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Methods Validation of Pork Authentication in Processed Meat Products (Sausages) Through Densitometry Analysis

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Abstract: *Analysis of the halalness of food products based on animal origin needs to be carried out, especially for critical food products such as processed meat products (sausages). Some processed meat products are found that they are contaminated by pork in the processing. This research was conducted to determine the validity of the analysis method of pork contamination in one of the processed products (beef, pork, and goat sausages) based on the characteristics of the protein profile. Beef, pork, and goat sausages that have been purchased from supermarkets in Jakarta and South Tangerang are used as the sample.The praparation of sample was preceded by extraction of the sausage protein in PBS buffer pH 7.2. Then, the dissolved protein content was tested, and the protein extract was separated by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis). The protein profile result from SDS-PAGE separation was followed by densitometric analysis (ImageJ) to obtain biomarker protein candidates. The validation of the analytical method includes the precision (repeatability), test for accuracy, linearity, and robustness of the method by varying the extraction pH. The results show that the protein extract has the differences in protein content and composition for each sample. The SDS-PAGE analysis results show that biomarker protein candidates appeared below 50 kDa which were thought to be the protein fraction of actin. The precision and accuracy test results obtained for each sample have met the required standards, namely with a KV value <5% and a percent recovery value> 95%. The results of the linearity test and the toughness of the method also show that the test method is quite effective in testing the halalness of animal food products, especially in sausage products which are contaminated by pork.*
Keywords: *densitometric, protein sausage, SDS-PAGE validation method.*

1. Introduction

The needs for halal products have become an important issue in Indonesia which has majority of moeslem population. It is almost 87.18 percent from the total population (BPS, 2010). The supply of halal food is very essential for Indonesian as moeslem majority. One of the food products which halal status is doubtful whether the food products derived from animal sources or not. Based on the prohibition, there are three groups of fresh animal food that are haram named the edible part (especially meat and fat) which comes from pigs, carcasses, and animals that are not slaughtered according to Islamic law. These groups especially carcasses and animals that are not slaughtered according to Islamic law, will be very difficult for ordinary people to recognize, especially if they are mixed with halal meat (Hermanto et al., 2016).

Sausage is an example of animal food which is prefered by Indonesian, especially for the youth generation. These foods are generally made from beef, chicken, or pork. There are cases of mixing or falsifying pork for beef product processing that often occur in the community. This is possible because the price of pork is relatively cheaper than beef. Besides, the weak supervision from related institutions such as the POM still allows business actors to do things that are not desired by acting dishonestly. For example, research in Yogyakarta has found that meatballs are mixed with pork (Erwanto et al., 2014). The same thing also happened in Salatiga city, based on the analysis that from 10 samples of corned beef, there is one sample contains pork positively (Fibriana et al., 2012). The mixed beef with the pork can also occur in another processing food products on the grounds that the

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production costs are more affordable. Based on this, it is need to analyze pork contamination in processed meat products such as beef sausage and goat sausage.

One way to analyze the halalness of animal food products, especially for food products that has undergone mixing through a genomic approach, named PCR (*polymerization chain reaction*). The PCR method is usually carried out using a specific primer that can distinguish the presence of pork derivatives from beef or other animal meat (Rahmawati et al., 2016; Rohman et al., 2017; Rokhim et al., 2021). The basic principle of identifying meat molecules at the DNA level is the amplification of a specific sequence for the corresponding animal genome. Currently, the modern kits for the identification of meat DNA are widely available, but at high price. An amplification of meat DNA can be carried out using specific primers, for example the part of the RYR1 gene that is common in mammals (Popovski et al., 2002).

Another method of testing the halalness of animal products besides the PCR method is through the proteomic approach which is done by comparing the protein composition of each sample, both pure and mixed samples. Research conducted by Susanto (2005) states that the identification of mixed beef with pork can be done by identifying the characteristics of the protein fraction in each sample both fresh and after boiling and the characteristics of the protein fraction at several levels of mixing meat pork into the sample. The results of this study indicated that fresh pork contained unknown protein with a molecular weight of 112.13 KDa which was not found in fresh beef samples. Heating at 90°C for 15 minutes caused a decrease in the thickness of protein bands in each sample. The specific difference in beef samples is the presence of troponin T protein which is presented in large quantities, while the mixing level of pork in beef meatballs is 25, 50, and 100 %.

