Optimization of Protein Isolation Technique on Pig Hair

Yatri Drastini[1], Sumantri[1], Nur Indah Kumalaratri[1], Agata Bella Lakshmita[1], Tridjoko Wisnu Murti[2]
[1]The Assessment Institute for Foods, Drugs and Cosmetics, the Indonesian Council of Ulama (AIDC ICU/LPPOM MUI)-Yogyakarta, Jl. Kapas 3 Yogyakarta 55156, Indonesia
[2]Faculty of Animal Science, Universitas Gadjah Mada
Corresponding email: drastini@ugm.ac.id

ABSTRACT

In the earlier research, the technique of protein isolation on pig hair took some weeks. The aim of the research was optimizing of the technique of the protein isolation on pig hair, so the protein could be used directly for detection of pig species. Samples were pig hairs and paintbrush. The protein isolation technique was optimized by cutting the hair in smaller pieces, using SDS in 2% and 3%, H_2SO_4 in 5% and 10%, and increasing sum of NH_4HCO_3 50%. The concentration of protein product was analyzed by Kjeldahl method, while pig species was detected by pork Xema kit. The result showed the optimum technique used SDS 2%, H_2SO_4 10%, and NH_4HCO_3 50%. Protein of pig hair were 16.30% b/v of uncentrifuged protein, 18.02 % b/v centrifuged protein (top part), 17.16% b/v entrifuged protein (bottom part). Protein of painbrush: 14.18 % b/v uncentrifuged protein; 29.70 uncentrifuged protein (top part), 35.72% centrifuged protein (bottom part). The samples of pig hair and the brush were detected positively by pork Xema test.

Keywords: Optimization, Protein isolation, Pig hair, Pork Xema test.

INTRODUCTION

Pig hair can be used for brushes. In February 2017, Malaysia seized 2,000 brushes from pig hair labeled halal (Kompas.com, 2017). In Indonesia, August 2002, the Republika Daily reported on the findings of the AIDC ICU on brush labelled "Bristles" (pig hairs) at bakery industries. Some auditors in AIDC ICU Yogyakarta also often found the use of hair brush in bakery industries. Determination of brushes made of hair or plastic/synthetic is done by burning some brush hairs. The hair brush will give a distinctive smell of burn hair comparing to that of plastic or synthetic brush. However, the hair have not been proven whether as pig hair or other animal hair. This phenomenon made a problem for Indonesian Muslim societies and encouraged researchers to conduct preliminary research on hair protein isolation (Drastini *et al.*, 2016; Drastini *et al.*, 2017).

Isolation was performed on proteins, but not DNA normally used in the Polymerase Chain Reaction (PCR) method to distinguish animal species. The DNA of hair rests on the root of the hair, while the hair of brush is the end of hair without roots. The end of the hair contains relatively protein. Based on protein, one of the rapid techniques to detect animal species is Xema

Pork Test. Xema Pork Test is used to detect fat tissue connective tissue, internal organs and pig blood in foods, cosmetics and others. Good antigens (antigenic) must be large, rigid and chemically complex molecules (Tizard, 1987). Protein is a macromolecule with a complex structure, so protein is a much better antigen than nucleic acid, fat, carbohydrate, or polymer of one amino acid. Isolation of protein from pig hair has not been reported. The specific purpose of this study was to find a hair protein isolation technique that can produce a relatively large amount of protein as a base material in further research. Initial research that researchers have done is isolating pig hair proteins using a modified method of techniques performed by Lee *et al.* (2006) by replacing the chemical ingredients ((Drastini *et al.,* 2016).

MATERIALS AND METHODS

Sampling

Samples were pig hair for optimizing isolation of protein, hair of brush, and pork as a positive control for Xema test.

Methods

Research were isolation of protein, a qualitative test of protein with ninhydrin, a quantitative test of protein by Kjeldahl method, and testing of hair species with Xema Test. Optimization of protein isolation is done by changing the concentration or volume of SDS, H2SO4, NH4HCO3 (Table 1). The protein isolation was a modification of protein isolation techniques by Lee *et al.* (2016).

Isolation of protein of hairs. Four methods of isolation of protein of pig hairs were performed to get the best result (Table. 1). First, two samples of 0.2 g hair were soaked in 25 ml of Sodium Dedocyl Sulphate (SDS) 2% and 3% for 30 minutes, and crushed in the mortar. The mixture was transferred to Erlenmeyer tube through a filtre, then added with 25 ml of Phosphate Buffer Saline (PBS) pH 7.8, and incubated at 65 °C for 18 hours. Erlenmeyer was taken from the incubator, then the solution was homogenized with a stirrer at room temperature. The extract was taken, then measured its' volume, and poured into a new Erlenmeyer. The extract was digested with 5 ml H2SO4 5%.

