercial chicken B with a protein content of 0.934 mg/mL. In previous studies, it was stated that protein levels in nugget relatively low compared to other processed products, due to a significant change, namely a decrease in protein content nugget. A product's decrease in protein content (such as a nugget) can be attributed to adding other

ingredients during manufacturing. These added ingredients may dilute or replace some of the protein, resulting in a lower overall protein content in the final product (Riyanto, 2010).

CONCLUSION

There are significant differences based on the protein profile which can be identified by the method Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), based on protein bands and their molecular weights, using linear regression statistical analysis. Protein bands in pork appear more, namely 12 protein bands, chicken meat 10 protein bands, nugget reference 2-7 protein bands, and nugget commercial 3-4 protein bands. In pork there are specific protein bands that are not found in chicken meat, namely 4 protein bands with a molecular weight of 62.95 kDa, 41.86 kDa, 31.38 kDa and 17.46 kDa. Nugget reference shows similarity to the protein bands in pork, except in sample 6 which is present unknown protein. Meanwhile on nugget Commercially there is no specific similarity with pork.

Further research is needed on the protein profiles of pork and chicken meat to better understand the differences in the protein profiles contained therein. Another method that can be used for further research is the method immunoblotting, using protein antigens and antibodies in order to obtain better and specific results.

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