Identification of Protein Bands Characteristic of Raw and Boiled Beef and Pork Meat Using the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Method

Anna P. Roswiem [a,1,*], Pedrianto [b], Firman Arifandi [c]

[a] Halal Research Center, YARSI University Reseacrh Institute, Jakarta, Indonesia
[b] Proteomic Lab, Telomere, Longevity Oxidative Stress (Telos), YARSI University Reseacrh Institute, Jakarta, Indonesia
[c] Faculty of Medicine, YARSI University, Jakarta, Indonesia
[1] annap_ros@yahoo.com
* corresponding author

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ABSTRACT

The second most abundant nutrient content in animal meat is protein, which is in the range (16-22) %. Proteins are denatured by heat. Protein denaturation will changes in protein structure. The Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method aims to determine the composition of proteins based on their molecular weight (MW). Therefore, the aim of this research is to determine the differences in protein band profiles that characterize pork and beef meat, raw or boiled, based on their MW. The research began with the isolation of protein from pork and beef meat, both raw and boiled. Next, the protein isolate produced from each sample was precipitated by adding ammonium sulfate with saturation of 20, 40, 60, and 80%. Then the protein formed was purified by dialysis method and the protein bands were characterized with 10% (v/v) SDS-PAGE. To determine the MW of protein, MW calculations were performed using PhotoCaptMW software, which using a protein with a MW of 10 kDa to 250 kDa as a marker for the MW of the protein. The results showed that the number of protein bands from raw pork and beef meat was greater than the number of protein bands from boiled meat. There are 23 protein bands that characterize raw pork which are not present in raw beef, with a range of MW (3.462-69.068) kDa; and the characteristic protein bands of raw beef which are not present in raw pork are 18 bands, with MW range of (15,445-291,176) kDa. The characteristic protein bands for boiled pork which are not present in boiled beef are 9 bands with a MW range of (2.692-19.659) kDa. There are 4 protein bands that characterize boiled beef which are not present in boiled pork with a MW of: 15,942; 16.133; 16,041 and 16,272 kDa.

1. Introduction

Foods made from animal sources that are widely consumed by consumers in Indonesia are beef and chicken. In Indonesia, until now, the price of beef is still very expensive. As a consequence of the high price of beef, it is possible for producers/sellers of meat and processed food to mix or replace beef with other cheaper animal meat, such as pork or boar meat, or rat meat. Indonesia is a country

whose majority population is Muslim. Therefore, this case can disturb the inner peace of the Muslim community, because meat and/or processed meat products are haram for consumption. Food products based on animal protein (meat) that are widely consumed are unprocessed animal meat (raw meat, such as Japanese cuisine) and processed ones, including by boiling, grilling or frying.

The most abundant component in meat is protein compounds. One method for protein identification is the Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) method. (Bintang, 2010; Roy, et al, 2012). The SDS-PAGE method is a very specific method that can be used to differentiate proteins from various animal species based on the presence of certain protein bands (also it is called as the characteristic protein bands) which are not found in other animal protein bands. Therefore, the problem of this study is whether the SDS-PAGE method can be used to identify the presence beef, and pork in raw and boiled samples based on their characteristic protein band profiles. Based on the background above, the aim of this research is to obtain a method for detecting the presence of raw and/or boiled beef, and pork protein through identifying the presence of characteristic protein bands.

2. Materials and methods

The materials in this study were raw (local) beef purchased from Superindo Cirendeu, South Tangerang and raw (local) pork purchased at Kem Chic-South Jakarta.

2.1. Materials

Raw (local) beef, raw (local) pork, NaCl, Ammonium sulfat, Tris HCL pH6,8; EDTA, BaCl2, Na2SO3, HCl 2N, Bovine Serum Albumin, CuS04, Sodium Potassium tartrat, NaOH, Kalium Iodida, Aquabides, Ninhidrin, Glisin, Pierce™ Coomassie Plus (Bradford) Assay Kit Scientific™, Laemmli Sample Buffer, SFX ACRYL SOLN 10% 50 gel Kit, 2-Mercaptoethanol, TEMED, Ammonium Persulfate, TGS BUFFER, TBS BUFFER SOLUTION, TWEEN 20, PBS 10 X pH7.4, milliQ water, Alkohol 70%, destaining buffer, ddH2O / Aquades, Protein Sampel, Marker Protein.