Another study conducted by Susanti (2019) showed that mutton, beef, buffalo, free-range chicken and chicken that had been treated with immersion in papaya leaves showed different results compared to controls named all meats had many minor protein bands. Meanwhile, there are only 6 to 9 protein bands in the major band. Thus, the mixing of pork in the beef sample can be seen from the thickness of the protein bands that appear, including troponin T, which decreases with an increase in the amount of added pork. However, this method is not effective enough to analyze pork contamination quantitatively which the protein bands are contained. They also are still relatively biased, so it is difficult to quantify, especially for small samples and samples that have gone through the heating process (Hermanto & Meutia, 2009). For this reason, it is need to validate the analysis method of pig contamination so that the protein bands that appear can be better strength.

In this study, the used of beef, pork, and goat sausages and their mixtures were extracted in phosphate buffer with varying pH. Then, this extraction results were identified the protein profile by SDS-PAGE and carried out densitometric analysis. From the results of this electrophoresis, the specific biomarker protein candidates will be raised and might be used for method validation purposes. The protein profile of biomarkers were carried out based on the bands that appeared and were converted into Rf and peak area values. The protein bands with specific Rf values are used as protein biomarkers and the ribbon density is quantified with the help of densitometry applications (ImageJ). The calculation of precision is carried out through repeated testing (repeatability) by using the same sample concentration. The accuracy value is determined by using the addition standard method (addition of positive control). The results of this validation are expected to provide a comprehensive picture of the precision and accuracy of the method of measuring pork contamination in sausage products through electrophoresis techniques and densitometric analysis.

2. Materials and Methods

In this study, the materials used are Mini-Protean Gel Electrophoresis (Biorad), power supply (200 Volt), homogenizer (Yooning), stainless steel cutter blades, digital cameras, microcentrifuge (Sorvall), Eppendorf tube, SS34 high centrifuge (Sorvall), micropipettes (1, 10, 100 and 1000 µL), ImageJ software for densitometric analysis, and dialysis cassettes for dialysis samples.

The sample of this research was beef and goat sausage samples that bought from supermarkets and local markets in Jakarta. The pork sausages are bought from supermarkets BSD area (Tangerang), PBS buffer (phosphate buffer saline) from Sigma Aldrich; acrylamide and bis-acrylamide; SDS (sodium dodecyl sulphate (Merck)); ammonium persulfate (APS) Merk; TEMED (N,N,N',N'-tetra methyl ethylene diamine) Sigma Aldrich; sodium chloride 0.5 M (Merck); tris hydrogen chloride buffer 1.5 M pH 8.8 (Biorad); tris buffer hydrogen chloride 0.1 M pH 6.8 (Biorad); Coomassie brilliant blue R250 (Biorad); glacial acetic acid (Merck); methanol (Merck).

2.1. Sampling

The sampling technique used is purposive sampling technique. Each sample is taken from the population that has the same representation to be used as the test sample. Sausage samples consisted of beef, pork, and goat sausages. Beef and goat sausages are bought from supermarkets and traditional markets in South Jakarta area. Meanwhile, pork sausages are bought from supermarkets in BSD Tangerang area. Before conducting the test, the sample is stored at -20°C to avoid damage to the sample.

2.2. Protein Extraction and Purification

Sausage sample (10 g of beef, pork, and goat) was separated from their casings and sliced into small pieces. The sample was added with 50 mL of PBS buffer (phosphate buffer saline) 0.01 M containing 0.5 M NaCl with a pH of 7.2. Next the samples were blended for + 5 minutes at 4°C. The sample was homogenized with a homogenizer for two minutes at speed of 11,000 rpm. The samples were stored at 4°C for two hours and the centrifuged at 5000 rpm at 4°C for 30 minutes. The separated supernatant was filtered with Whatman No. filter paper. 1 (125 mm diameter), aliquots were inserted into the dialysis cassette 5 mL MWCO <1 kDa. The aliquots were dialyzed for 1 × 24 hours using distilled water until the aliquots were free of salt ions. The protein content of each sample was determined by the Bradford method (Hermanto & Meutia, 2009).

