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Detection of Porcine DNA in Processed Beef Products Using Real Time – Polymerase Chain Reaction

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ABSTRACT

Meat is one of food materials which has protein source and mostlyconsumed by non-vegetarian. Consuming halal food is an obligation for every Muslim. Meat processed products usually contaminated by pork. One of technique that is often chosen as an authentication process for proofing halalness of the product is PCR technique, one of PCR technique which most commonly used is RT-PCR. RT-PCR technique was chosen as identification method because it has high accuration for detection of porcine DNA in fresh meat and processed products. RT-PCR is the amplification technique in the specific regions that are restricted by two oligonucleotide with the help of polymerase enzymes. Annealing is the first process of RT-PCR analysis who was primary attachment to the DNA template that determines the specificity and amount of DNA produced. In this study, extraction kit and detection kit were used for analysis Porcine DNA in meatballs. The results obtained from this study were from whole DNA samples, which had DNA purity ranging from 1.82 to 1.93. From the all samples three of them containing porcine DNA. The positive samples shown from amplification curves who was specifically formed when probes reacts with porcine gene.
Keywords: *DNA, Meatballs, Porcine, RT-PCR*

1. Introduction

Halal food is good for all people, both physically and spiritually. Halal food is food that is permitted to be consumed or not bound by provisions prohibiting it, both thayyib and appeasement (Girindra, 2006). In the present, the number of cases of halal food contamination with ingredients that are not halal is the problem faced by Muslims. Processed food products that must be concerned its sausages, nugets, ground meat, corned beef, meatballs, mayonnaise, chewy candy, chocolate, jelly, flavor. In addition to processed food products, there are food additives to oversee for halalness, such as gelatin, lecithin, collagen, glycerol/glycerin, improver, shortening, renin or pepsin and animal derivatives. Muslim consumers have strict orders regarding the rules of food that can be consumed. Every Muslim has different compliance regarding orders, this difference can be seen from the halal application (Salehudin, 2010).

The advancement of technology in the molecular biology is the best option to identify material contamination that is not halal. For example pork contaminants can be detected by PCR or RT-PCR. The Taqman RT-PCR system with minor groove binding has also been used in the detection of quantification of DNA of cows, pigs, sheep, chickens, turkeys and ostriches. Some testing of meat contamination in Indonesia has also used molecular technology. According to Margawati & Ridwan (2010), the tests conducted did not show any contamination of beef meatballs using the species specific PCR method using primers from the pig leptin gene.

The main focus of authentication for Muslim consumers in processed meat is the porcine substitution of pigs, blood clots, organs in pig animals, and food processing plants that using derivatives from pigs (such as enzymes) because they have cheaper price and guaranteed availability. DNA molecules are the target components for species identification compared to proteins because of the different stability of these two components and their amount in tissue (Hamzah, 2014).

A number of published works have reported the advantages of using Real Time – PCR compared with conventional PCR for species identification. The ability to detect very small size products, since it eliminates the need

for gel visualization, the reduced risks for crossover contamination, as reaction are kept confined during amplification and analysis and capability for large scale processing and high automated throughput. Probe is using among the most broadly applied Real Time – PCR technologies to detect banned animal material in feedstuffs (Pegels, 2012).

2. Materials and Methods

The materials used were various of processed meat products. Meat products which are estimated to contain pork or their derivatives such as sausages and meatballs that have been labeled halal or which have not been labeled halal. The other materials used were DNeasy Mericon Food (QIAGEN), Mericon Pig Kit (QIAGEN) (Figure 1), Chloroform and Ethanol.

Number of reactions		24	96
Yellow	mericon Assay*	2 x 12 reactions	1 x 96 reactions
Red	Positive Control DNA	20 reactions	20 reactions
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml	1.5 ml
	RNase-Free Water	1.9 ml	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl	1040 µl
	50x ROX Dye Solution	45 µl	210 µl

Figure 1. Content of Mericon Pig Kit

DNA Extraction

The 200 mg of sample was prepared by the addition of 1 mL food lysis buffer (either directly 1 mL or gradually 500 µL and 500 µL, depending on the type of sample). Followed by a purification process by taking 1 mL of sample and adding 2.5 proteinase-K, vortex and incubation at 60 0C for 30 minutes. Centrifuge at 2500xg for 5 minutes. Add 500 µL of Chloroform to the new 2 ml tube. The clear layer from the lysis tube was removed, without touching precipitation on the bottom of the tube. 500 µL of samples were put into tube containing Chloroform. Vortex for 15 seconds and centrifuge at 14000xg for 15 minutes. A clear layer was taken and the volume was measured, then PB buffer was added with the ratio 1:1 then vortex for 15 seconds. Placed all liquid into the Qiaquick spin column and centrifuge 17900xg for 1 minute. The liquid was disposed and stored in the collection tube. 500 µl of Buffer AW2 were added, 17900xg centrifuge for 1 minute and the supernatant was removed. Place Qiaquick spin column in 2 mL of the new collection tube and centrifuge it again at 17900xg for 1 minute on the dry membrane. The collection tube was discharged and placed into the Qiaquick spin column in the new 1.5 ml tube. 150 µl of EB was added and incubate for 1 minute at room temperature then centrifuge for 1 minute. The eluted DNA can be directly used for PCR or stored at -20°C.