2.2. Tools

A set of SDS-PAGE Bio Rad PAC 300 electrophoresis tools; Refrigerated Centrifuge Hitachi Himac SCR 20 B; Eppendorf micropipette 1.5 ml; Micro tube rack, Eppendorf tube 1.5 ml; Water bath shaker (Taiko Personal–11); dialysis tubes; Freeze drying EYELA FD–1; CS–930 Dual Wavelength Scanner; and UV-Vis Spectrophotometer (Shimadzu), Analytical balance, pH meter, Hot plate, Magnetic stirrer, Microfuse, Backman Nano drop centrifuge, Maestrogen Spectro Nanodrop, 100 microliter and 1000 microliter micro pipette, Mini-PROTEAN® Tetra Cell, PowerPacTM Power Supply, Head Block / water bath, Trans Blot Turbo System, Micropipette set + Tip, Roller, Microtube 1.5 ml, Gloves, Shaker Glove, Tips 0.5-10 µL, Tips 20-200 µL, Tips 1000 µL, Tips 5000 µL, Centrifuge tube 15 ml conical 40/bag, Centrifuge tube 50 ml conical 20/bag, Computer Dell (Connected with ChemisDoc Imaging System)

3. Procedure

3.1. Sample Preparation

Sample preparation in this study used modified method of Harrow, et al (1960) in Margriet, (2003).

3.2. Isolation of Protein

Pork and beef meat (without fat) cut into small pieces, add a little ice water then blend until smooth. The meat pulp formed is filtered. The solid is discarded, the filtrate is centrifuged for 15 minutes, 6000 RPM (sorvall refrigerated centrifuge SS - 34 pellet rotor). The pellet (biomass) was discarded, the filtrate formed was "crude protein extract". Margriet, (2003). And then the extract was fractionated by adding ammonium sulfate with a saturation of 20, 40, 60, and 80% (Goletti, M. and Purwanto, M. 2007)

3.3. Fractionation of Crude Protein Extract Samples by Ammonium Sulphate

Fractionation of crude protein extract samples. Make a fractionation of the ammonium sulfate precipitation 20, 40, 60 and 80% by adding a number of ammonium sulfate salts according to Table 1-1, add ammonium sulfate salt little by little while stirring slowly (do not foam) at cold temperature (approximately within a minimum time of 1 hour). After adding salt, centrifugation was carried out for 30 minutes at 10,000 rpm. Separate the supernatant from the precipitate. The precipitate is the protein fraction that precipitates at a saturation level of 20% ammonium sulfate while the supernathan is a protein salt solution which has a saturation of 20%. To the supernathan 20% add ammonium sulfate as above for the next precipitation fraction 20-40% and so on, the first precipitate is named the precipitate fraction 20%, namely for the precipitation of 20% ammonium sulfate. The second precipitate fraction of 40% is the 40% ammonium sulfate precipitation fraction, and so on. Store the precipitate at 20 °C. Dissolve each precipitate with 20 mM Tris HCI pH 6.8 as little buffer as possible. Measure the total volume of each fraction. Take 1-2 ml for protein test (Yu, Z. et al 2014)

Table 1. Fractionation of Crude Protein Extract Sampel with Ammonium Sulphate\

Precipitation (%)	0-20	20-40	40-60	60-80
Ammonium sulfate amount (grams) for 1 L of solution	106	113	120	161
Name of the precipitate fraction	20 %	40 %	60 %	80 %

3.4. Dialyze The Protein Extract In The Sample

Dialysis is used to separate large molecules from smaller molecules with the aid of a semipermeable membrane. This membrane has pores of a certain size which will pass molecules of smaller size and retain molecules of a larger size. The large molecules remain in the dialysis tube, the small molecules will come out through the membrane into the solvent or buffer until an equilibrium occurs between the concentration of the fluid inside and outside the dialysis tube. The dialysis process can be carried out several times on the same sample and membrane repeatedly. Changing the solvent or buffer in this study dialysis is used to remove ammonium sulfate salts or other small molecules that remain in the protein extract and sample (Bintang, M. 2010)

3.5. Method of Determination Protein Bands and Molecular Weight in the Protein Samples

Identification of the presence of protein bands from various animal species (samples above) was carried out using the SDS-PAGE method. The molecular weight (MW) of the protein sample was determined based on the standard curve of the line equation between the log MW of protein markers and the Rf value of each protein marker band. In this study the MW protein samples were calculated automatically using PhotocaptMW software. (Chanchaithong, P. and Prapasarakul, N. (2011).