2.3. Measurement of Protein Content

Protein content determination was carried out according to the BCA (Bicinchoninate Assay) method. BSA standard solution (Bovine Serum Albumin) was used as standard. Sample preparation and standard solutions were carried out using BCA reagents consisting of Reagent A: sodium bicinchoninate (0.1 g), $Na_2CO_3 \cdot H_2O$ (2.0 g), sodium tartrate (dihydrate) (0.16 g), NaOH (0.4 g), $NaHCO_3$ (0.95 g), dissolved in 100 mL distilled water and adjusted the pH to 11.25 with the addition of $NaHCO_3$ or NaOH. Reagent B: $CuSO_4 \cdot 5H_2O$ (0.4 g) in 10 mL of distilled water. The working solution is made by mixture of reagent A and B with a ratio (100: 2) which will form a green complex compound that can be read at a wavelength of 562 nm (Smith et al., 1985).

2.4. Analysis of Protein profile using SDS-PAGE

Protein extract were separated by protein profiles using SDS-PAGE electrophoresis using the Laemmli method (Laemmli, 1970). 10% resolving gel and 4% stacking gel solutions were prepared in a buffer solution of tris HCl 1.5 M, pH 8.45. The samples were denatured using a sample buffer (Coomassie brilliant blue 1%, glycerol 25%, tris-HCl 1M pH 6.8, SDS 20% and β-mercaptoethanol) with a buffer and protein ratio of 1: 2. Furthermore, it is boiled at 90°C for 10 minutes and centrifuged for 5 minutes. The electrophoresis device was prepared with resolving buffer (tris-HCl 1.5 M, pH 8.8), stacking buffer (tris-HCl 0.5 M, pH 6.8), bis-acrylamide solution (1.5%), and acrylamide (48%). Electrophoresis was carried out for 60 minutes at 150 volts with protein marker as a comparison (Range 1.06-26.6 kDa). Protein staining used 0.1% (w/v) coomassie brilliant blue solution. The results of the staining were washed by using the distaining solution (7.5% acetic acid and 40% methanol). The proteins that have been tested were captured by using digital camera. The protein profiles from SDS-PAGE electrophoresis were further analyzed to determine the Rf value of each protein band by densitometric analysis by using ImageJ software. Then it was analyzed quantitatively by determining the thickness of the protein band (intensity), volume, and retention time. The analysis results were compared for each type of sample using ImageJ 7.0 software application.

2.5. Method Validation (Precision, Accuracy, Linearity, and Method Strength Test)

2.5.1. Precision Test

The precision test is carried out by repeated measurements (repeatability) by using a sample of pork sausage that is as same as the protein profile which had been analyzed previously and the biomarker protein is determined to be used as the target in calculation of the precision test. The biomarker protein bands were quantified by using densitometric analysis by calculating the Rf value and the peak area then calculating the standard deviation of each using the equation (Harmita, 2004):

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(X_1 - \bar{X}}{n-1}} \quad (1)$$

$$KV(\%) = \frac{SD}{\bar{X}} \times 100\% \quad (2)$$

SD = standard deviation of measurement results
KV = relative standard deviation

2.5.2. Accuracy Test (Percent Recovery)

The calculation of percent recovery was carried out by the addition standard method which the protein hydrolysate of pork sausage samples containing protein biomarkers were tested separately in a pure state. Then re-tested after being mixed into the hydrolysate samples of beef and goat sausages. Then the result of biomarker protein bands are calculated for their respective areas and entered into the equation (Harmita, 2004):

$$\%\ Recovery = \frac{Area\ of\ pure\ biomarker}{Area\ of\ mix\ biomarker} \times 100\% \qquad (3)$$