RT – PCR Analysis

The 130 µL multiplex PCR master were mixed into mericon assay tube, vortex and centrifuge. The positive was dissolved then 200 µL quantitec nucleic acid dilution buffer was added, vortex and centrifuge. Set up all the sample and control of reaction.

Table 1. Set Program of RT – PCR

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq *Plus* DNA polymerase
3-step cycling:			
Denaturation	15 s	95°C	Data collection at 60°C
Annealing	15 s	60°C	
Extension	10 s	72°C	
Number of cycles	45		
Detection	**Reporter**	**Excitation/emission**	**Channel**
Target	FAM	495/520 nm	Green
Internal control	MAX	524/557 nm	Yellow

The program of RT – PCR was set and the tubes containing the sample and control were inserted into the machine then run according to the RT-PCR program.

3. Results and Discussion

Results of this study presented in Table 2. The amplification curves that indicates positive samples might show the safety of food we are consumed. Food that are sold in the market may be contaminated by pork. This test aimed to conduct testing directly on the market. The result of positive samples will be given to the competent authority as a reference for futher action.

Table 2. Extraction Result of Beef Processed Product

	260	280	Conc ng/µl	Ratio	Result
A1	0,0027	0,0014	2,7	1,93	+
A2	0,1159	0,0633	115,95	1,83	-
B1	0,1044	0,0574	104,45	1,82	-
B2	0.0467	0.0257	46.75	1.82	+
C1	0,1143	0,0631	114,35	1,81	+
C2	0,0372	0,0198	37,25	1,88	-
D1	0,0551	0,02995	55,1	1,84	-
D2	0,0646	0,03485	64,6	1,85	-
E1	0.0013	0.0007	1,3	1.86	-
E2	0,0511	0,02735	51,1	1,87	-

In this study, all the samples was isolated, then analyzed the purity using Tecan Nanophotometer at a wavelength of 260/280 nm which is the wavelength used specifically for DNA. The results obtained from the all samples have purity in the range 1.81 - 1.93, the range has passed for further testing using Real-Time PCR.

From all samples that had DNA purity in the range 1.8 - 1.9, the identification of porcine with RT-PCR was conducted to determine porcine contamination in processed meat products, the results of RT-PCR analysis can be seen in Figure 2.

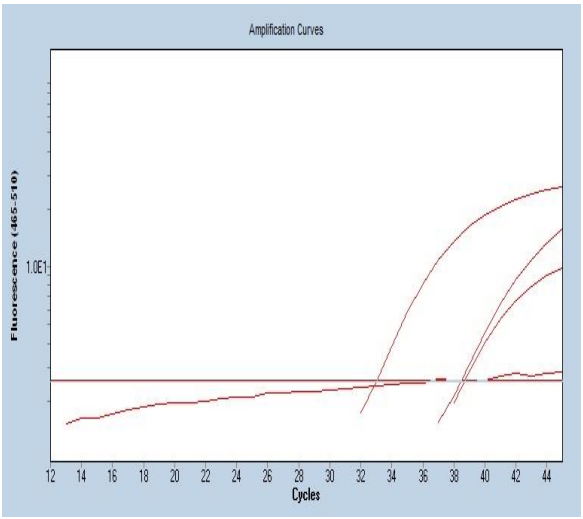

Figure 2. Analysis Result of Porcine DNA Contaminant Using RT – PCR

Compounds with basic components of DNA or RNA will be absorbed maximally at ultraviolet wavelengths at 260 nm, while amino acids with aromatic rings absorb light with maximum absorbance at 280 nm wavelengths. In the analysis of DNA purity, it appears that the entire sample has DNA purity in the range 1.8 - 2.0. According to Yoon (2016), the ratio used is the absorbance ratio of 260 and 280 nm to see the purity of nucleic acids with a ratio between 1.8 - 2.0, so that it can be obtained purely. The DNA produced in this study had fulfilled the purity criteria for porcine DNA analysis testing using PCR. DNA extraction was carried out using species-specific methods. The aim of this method was to identify contaminants in food products that can cause food poisoning and damage the human immune system.

In the testing of porcine DNA contaminants using the RT-PCR method, obtained the results that among all samples analyzed found three samples which was positive contain porcine DNA. The three samples were A1, C1 and B2. This can be seen from the CP (crossing point) value of the three samples which are at <35 when compared with positive control with the CP value 32.63. The content of porcine DNA in meatball and beef sausage products can be caused by cross-contamination in the production process, because there are processed meat products that have obtained halal certificates from LPPOM MUI.

Similar results were also obtained by Khatani (2017), where tests conducted on various of processed meats showed that some products found positive containing porcine DNA with of Ct values at 34 - 37.45, it could also caused by cross contamination during processing due to porcine DNA concentration found in samples showed a low concentration of ≤ 0.0001 ng / µL.

4. Conclusion

The RT - PCR method is the accurate and fast method to be used to identify porcine DNA contamination in a processed product. These method using probe which was species specific analysis for detection of porcine DNA. From all the samples that has been analysed, three samples which positive contain porcine DNA were found.

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