4. Result and Discussion

4.1. Isolation and Protein Content in Samples

The isolation results and protein content of raw and boiled pork and beef meat are shown in Table 2 below :

Table 2. Isolation Results and Protein Content in the Sample

NO	SAMPLES	Protein content in the sample (mg/ml) with the addition of ammonium sulfate with a saturation of:			
		20%	40%	60%	80%
1	raw pork	26,357	36,542	34,923	17,330
	boiled pork	23,891	42,507	10,751	6,380
2	raw beef	11, 354	21, 532	18,306	9,755
	boiled beef	18,446	5,676	3, 184	2,161

From the table above it can be seen that the protein content of raw pork and beef meat is higher than the protein content of boiled pork and beef meat. These results indicate that the protein is denatured by heat. Denaturation causes the degradation of protein structures in raw meat which include secondary, tertiary and quaternary structures into primary structures, resulting in bands of primary structured proteins with smaller molecular weights and others compounds that are not proteins such as peptides (non-protein) and amino acids (Nelson and Cox, 2015; Bintang, M. 2010) (Yu, Z. et al. 2014)

Apart from that, from the table above, the protein content of pork is higher than the protein content of beef. However, the benefit of "ukhrawi" and the command to comply with absolute orders in the Shari'a still limit humans from consuming pork. Because in principle, protein to meet the body's needs can still be obtained from other animal species and halal food

4.2. Profile and Molecular Weight (MW) Protein Bands From Raw and Boiled Pork and Beef Meat Using the SDS-PAGE Method

4.2.1. Profile and Molecular Weight (MW) Protein Bands From Raw and Boiled Pork meat

Profiles protein band from raw and boiled pork resulting from the SDS-PAGE method are shown in Table 3 below.

Table 3. The Protein Bands and Molecular Weight (MW) of Protein in Raw and Boiled Pork Samples Using Software PhotoCapt MW.

	Molecular Weight (MW) / Retention Flow (RF)								
	L1	L2	L3	L4	L5	L6	L7	L8	L9
1	250.000	42.411	69.068	60.576	31.094	8.846	19.659	8.077	8.077
2	150.000	10.417	40.431	22.218	22.944	3.077	9.615	3.462	2.692
3	100.000	5.385	14.332	19.740	19.759		4.231		
4	75.000		10.000	18.172	19.525				
5	50.000		3.462	15.000	17.314				
6	37.000			10.000	14.332				
7	25.000			4.615	10.000				
8	20.000				4.231				
9	15.000								
10	10.000								

Where :
L1. MW of Proteins Marker
L2. MW of Raw pork meat with ammonium sulfate concentration of 20%
L3. MW of Raw pork meat with ammonium sulfate concentration of 40%
L4. MW of Raw pork meat with ammonium sulfate concentration of 60%
L5. MW of Raw pork meat with ammonium sulfate concentration of 80%
L6. MW of Boiled pork meat with ammonium sulfate concentration of 20%
L7. MW of Boiled pork meat with ammonium sulfate concentration of 40%
L8. MW of Boiled pork meat with ammonium sulfate concentration of 60%
L9. MW of Boiled pork meat with ammonium sulfate concentration of 80%

From the data above, the number of protein bands in raw pork is different from the number of protein bands in boiled pork, namely 23 protein bands in raw pork and 9 protein bands in boiled pork. There are 2 protein bands with the same MW between raw pork protein bands and boiled pork protein bands, namely protein bands with MW of 4.231 and 3.462 kDa. The amount of boiled pork protein band is less than raw pork protein band. This shows evidence that boiling causes the protein to be denatured or the protein structure of raw pork to be degraded, so that only the primary structure is produced.