2.5.3. Linearity Test (Blending)

The hydrolyzate samples were mixed with different variations of hydrolyzate concentrations. The mixing was carried out at protein concentration ratio of sausage: 50% beef: 50% pork, 60% beef: 40% pork, 70% beef: 30% pork, 80% beef: 20% pork, 90% beef: 10% pork. Furthermore, each hydrolyzate mixture was tested again for its protein band with SDS-PAGE followed by densitometric analysis. The specific protein bands were quantitatively calculated and compared for all treatments. Furthermore, the linearity of the measurement results is calculated by using the linear regression equation (y = mx + b) by plotting the concentration value (x) against the peak area value of the target protein (y) (Harmita, 2004).

2.5.4. Robustness

The validation of the strength of the method was carried out by testing changes in the hydrolysis conditions of the sample by varying the pH of the extraction using phosphate buffer pH 6.5 and pH 8. The extraction results at each pH were compared with the extraction results at an initial pH of 7.2. They were separated by using SDS-PAGE. The resulting specific protein bands were compared quantitatively with the R_f value and the area by taking into account the occurrence of significant changes (Harmita, 2004).

3. Results and Discussion

3.1. Sausage Protein Extract

The extraction of sausages protein was carried out by using the homogenization method carried out at low temperature. It is intended that the resulting protein is not degraded by protease enzyme activity or oxidized due to too high temperature. It was carried out at neutral pH conditions (7.4) by using 0.05 M phosphate buffer. The muscle tissue protein usually can be extracted at higher pH, because it is possible that the effect of pH can increase the gelatinization process of myofibril proteins such as actin and myosin. The results of protein extraction were then measured for protein levels using the BCA method (Smith et al., 1985), where the protein extract solution was reacted with the BCA reagent (Bichinconinic acid in Alkaline Conditions) to produce a green compound complex which could be measured in the pada 562 nm area (Table 1).

Table 1. Concentration of sausages protein

Sausages	Concentration (µg/mL)
Beef[1]	2115.61
Beef[2]	2446.19
Goat[1]	617.875
Goat[2]	752.69
Pork[1a]	1487.71
Pork[2a]	1541.27
Pork[1b]	1864.45
Pork[2b]	1892.15

Based on the measurement results of protein content in each sausage sample, the concentration range was obtained between 617,875 - 2446.19 µg/mL. The highest protein content was obtained in beef sausage samples while the lowest protein content was obtained in goat sausage samples. The differences in protein content may occur due to differences in the type of meat and treatment for each sample. In addition, the solubility of each protein is different, and it is influenced strongly by the protein composition of myofibrils in each sample. This greatly affects the gelatinization process which causes the protein is difficult to be dissolved and will form aggregates that are easier to settle.

Based on the two myofibril proteins, myosin and actin contribute the most to the formation of the desired gel characteristics in processed meat products. Heat-induced myosin gelation results in the formation of 3-dimensional tissue structure that holds water in a less mobile area. (Listrat et al., 2016)

have suggested that the rheology and physical properties of globular protein gels depend on molecular size and are slightly influenced by amino acid composition and distribution, and this may also apply to fibrillar proteins. During tissue formation, fat and water retention is enhanced and this will affect the texture and cohesion of the final product as well as determine the gelling capacity of myofibril protein (Maqsood et al., 2018).

The protein concentration in the beef sausage sample is greater than the protein concentration of pork and goat sausages. However, the difference in total protein concentration between beef and pork is not significantly different. The difference in these concentrations is thought to be due to the difference in muscle tissue taken when making the sausages. Muscle tissue taken in cattle is the hamstrings and in pigs taken is the back. In addition, it is also suspected that the treatment at the time of protein extraction was not optimal. Santoni et al. (2000) said that the destruction of meat aims to break down the cell walls of muscle fibers so the protein can be extracted with salt solution. Friction with the grinding tool can result in inhibition of protein extraction resulting in protein coagulation (Listrat et al., 2016). Thus, heating, and drying treatments can result protein denaturation. It makes the total dissolved protein concentration is lower (Wahniyathi & Ali, 2005).

