Purification of polyclonal antibody against pork extracts antigens using Protein A Column as material for developing halal food detection kit

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Purification of polyclonal antibody against pork extracts antigens using Protein A Column as material for developing halal food detection kit

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Abstract. The main objective of this study was to purify polyclonal antibodies against pork extract as raw material for developing diagnostic kits for halal food made from animal products. Antibodies were obtained from rabbit serum which was immunized with raw pork extract, and which had been heated at 70° C, 80° C and 100° C. The purification process was carried out in two stages namely using 33% ammonium sulfate and continued with Protein A column affinity. The antibody purification results were analyzed using SDS-PAGE, while the antibody activity was tested using the western blot method on raw meat extracts and on those heated at 70° C , 80° C, and 100° C. The results of this study showed that antibodies purified with a combination of ammonium sulfate and Protein A column affinity produced relative purity of antibodies up to 97.6 per cent compared to those purified using ammonium sulfate alone. Western blot analysis showed that the purified antibodies were able to detect raw as well as heated pork extracts up to 70° C at a total protein content of 20 micro grams of meat extract. Antibodies obtained from immunizations using meat extracts that have been heated at 70° C gave the same potential as antibodies from rabbits immunized using raw meat extract, i.e. can only detect antigens of raw meat and antigens of pork heated at 70° C. From the results of this study it can be concluded that purification of antibodies using a combination of ammonium sulfate and Protein A column is effective in producing polyclonal antibodies with relatively high purity levels. In the future, further research needs to be carried out on the development of immunodiagnostics using polyclonal antibodies purified from animals immunized with pork extract that has been heated at a temperature of 70° C or more.

INTRODUCTION

The issue of halal food products is crucial and has caused concern among Muslim consumers around the world. This is because the counterfeiting of halal components with non-halal components in food products has been widespread and is difficult to identify using ordinary senses. Detection and quantification of the swine components in food are needed not only because of religious beliefs but also because of health reasons.

According to Soedjono [1] various techniques to identify meat species commonly used in food products have been developed extensively. Advances in the molecular technologies have developed methods/technologies to identify unwanted meat contamination in a food ingredient. Some of them are analytical methods to identify pig (swine) derivatives in food, such as identification of porks in meatballs by PCR-RFLP or polymerase chain reaction and restriction fragment length polymorphism [2], detection of swine or pork substances in beef sausage products by means of polymerase chain reaction method or PCR [3], as well as identification of swine DNA, by means of Capillary Gel Electrophoresis, Duplex-PCR, and Multiplex-PCR [4]. The problem is that these methods require sophisticated equipment and special skills.

Other methods that have been developed for the detection of pork in food were protein-based methods by exploring polyclonal anti-pig protein antibodies, including for the detection of pork gelatin [5], also the development of rapid immunodiagnostic tests for pork components in beef- and raw chicken [6]. The problem with the kit developed by Depamede [6] was that it cannot detect cooked pork, because the material used in the kit was antibodies to raw meat. In our group we have succeeded in producing antibodies against heated pork extracts [7]. In this study we report that we are able to isolate the antibodies using ammonium sulfate combined with the Protein A column.

MATERIALS AND METHODS

This study used serum immunoglobulin (IgG) from rabbits that had previously been immunized repeatedly using antigens from pork extract that had been boiled at temperatures of 70ºC, 80ºC, and 100ºC; while as a control the immunoglobulin from raw pork extract was employed (Rizal 2018 and Ridho 2018). The antibodies from each serum were gradually isolated. Firstly through 33% ammonium sulfate precipitation then followed by the purification

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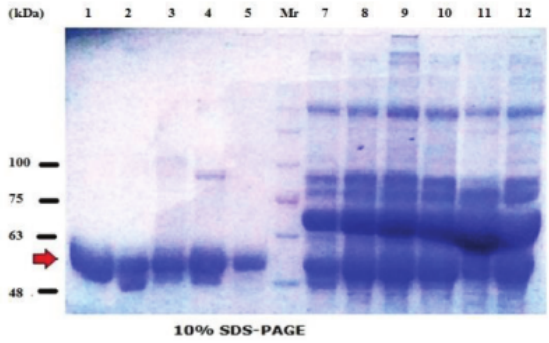
process using the Protein A column affinity. Pure antibodies were eluted using glycine at pH 3 [6]. The purity level of each antibody was analyzed based on the results of SDS-PAGE, while each of their immuno-reactivity was analyzed by the westernblot method. All data were analyzed descriptively.