The results above are different from the results of a study by Hermanto (2009) which stated that there were 14 protein bands in raw pork with MW in the range (31.0-66.2) kDa. Meanwhile, the MW of raw pork protein band in the study was in the range (3.462-69.068) kDa. This difference was caused in this study, the isolation of protein content in raw pork was carried out by adding ammonium sulfate with saturations of 20, 40, 60, and 80%; whereas in Hermanto's (2009) research, protein isolation in raw pork was only carried out by adding 10% ammonium persulfate. So the number of protein bands in raw pork in this study is greater than the results of Hermanto's (2009) study.

4.2.2. Profile and Molecular Weight (MW) Protein Bands From Raw and Boiled Beef

The Protein band from raw and boiled beef from SDS-PAGE and molecular weight (MW). Was calculated using PhotoCaptMW software its are showed in Table 4. The Protein bands and MW of Protein in Raw and Boiled Beef sample using software PhotoCaptMW.

Table 4. The Protein Bands and MW from Raw and Boiled Beef.

	Molecular Weight (MW) / Retention Flow (RF)								
	L1	L2	L3	L4	L5	L6	L7	L8	L9
1	250.000	38.288	291.176	75.898	38.288	15.942	16.133	16.041	16.272
2	150.000	27.786	193.588	29.728	20.466				
3	100.000	21.235	38.288	24.524	15.756				
4	75.000	15.445	26.436	20.466					
5	50.000		15.834	19.103					
6	37.000			16.103					
7	25.000								
8	20.000								
9	15.000								
10	10.000								

Where :
L1. MW of Proteins Marker
L2. MW of Raw pork meat with ammonium sulfate concentration of 20%
L3. MW of Raw pork meat with ammonium sulfate concentration of 40%
L4. MW of Raw pork meat with ammonium sulfate concentration of 60%
L5. MW of Raw pork meat with ammonium sulfate concentration of 80%
L6. MW of Boiled pork meat with ammonium sulfate concentration of 20%
L7. MW of Boiled pork meat with ammonium sulfate concentration of 40%
L8. MW of Boiled pork meat with ammonium sulfate concentration of 60%
L9. MW of Boiled pork meat with ammonium sulfate concentration of 80%

From the table above, it can be seen that the number of protein bands in raw beef is 18 protein bands and in boiled beef 4 protein bands. Similar to the protein band of boiled pork, the protein from boiled beef also to be denaturation or the protein structure of raw beef is degraded so that when boiled it produces only its primary structure.

From the results above, it can be seen that the number of protein bands from raw beef is slightly different from Hermanto's (2009) study, namely 17 bands, but the range of MW protein is quite different, namely in the range (21.5-116) kDa; whereas in this study the number of protein bands was 18 bands, but in the MW range (15,445-291,176) kDa. This difference was due to the fact that to isolate protein in beef in Hermanto's research only ammonium persulfate was used with only one concentration, namely 10%. Meanwhile, in this study, ammonium sulfate was used with varying saturation, namely 20, 40, 60, and 80%.

4.2.3. Differences in Identifying Protein Bands Between Raw Pork and Raw Beef

Until now, in Indonesia the price of beef is still very expensive, so adulteration products often occur with beef substituted or mixed with pork as raw material.

From the results of the research above, with the differences in the protein bands from either raw or boiled pork or beef, it can be seen the profile of the identifying protein bands in products with raw beef mixed with or replaced by pork which can be seen from the number of different protein bands (MW).

4.2.4. The Characteristic Protein Bands Between Raw Pork And Raw Beef Meat Using the SDS-PAGE Method

The Characteristic Protein Bands of raw pork is the protein bands with a certain molecular weight in raw pork which are formed on polyacrylamide gel, but those protein bands are not formed in raw beef.

The Characteristic Protein Bands of raw beef are protein bands with a certain MW in raw beef which are formed on polyacrylamide gel but those protein bands are not formed in raw pork.

4.2.4.1 The Characteristic Protein Bands of Raw Beef and Pork

From Tables 5 and 6 above, there are 23 Protein Bands Characteristic Raw Pork with MW: 69.068; 60,576; 42,411; 40,431; 31,094; 22,944; 22,218; 19,759; 19,740; 19.525; 18.172; 17.134; 15,000; 14.332; 14.332; 10.417; 10,000;53,385; 4.615; 4,231; and 3.462 kDa; which is not present in raw beef.