3.2. Protein Profile from Electrophoresis SDS-PAGE

Electrophoresis is performed to separate the protein components in each extract and identify possibility of biomarker proteins. Biomarker protein is a specific protein that can only be found in pork sausage samples that are not found in beef or goat sausages. This biomarker protein will later be used in the validation of the pork contamination analysis method through densitometric analysis. SDS-PAGE electrophoresis was carried out in a gel concentration of 14% in a resolving buffer pH 8.5. The result of electrophoresis produces ribbons of proteins as shown in Figure 1. Based on the results of the separation of protein bands, in all sausage samples the same protein band appears in the 50 kDa range which is thought to be the actin protein fraction and 2 other protein bands in the 220 kDa and 150 kDa ranges which are thought to be myosin heavy chain proteins (myosin heavy chain/MHC). The composition and intensity of protein in each sample were strongly influenced by the type of meat and the treatment during the sausage processing.

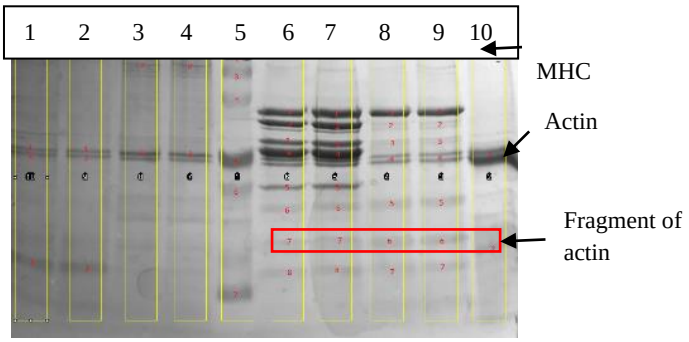

Figure 1. Protein profile of the extracted sausage sample in phosphate buffer pH 7.4. (1-2: beef sausage, 3-4: goat sausage, 5: protein marker, 6-7: pork sausage A, 8-9: pork sausage B, 10: control pork sausage.

Water-soluble protein consists of myoglobin and enzymes that play the role in metabolic processes of muscle cells. This protein is separated easily by extraction with a weak salt solution (ionic strength <0.1). Myosin is a muscle protein with the largest amount, and it is an asymmetric molecule with a molecular weight of about 500 kDa, with a α-helic content of 60-70%. Myosin can be separated by ultracentrifugation into two sub-units, heavy meromyosin (220 kDa) and mild meromyosin (20 kDa). Another fibril protein, actin comes in two forms, the first is being a monomer called actin-G with a molecular weight of 47 kDa and actin-F (fibrous) with more molecular weight. Actin units combine to form a double helix of indeterminate length. Actomyosin is an actin-F complex with myosin, and it is responsible for the contraction and relaxation of muscles. Other microfilaments are found in zone H. There are tropomyosin and troponin which consist of three types of molecules, troponin I, C and T. The presence of tropomyosin and troponin in muscle cells play the role in the binding process of myosin (Listrat et al., 2016).

3.3. Biomarker Protein Identification

The results of the SDS-PAGE electrophoresis process optimization can be seen in Figure 2. From this figure, by varying the hydrolysis time and extraction pH, several bands with specific molecular weights are obtained.

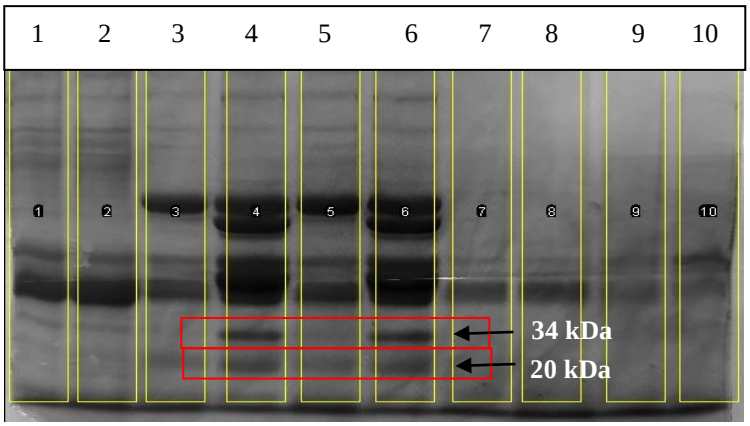

Figure 2. SDS-PAGE electrophoresis optimization results (1-2: control pork protein (pH 6.5), 3 and 5 commercial pork sausages (pH 6.5), 4 and 6: commercial pork sausage (pH 8), 7-8: sausage commercial beef, 9-10: commercial goat sausage

