Characterization of sarcoplasmic protein in the meat using SDS-PAGE method

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Abstract. A characterization of the sarcoplasmic protein in the meat had been investigated. The method that was utilized in this study was SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) which played a role in analyzing the molecular weight of the meat protein. The characterization of the molecular weight depended on the concentration of acrylamide gel that has been used. The result reported that using of 10% acrylamide gel showed difference of the molecular weight characterization from the sarcoplasmic protein. The difference of protein in the meat is on sarcoplasmic proteins. Protein with molecular weights of 50.8 kDa was not found in pork. Therefore, this molecular weight of the protein can potentially serve as a key for differentiating pork and other meats.

1. Introduction
Human necessary in consuming meat increases rapidly. The enhancement makes them paying more attention to the quality of meat than its quantity [1]. The meat has nutrient composition that is well enough for the human body. In addition, when the food is processed, it has a delicious taste. However, the high price if meat and uncontrolled supply are currently considered to be a lot of trouble for processed meat product producers. Therefore, meat producers substitute beef with other types of meat that are cheaper. As reported in 2013, there has been adulteration of beef with European horse meat [2]. Some producers want to reap greater profits by substituting using this meat. The substitution is worked as the price of other meats such as pork are cheaper but has the closer characteristics to beef. It poses a serious threat to the safety and halalness of the processed meat products. The existence of this case has damaged consumer confidence [3]. The raises concern in the community because the beef fraud is spreading in the market [4].

The protein content in the meat can be used as a reference to know characteristic of the meat type in food preparations. Meat that has a lot of muscle tissue has the highest quality to be processed as food. Protein is a key component of meat and a critical determination of its structure, nutritional value, and
texture. Moreover, specific protein inspected in the post-mortem process have a role as meat quality as softness [6].

SDS-PAGE is used to separate protein based on molecular weight. Tropomyosin, one of the proteins, has been identified its presence in poultry and mammals. The protein in each species can be distinguished according to distribution of thickness band which yielded by SDS-PAGE analysis [7]. It was found several protein fractions that detected thickly in fresh pork samples but detected thinly in fresh beef samples. These fractions are proteins with molecular weight (MW) 116.47 kDa, 54.45 kDa, and 40.67 kDa respectively. Other differences of protein band could also be seen from number of protein band that detected in the sample respectively. Protein with MW about 112.13 kDa was found in fresh pork sample, but not found in the fresh beef sample [8]. A study declared that the adulteration of meat species at concentration as low as 5-10% in the binary mixture sample could be distinguished and detected without risk using SDS-PAGE method [9]. The other study reported that SDS-PAGE method could be served in identifying meats such as cattle, sheep, lambs, goat, red deer, and rabbits. It happens because the method allowed to investigate the following myofibrillar and sarcoplasmic muscle proteins: myosin and actin, α-actinin, tropomyosin, troponin [10]. Based on recent study, analysis of protein using SDS-PAGE method could be utilized as an alternative method to distinguish the origin of meat. The aim of the study is to know protein bands profile in the meat as it can differentiate origin meat.

2. Method

2.1. Materials

Materials that were used in this study were meat (it was originated from traditional market on K. H. Ramli Street, Makassar City, South Sulawesi), ammonium persulfate (APS) 10%, Tris-HCl electrophoresis buffer, phosphate buffer 0.1 mol L⁻¹ pH 7, Comasie Brilliant Blue (CBB) G-250, Coomassie Brilliant Blue R-250, dinitrosalicylic acid (DNS), glicine, pH paper, NaCl 0.5%, Jena Bioscience Marker, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), SDS, filter paper, electrophoresis instrument Bio-Rad, spectrophotometer UV-Vis Varian Cary 50 cone, sentrifuge Haereus, wizard advanced IR vortex mixer VELP SCIENTIFICE, waterbath shaker Thermo Scientific MAXQ 7000.