Protein Bands Characteristic Raw Beef, there are 17 protein bands in raw beef with a MW of 38,288; 27,786; 21.3235; 15,445; 291,176; 193,588; 38,436. 26,436; 15,834; 75,898; 29,728; 24,524; 20,466; 19,103; 16,103; 38,288; 20,466; and 15,756. kDa; which is not present in raw pork.

Table 5. Molecular Weight of Raw Pork Protein Bands

	L1	L2	L3	L4	L5
1	250.000	42.411	69.068	60.576	31.094
2	150.000	10.417	40.431	22.218	22.944
3	100.000	5.385	14.332	19.740	19.759
4	75.000		10.000	18.172	19.525
5	50.000		3.462	15.000	17.314
6	37.000			10.000	14.332
7	25.000			4.615	10.000
8	20.000				4.231
9	15.000				
10	10.000				

Table 6. Molecular Weight of Raw Beef Protein Bands

	L1	L2	L3	L4	L5
1	250.000	38.288	291.176	75.898	38.288
2	150.000	27.786	193.588	29.728	20.466
3	100.000	21.235	38.288	24.524	15.756
4	75.000	15.445	26.436	20.466	
5	50.000		15.834	19.103	
6	37.000			16.103	
7	25.000				
8	20.000				
9	15.000				
10	10.000				

Where :
L1. Molecular Weight (MW) of proteins marker
L2. Molecular Weight (MW) of raw pork/beef protein bands with ammonium sulphate concentration of 20%
L3. Molecular Weight (MW) of raw pork/beef protein bands with ammonium sulphate concentration of 40%
L4. Molecular Weight (MW) of raw pork/beef protein bands with ammonium sulphate concentration of 60%
L5. Molecular Weight (MW) of raw pork/beef protein bands with ammonium sulphate concentration of 80%

4.2.4.2 The Characteristic Protein Bands of Boiled Beef and Pork

From tables 7 and 8 it can be seen that there are 9 protein bands for boiled pork and 4 protein bands for boiled beef. However, neither of the two boiled meat protein bands (pork and beef) have the same MW.

Therefore, there are 9 protein bands that characteristic boiled pork which are not present in boiled beef with MW respectively: 8,846; 3,007; 19,659; 9,615; 4,231; 8,007: 3,462; 8,007; and 2,692 kDa, and there are 4 protein bands characteristic of boiled beef which are not present in boiled pork, with MW respectively 15,942; 16,133; 16,041 and 16,272 kDa.

Table 7. Molecular Weight (MW) of Boiled Pork Protein Bands

L6	L7	L8	L9
8.846	19.659	8.077	8.077
3.077	9.615	3.462	2.692
	4.231		

Table 8. Molecular Weight (MW) of Boiled Beef Protein Bands

L6	L7	L8	L9
15.942	16.133	16.041	16.272

Where :
L6. Molecular Weight (MW) of boiled pork and beef protein bands with ammonium sulfate concentration of 20%
L7. Molecular Weight (MW) of boiled pork and beef protein bands with ammonium sulfate concentration of 40%
L8. Molecular Weight (MW) of boiled pork and beef protein bands with ammonium sulfate concentration of 60%
L9. Molecular Weight (MW) of boiled pork and beef protein bands with ammonium sulfate concentration of 80%

5. Conclusion

The results showed that the number of protein bands from raw pork and beef meat samples was greater than the number of protein bands from boiled pork and, beef meat. Protein bands that characterize raw pork that are not present in raw beef are 23 bands with a MW range of (3.462-69.068) kDa; and there are 18 protein bands that characterize raw beef which are not present in raw pork. with a range of MW (15.445-291.176) kDa. Protein bands that characterize boiled pork which are not present in boiled beef are 9 protein bands with MW respectively: 8,846; 3.007; 19,659; 9,615; 4,231; 8,007; 3,462; 8,007; and 2,692 kDa. Protein bands that characterize boiled beef which are not present in boiled pork, there are 4 protein bands with a MW of: 15,942; 16.133; 16,041 and 16,272 kDa.

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