Based on Figure 2, the pork sausage protein as a whole produce band with relatively different intensities, where in the hydrolyzed protein extract shows that there are two specific protein bands that appear around 34 kDa and below 20 kDa. In protein extract, it was extracted from beef sausage and goat sausage. There is no protein that were contained from both of beef and goat. However, there were other protein bands that appeared above 34 kDa. The two specific proteins that appeared with a relatively thick intensity in pork sausage were obtained under the conditions of separation by using Tris HCl pH 8 as buffer. Both proteins can be used as biomarker protein candidates for the validation process used of the analysis method of pork contamination in beef and goat sausage. The SDS-PAGE results on the gel were carried out to determine the molecular weight of each protein band (Table 2) using a straight-line equation obtained from the standard curve of comparator molecular weight (protein marker) from Bio-Rad.

Table 2. Molecular weight determination

R_f	Log MW	MW
54	2.325259034	211.475
139	2.074007784	118.579
211	1.897599603	78.995
430	1.724644454	53.045
626	1.566802688	36.881
772	1.457018503	28.643
1031	1.250639534	17.809

SDS-PAGE gel results showed the presence of 25 protein bands. Among the 25 protein bands are formed, there are the bands that look thicker than other proteins. This is explained by Alonso Villela et al. (2020) that the thickness of the recorded protein bands is an illustration of the amount of protein contained in each sample.

3.4. Validation of Analytical Methods

3.4.1. Precision Test

The precision test is carried out by repeated measurements (reparability) by using pork sausage samples with the same concentration and the biomarker protein is determined which will be used as a target in the calculation of the precision test. The biomarker protein bands were quantified by using densitometric analysis by calculating the peak area values. The results of the precision test can be seen in Figure 3.

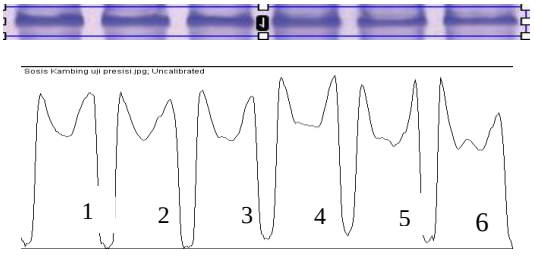

Figure 3. Peak elektrophoregram of biomarker (ImageJ)

From Figure 3, the area measurement is then carried out using the ImageJ application and the area values are obtained as listed in Table 3.

Table 3. Precision test

Peak	Area
1	20341.832
2	20372.711
3	18922.418
4	20613.054
5	18807.489
6	19513.439
SD	788.826667
Average	19761.82383
KV	3.99166936

From the calculation of the standard deviation value, the relative standard deviation is 3.99% (KV> 2%). It indicates that the precision test results exceed the required threshold value. However, for the test results, the relative standard deviation value (% RSD obtained is less than 5 percent. The precision value really depends on the accuracy factor in conducting sample preparation and pipetting the sample into the gel well which causes the reproducibility of the measurement results to be difrom one t. The similarity is measured as standard deviation or relative standard deviation (coefficient of variation). The similarity can be expressed as repeatability or reproducibility. The repeatability is the accuracy method if it is repeated by the same analyst under the same conditions at short time intervals. The repeatability is assessed through the implementation of complete separate assignment to separate identical samples from the same batch, thus providing a measure of similarity under normal conditions (Harmita, 2004).