RESULTS AND DISCUSSION

Figure 1 shows the results of SDS-PAGE analysis of antibody purified from rabbit serum that had been immunized with pork extracts. The purification process was carried out in two stages, beginning with ammonium sulfate precipitation and followed by means of Protein A column. It is clear here that the purification using protein A column (lane 1-5) resulted in purer (~ 97%) antibody as indicated by almost only one protein band (arrow) with a sufficient homogeneous without being contaminated by other protein bands. While the results of purification with ammonium sulfate (lane 7-12), it can be seen that there are still other bands, besides the target protein band. These results are in accordance with what was revealed by Murphy et al. [8] that the antibodies produced from purification using the Protin A column are purer compared to those of the ammonium sulfate precipitation method.



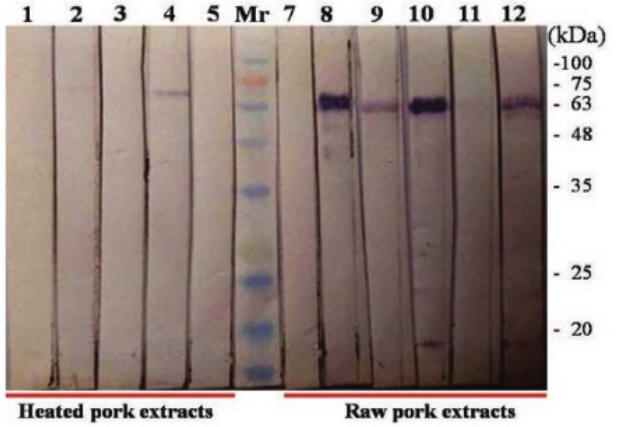
1-5 Purification using Protein A column
1. Unimmunized serum
2. Ab.1 (immunized with raw pork)
3. Ab.2 (immunized with 60ºC pork)
4. Ab.3 (immunized with 70ºC pork)
5. Ab.4 (immunized with 80-100ºC pork)
6. Mr (Protein Marker)

7-12 Purification using ammo. sulfate (AS)
7. Ab. 1 AS
8. Ab. 2 AS
9. Ab. 3 AS
10. Ab. 4 AS
11. Unimmunized serum IgG
12. Unimmunized serum IgG after dialysis

FIGURE 1. SDS-PAGE results

Based on the SDS-PAGE analysis, antibody activities were carried out on the antibodies purified using a combination of ammonium sulfate precipitation and protein A column only. The method used was westernblot against raw pork and pork heated at 70° C. Boiled pork at a temperature of 70° C was chosen due to it is related to the process of making sausages by heating at a temperature of 155° F or around 68 to 70° C [9]. Raw pork extract and the boiled pork extract of 70° C were run on SDS-PAGE at a concentration of 20 micro grams of each lane, and then transferred on the nitrocellulose membrane. After that the membrane was cut into pieces or strips, and reacted with the purified antibodies (Ab1, Ab2, Ab3 or Ab4). The results are presented in Figure 2.

Western blot analysis (Figure 2) shows that the purified antibodies were able to detect mainly raw pork extracts and the heated pork extracts up to 70° C with a molecular weight around 63 kDa, at a total protein content of 20 micro grams of meat extract. Interestingly, none of the antibodies was able to detect heated pork, except the antibodies obtained from rabbits that immunized using meat extracts that had been heated at 70° C. It can be seen that the antibody gave the same potential as antibodies from rabbits immunized using raw meat extract, i.e. able to detect both antigens of raw meat and antigens of pork heated at 70° C (lanes 4 and 10, Fig.2). On the other hands purified antibodies obtained from rabbits immunized using antigens that had been heated at temperatures above 80°C were unable to the detect even the raw pork extract (lane 11, Fig.2).



Lane 1-5: Strips containing heated pork extract of 70° C		**Lane 7-12** Strips containing raw pork extract	
1	Treated with unimmunized IgG (negatif)	7	Treated with unimmunized IgG (negatif)
2	Treated with Ab1	8	Treated with Ab1
3	Treated with Ab2	9	Treated with Ab2
4	Treated with Ab3	10	Treated with Ab3
5	Treated with Ab4	11	Treated with Ab4
M: Marker		12	Pooled Ab as a positive control

FIGURE 2. Results of immunogenicity test of purified polyclonal antibodies against heated/boiled (70°C) and raw pork using Western blot method.

CONCLUSIONS

From the results of this study it can be concluded that purification of antibodies using a combination of ammonium sulfate and Protein A column is effective in producing polyclonal antibodies with relatively high purity levels. Antibody obtained from heated pork of 70° C was the only antibody that able to detect pork which heated at 70° C, hence further studies need to be carried out on the development of immunodiagnostics using polyclonal antibodies purified from animals immunized with pork extract that has been heated at a temperature of 70° C or more.

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