2.2. Preparation of protein

Meat samples about 10 g respectively were pondered. Then, they were mixed with 30 mL NaCl 1% in 0.2 mol L⁻¹ phosphate buffer pH 7. After that, the mixture was mashed up using blender. The compound was separated with centrifuge. The treatment was repeated until three times so that a precipitate would be obtained. Protein content analysis was carried out by Bradford Method. The method uses Bradford reagent which contains Comasie Brilliant Blue (CBB). It also utilizes bovine serum albumin (BSA) as standard solution.

Standard solution (0.2; 0.4; 0.6; 0.8; 1.0) mg mL⁻¹ was entered in the tube and was added with 5 mL of Bradford reagent. The mixture was incubated at the room temperature for 5 minutes. Blank solution was done with mixing 0.1 mL of aquades and 5.0 mL Bradford reagent [11]. After that, measurement of absorbance used UV-Vis spectrophotometer at 595 nm. Protein solution as sample about 0.2 mL was added with Bradford reagent. Then, it was incubated at the room temperature for 5 minutes. After that, measurement of absorbance used UV-Vis spectrophotometer at 595 nm [12].

2.3. Extraction of sarcoplasmic protein

The extraction of this study was done in two steps. First step, sample was mashed up using blender. For the second step, the solution was separated using centrifuge. Refining of the sample using a blender aims to break down the cell wall so that the protein can be extracted. After mashed up, sample was centrifuged to separate between crude protein in the supernatant and the other compound in the pellet. The mixture about 2 g NaCl 0.5% in 20 mL phosphate buffer 0.2 mol L⁻¹ pH 7 were entered in blender. They were then mashed up at 4-6°C for 3 minutes. The sample was separated with centrifuge at a speed
of 4500 rpm at 4°C for 30 minutes. The precipitate was then homogenized with the same solution then re-centrifuged. The filtrate obtained was called the sarcoplasmic protein fraction [13].

2.4. Characterization using SDS-PAGE
The sarcoplasmic protein fraction about 20 µL was mixed with 5µL of phosphate buffer. The mixture was then homogenized using vortex, was boiled for 5 minutes, and was entered in gel well. The protein was separated with 100 mA and 50 V. The gel was colored using Coomassie blue [14].

3. Result and discussion

3.1. Protein level and sarcoplasmic protein extract
Protein solubility depends on the concentration of the salt solution and the pH of the solution. It is such as sarcoplasmic proteins which can be dissolved in water and runny salts at the neutral pH.

Table 1. Protein level of concentration in salt solution.

| Concentration of salt solution | Chicken (mg mL⁻¹) | Lamb (mg mL⁻¹) | Beef (mg mL⁻¹) | Pork (mg mL⁻¹) |
|--------------------------------|-------------------|----------------|----------------|----------------|
| 0.5 mol L⁻¹                    | 0.4000±0.06000    | 0.3620±0.07334 | 0.4716±0.03745 | 0.3792±0.05425 |
| 0.7 mol L⁻¹                    | 0.4593±0.04282    | 0.4298±0.09202 | 0.5326±0.04151 | 0.3655±0.09960 |
| 0.9 mol L⁻¹                    | 0.480±0.09644     | 0.2812±0.06069 | 0.4819±0.03074 | 0.2511±0.02766 |
| 1.1 mol L⁻¹                    | 0.4727±0.04623    | 0.4156±0.01623 | 0.4324±0.03413 | 0.2688±0.03183 |

Table 1 shows that chicken and beef meat have a more soluble type of protein in concentrations range from 0.5 mol L⁻¹ - 1.1 mol L⁻¹ than lamb and pork. This was also shown in the results of the SDS-PAGE analysis (Figure 1) where the results indicated the thin band on lamb and pork. It happens because a few types of protein can only be dissolved at the concentrations of salt solution. The concentrations were 0.5 mol L⁻¹ - 1.1 mol L⁻¹. In comparison, beef and chicken have high protein content. As the result, the thicker bands were shown on the SDS-PAGE gel.