The similarity experiments are carried out on at least six sample replicas which are taken from the sample mixture with a homogeneous matrix. It is better if the similarity is determined for the actual sample, which is in the form of a mixture with the pharmaceutical preparation material (placebo) to see the effect of the carrier matrix on this similarity. Careful criteria are given if the method provides a relative standard deviation or a coefficient of variation ohe coefficient of variation increases with decreasing levels of the analyte result. It was found that the coefficient of variation increased with decreasing concentrations of analytes. At levels of 1% or more, the relative standard deviation between laboratories is about 2.5 percent while one per thousand is 5 percent. At the level of one per million (ppm) the RSD is 16 percent, and at the level of per billion (ppb) is 32 percent (Harmita, 2004).

3.4.2. Accuracy Test

The calculation of recovery percent was carried out by the addition standard method which the pork sausage protein hydrolyzate samples containing protein biomarkers were tested separately in a pure state and then re-tested after being mixed into the hydrolyzate samples of beef and goat sausages. The results of the accuracy test can be seen in Table 4.

Table 4. Accuracy test

Peak area (pork)	Peak area (mix of sample)	Persen recovery (%)
18281.26	18765.53	97.4194
19145.12	19871.89	96.3427

The results of the calculation of % recovery above, show that the results of the accuracy test generated the percent recovery value is above 95 percent. This result shows that this analytical method is quite accurate in measuring pork contamination in beef and goat sausage samples. When it was compared with Sarno et al. (2020) that the accuracy of measuring pork contamination in halal beef using the optimized Electronic Nose System method results an accuracy value. It was 93.10 percent. It shows that the relatively of it is better than what they get. However, for ensuring this result, it is need to retest the reliability of this method. It is expected to be more accurate. The accuracy of the results of the analysis is highly dependent on the distribution of systematic errors in all stages of the analysis. Therefore, for achiving high accuracy, it can be done by reducing the systematic error such as using calibrated equipment, using good reagents and solvents, controlling temperature, and implementing it carefully, adhering to the principles of following procedures.

3.4.3. Linearity Test (Blending)

The hydrolysate samples were mixed with different concentrations including for protein 1 (34 kDa) and protein 2 (20 kDa). The mixing was carried out at a protein concentration ratio of sausage: 50% beef: 50% pork, 60% beef: 40% pork, 70% beef: 30% pork, 80% beef: 20% pork, 90% beef: 10% pork (Table 5). Furthermore, each hydrolysate mixture was analyzed for its protein band by SDS-PAGE followed by densitometric analysis. Peak area of specific protein bands was quantitatively calculated and compared for all treatments. The results of the linearity test can be seen in Table 5.

Table 5. Linearity test

Protein1 (beef/pork)	Peak Area	Protein 2 (beef/pork)	Peak area
90/10	7665	90/10	5929
80/20	9355	80/20	8715
70/30	10572	70/30	11715
60/40	13101	60/40	12568
50/50	15839	50/50	14302

From the calculation of the average OD value, a regression curve is arranged to determine the linearity value. The linear regression curve can be seen in Figure 4.