Table 1. Some methods of isolation protein of pig hairs

Isolation	Methods			
	I	II	III	IV
Extraction	Hair was not cut 2% and 3% SDS SDS was filtered	Hair was cut 2% and 3% SDS SDS was not filtered	Hair was cut 2% SDS SDS was not filtered	Hair was cut 2% SDS SDS was not filtered
Digestion	5ml H2SO4 5% 15ml NH4HCO3 50%	5ml H2SO4 5% 40ml NH4HCO3 50%	15ml H2SO4 10% 40ml NH4HCO3 50%	H2SO4 10% (1:1) 50ml NH4HCO3 50%
Centrifuge	No	No	No	Yes

The solution was then heated in waterbath at 40 ^{o}C for 1 hour and shaked every 5 minutes to optimize H2SO4 work. The remaining H2SO4 was removed by 15 ml NH4HCO3 50%

(Drastini *et al.*, 2017). Second, the method was similar to the first, but the hair was cut in pieces as small as possible, SDS was not filtered, and the volume of NH_4HCO_3 50% was increased to be 40 ml (Table 1). Third, the method was similar to the second, but it used only 2% SDS, and concentration and volume of H_2SO_4 were increased to be 15ml H_2SO_4 10%. The last method was similar to the third, but H_2SO_4 with proportional volume to the extract volume (H_2SO_4: extract = 1: 1) and increasing in the volume of NH_4HCO_3 50% (Table 1).

Qualitative test of protein with ninhydrin. The presence of hair protein in the extract was tested by addition of 3 -5 drops of 5% ninhydrin into 5 ml of isolated protein extract. Positive results are shown by purple ring formation between 2 layers (Rohman and Sumantri, 2007).

A quantitative test of protein by Kjeldahl method. Protein concentrations of hair were calculated by the Kjeldahl method consisting of 3 stages of the process namely destruction, distillation, and titration (Rohman and Sumantri, 2007). Destruction was performed by 5 ml of hair protein extract added 2.5 g of anhydrous Na_2SO_4, 5 g of $CuSO_4.5H_2O$, and 10 ml of concentrated H_2SO_4. The mixed solution was heated at 350 to 400 ^oC in a heating mantle for 2 hours until the solution was clear.

The destruction result was poured into a boiled tube of distillation, then added 3 drops of phenolphthalein indicator, 2 boiling stones, 50% NaOH up to over base, and 200 ml of distilled water. The distillate was then bond with 25 ml of 0.1 N HCl and heated in boiled tube until the distillate got to be 150 ml.

A total of 50 ml of distillate was then titrated with 0.05 N NaOH being standardized. The process was repeated up to 3 times. Blanko was made as well as the treatment without the sample. N levels were calculated by the formula:

$$\% N = \frac{[(\text{ml NaOH blanko X 5}) - (\text{ml NaOH sample X 5})] \text{ X } 14{,}008 \text{ X N NaOH X Fp}}{\text{g sample X 1000}}$$

Detection of hair species with Xema Kit. The protein extracts in which positive results by the ninhydrin test were detected for pig species with Xema Porcine Detection Kit. Xema paper was immersed in a hair protein extract for 5 to 10 seconds, until the extract was absorbed into all parts of Xema's white paper. Then, the Xema paper was placed on a flat spot and held for 10 minutes. The test results were positive for pig species, if two colored lines on Xema paper were formed (Anonimus, 2016).

Data Analysis

Qualitative and quantitative data were analyzed descriptively.

RESULTS AND DISCUSSION

All samples were positive on ninhydrin test. It mean the extract consisted of protein (Table 2). Method-I using 2% or 3% SDS (Table 1) gave negative results on the Xema test (Table 2, Figure 1). This was probably due to the hair was not cut into small pieces. Hair is coated by a thick cuticle. If the hair is not cut into small pieces, then the SDS can not work optimum. According to Tan and Yiap (2009) SDS serves to dissolve the protein membrane and lyse the cell.

Method-II (Table 1) was done by preparing the cut hair as small as possible, but the result of Xema test remained negative (Table 2). The experiment was continued by increasing the concentration and volume of H_2SO_4 which served to digesti protein (nitrogen decomposition)

(Wirahadikusumah, 1989), but Xema test results remained negative (Table 2). In Method IV, volume of H2SO4 was increased according to extract volume (1: 1), and finally Xema test results showed positive (Table 2, Figure 1). The results of Kjeldahl on the extract showed the amount of protein were 16.30% w / v in uncentrifuged extract, 18.02% w / v in the centrifuged extract (top layer), and 17.16% w / v in the centrifuged extract (bottom layer) (Table 2).

Table 2. Result of ninhydrin, xema and Kjeldahl tests on pig hairs

Methods	Explanations	Tests			Average	Sdev
		Ninhydrin	Xema	Kjeldahl (% b/v)		
	Pig hair samples:					
I	Added 2% SDS, no hair	Positive	Negative	No treatment		
I	cut, etc	Positive	Negative	No treatment		
II	Added 3% SDS, no hair	Positive	Negative	No treatment		
II	cut, etc	Positive	Negative	No treatment		
III	Added 2% SDS, hair	Positive	Negative	No treatment		
IV	cut, etc	Positive	Positive	16.30, 18.02,	17.16	0.86
	Added 3% SDS, hair			17.16*		
IV	cut, etc	Positive	Positive		26.98	10.22
	Added 2% SDS, etc	Positive	Positive	15.74, 29.47,	26.36	12.55
	Added 2% SDS, etc	Positive	Positive	35.72*	26.33	10.69
	Paintbrushes:			12.10, 31.26,		
	1.Added 2% SDS, etc			35.72*		
	2.Added 2% SDS, etc			14.70,		
	3.Added 2% SDS, etc			28.58,35.72*		
	Average			14.18, 29.70,		
				35.72		

*volume of not centrifuged extract, centrifuged extract (top layer), and centrifuged extract (bottom layer), respectively.