As we known (table 1), there are differences in protein solubility at the various meat. In addition to pH and salt concentration factors, the genetic factor, gender, muscle type, cattle, environmental factor (nutrition and cattle fodder including additive compound), handling factor before and after cattle cutting are also included in cattle physiology factors that can give an influence on meat chemical compositions. Generally, the compositions can be estimated, as follow: 75% of water, 19% of protein, 2.5% of lipid, 1.2% of carbohydrate, 2.3% of non-protein substance (1.65% of nitrogen substance and 0.65% inorganic substance), and relatively little lipid-soluble and water-soluble vitamins [15]. It was reported that chemical quality of meat could be affected by cutting effect, both before and after cutting. Pre-cutting effect that can affect meat quality is genetic, species, class, additive compound (hormone, antibiotics, and mineral), and state of stress [16].

3.2 Characterization of protein using SDS-PAGE
Protein in the meat that becomes a benchmark to analysis is protein in tendon. Generally, protein in tendon is divided into three parts. The first is water and watery salt soluble-protein (sarcoplasmic protein). The second is concentrated salt soluble-protein (myofibril protein). The last is insoluble protein in concentrated salt at the low temperature [17].

Based on the SDS-PAGE result (figure 1), it was obtained a lots of protein bands in the sample. In this study, sarcoplasmic fraction indicated the separation that was seen clearly at molecular weight of 10 kDa-75 kDa. The range of molecular weight that can be analyzed depends on the concentration of acrylamide gel. The concentration of acrylamide gel utilized on this study was 10% because the sample has low MW. Utilizing of the concentration was served as initiator which can activate acrylamide to react with other polyacrylamide gels forming long polymer chains to form a separating gel and gel
barrier solidifies. The composition of the separating gel and the holding gel was almost the same, except the amount of ingredients to be added. The protein marker used was *Jena Bioscience* which has a MW of 5-250 kDa.

![Electropherogram of meat proteins of the sarcoplasmic fraction](image)

Figure 1. The electropherogram of meat proteins of the sarcoplasmic faction. M: marker, P: pork, C: chicken, B: beef, L: lamb, PB: mixture of pork and beef, PC: mixture of pork and chicken, PL: mixture of pork and lamb.

Based on Sarcoplasmic fraction for pure meat (figure 1), mammal species (P, B, and L). In the sarcoplasmic fraction mixture, differences in intensity of band thickness occur in PB and PL. This showed that the lamb has the thinnest band compared to other samples.

The thickness of the protein band formed in the SDS-PAGE results showed that many proteins have the same MW so that they are in the same band position [18]. It corresponds with the principle of movement of charged molecules that can move freely under the influence of an electrical field. Molecules with the same charge and size will accumulate in the same or adjacent bands.

There were two bands with specific molecular weights in the sarcoplasmic fraction. The presence of protein with a molecular weight of 50.8 kDa was identified in all meat samples except pork. At a molecular weight of 36 kDa, it was not found in chicken and pork, but it was clearly seen in beef and lamb. Sarcoplasmic proteins with molecular weights of 50.8 and 36 kDa were enolase and glyceraldehyde-phosphate dehydrogenase (GAPDH) [19]. However, only protein with MW 50.8 kDa can be used as a reference in distinguishing pork from original meat.

Enolase protein has components of amino acids. They are aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, ammonia, arginine, and tryptophan [20]. Its constituent amino acids are up to 338 monomers [21]. Whereas GAPDH is composed of amino acids such as aspartin, threonine, serine, glutamine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine [22].

4. Conclusion

The difference of protein in the meat is on sarcoplasmic proteins. Protein with molecular weights of 50.8 kDa was not found in pork. Therefore, this molecular weight of the protein can potentially serve as a key for differentiating pork and other meats.
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