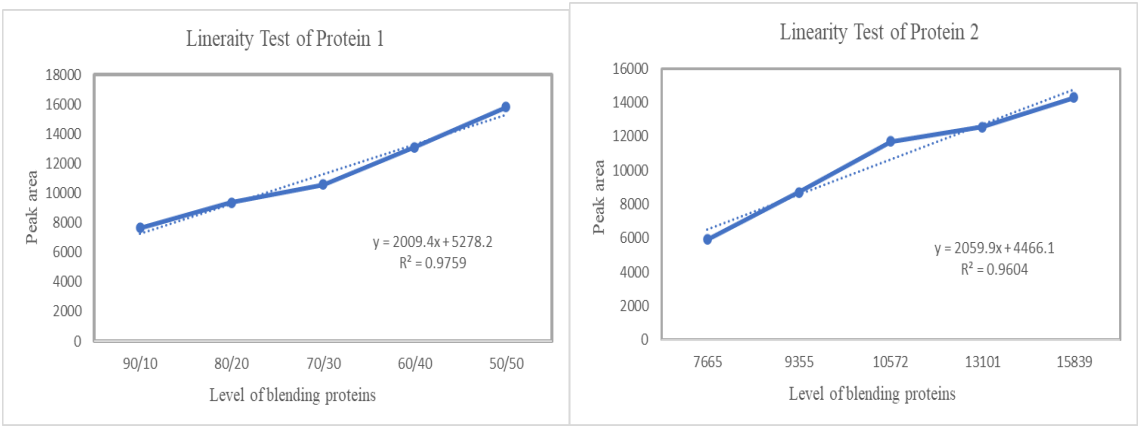

Figure 4. The results of the linearity calculation (blending) of protein 1 and protein 2

Based on the results of linearity, this method gives a good response, with the correlation coefficient (r^2) is 0.9759 (protein 1) and is 0.9604 (protein 2) which shows that the signal response generated is quite proportional for increasing the sample concentration. From the various variables of the verification test method, the linearity value meets the standards is obtained. In another word, this method is good enough in detecting contamination of pork in processed food products such as beef sausage products that have been tested.

3.4.4. Robustness Test Method

The robustness test method was done by testing the changes in the extraction conditions of the sample by varying the extraction pH by using phosphate buffer pH 6.5, 7.2, and pH 8. The extraction results of each pH were compared with the extraction results at the initial pH (7.2). The protein bands were observed, and their density were compared after separation by SDS-PAGE. The specific protein bands were compared to the Rf value and their area quantitatively by observing a significant change. The results of the effectiveness method test can be seen in Figure 5.

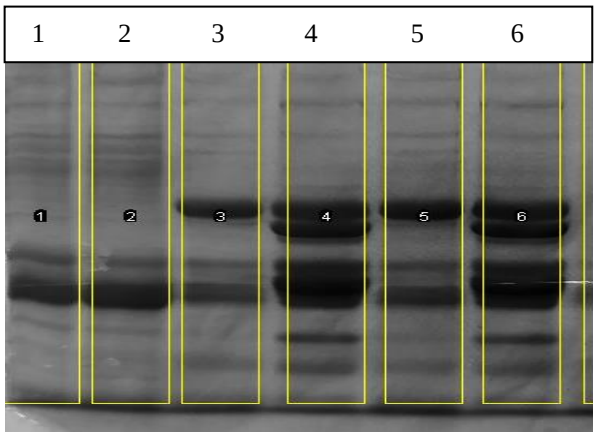

Figure 5. The results of the method strength test on pork sausage protein extract with a pH range of 6.5 (1-2), pH 7.2 (3 and 5) and pH 8 (4 and 6)

Based on the robustness test results, the difference in extraction pH did not have a significant effect on the Rf value but it did not have a significant effect on the thickness of the resulting protein bands. It indicates that this method is highly unstable by deliberate pH changes which were madeby using the parameters of the analytical method. The effectiveness test provides an indication of the reliability of a method in normal used. The stability of the observed values can be tested by changing several analysis conditions such as pH of the solution, reaction temperature, reaction time or the addition of reagents. When the observed value is unstable, the analysis procedure should be corrected. The system suitability tests were done to ensure whether a system is running properly and correctly or not. It was also to ensure that the systems and procedures used have to provide the correct data.

From all the method validation, it can be concluded that the halal authentication method of sausages food products based on the protein extract profile in processed meat has provide a quite valid and acceptable data based on the validity test parameters that are sufficient to meet the predetermined requirements except for robustness test. However, to ensure that this test method is reliable enough, it still needs further analysis by testing the suitability of the system under different conditions.

4. Conclusion

The validation of pork authentication in processed meat products (sausages) brings a significant result which the level of precision and accuracy that have met the required standards, with a KV value <5% and an accuracy value more than 95%. The linearity test results also show that the test method used was prospectively in testing the halalness of meat products-based on animal origin, especially on sausage products that are contaminated by pork. These results provide an alternative choice of reliable methods for finding pork contamination in sausage products and other processed meat products future.

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