Optimization technique of protein isolation of pig hairs is modificated from Lee *et al.* (2016) method. The methods used 2% SDS, 50 mM sodium phosphate (pH 7.8), 20 mM DTE, and digestion with 10% sulfuric acid. Our research was more simple and gave a total protein extracted (17.16) in one experiment, while Lee method comprised 13.3 ± 3.9% of the total protein in six experiments. Even, the average of total protein of paintbrushes had around 26 % b/v. It mean the method-IV is the optimum method for hair protein isolation. Comparing between uncentrifuged extract and centrifuged extract, the centrifuged extracts (29.70 or 35.72) had more total protein than the uncentrifuged extracts (14.18) (Table 2).

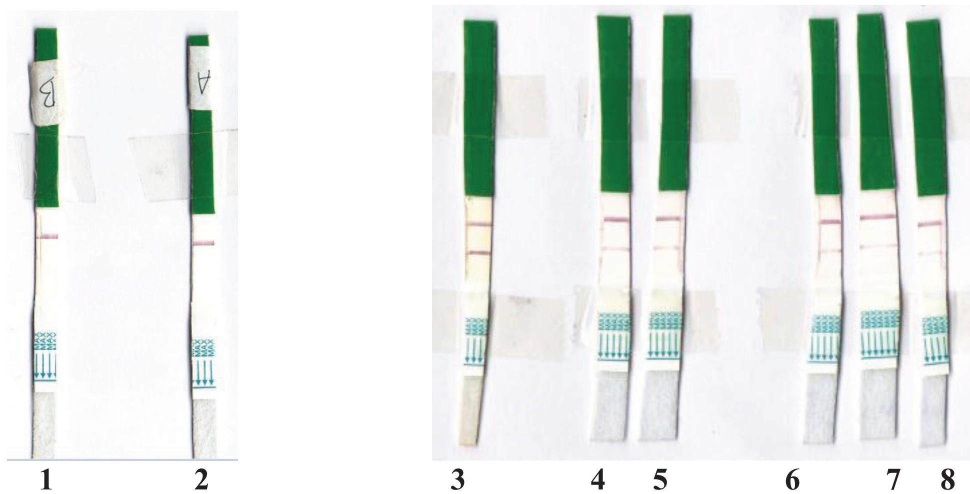

Figure 1. Results of Xema tests on protein producted with methods I/IV
1-2 = negative, method-I; 3-8 = positive, mrthod-IV
3=pork, 4,5=pig hair, uncentrifuged; 6, 7=pig hair, centrifugated (top)
8 =pig hair, centrifugated (bottom)

CONCLUSIONS

The best technique of isolation of protein in pig hairs of four methods was using SDS 2%, H_2SO_4 10% (H_2SO_4: extract = 1:1), and NH_4HCO_3 50% (method-IV). Total protein extracted of pig hair or paintbrush was relatively a lot, and the protein was distributed wether in top or bottom layer of the extract centrifugated. Application of protein isolation on paintbrushes showed the method produced 14.18 % b/v protein of uncentrifuged extract; 29.70 % b/v that of centrifuged protein (top part), 35.72 % b/v that of centrifuged protein (bottom part). The samples of pig hair and the brush were detected positively by pork Xema test.

REFERENCES

Anonimus. 2016. Xema Test Pork Fat/Blood. *www.xematest.com* (22 Desember 2016).

Drastini, Y., Sumantri, C.Y., Admantin, and T.W., Murti. 2016. New Technique to Detect Pig Hair by Immunochromatographic Rapid Test. Proceeding of The 6 th ISTAP*,* Yogyakarta, Indonesia.

Lee, Y.J., R.H. Rice, and Y.M. Lee. 2006. Protome Analysis of Human Hair Shaft from Protein Identification to Posttranslational Modification. The American Society for Biochemistry and Molecular Biology, Inc. Mol. Cell Proteomics. 5(5): 789-800.

Rohman, A., and Sumantri. 2007. *Analisis Makanan*. UGM Press, Yogyakarta.

Tan, S.C. and B.C., Yiap. 2009. Review article DNA, RNA, and Protein Extraction: The Pastand the Present. Journal of Biomedicine and Biotechnology. IDE 574398.

Tizard, I. 1988. Pengantar Imunologi Veteriner. Airlangga University Press.

Wirahadikusumah, M. 1989. Biokimia Protein, Enzim, da Asam Nukleat. Penerbit ITB. Bandung.