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BEEF MEATBALLS ADULTERATION TESTS WITH REAL TIME QUANTITATIVE PCR DETECTION FOR HALAL AUTHENTICATION - CASE STUDIES SELLERS AT TRADITIONAL MARKET AND SMALL MEDIUM ENTERPRISES (SMEs) MERCHANTS IN INDONESIA

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Abstract

The increase of beef price trigger meatballs adulteration which using pork. Protein specific identification in processed food like meatballs getting difficult because there are possibilities of protein breakdown caused by process. Therefore, molecular approach such as real time quantitative polymerase chain reaction was done to identify pork addition in beef meatballs and give halal authentication as safety warranty to the consumer. Forty eight samples taken purposively from 21 SMEs merchants and 21 sellers at traditional market around Jatinangor education centre. The result shown all the merchant and sellers sold halal beef meatballs, because no adulterated beef meatballs found.

Keywords*: real time PCR, pork adulteration, beef meatballs*.

INTRODUCTION

Adulteration recently become a serious problem and frequently found in meat and meat products. In 2013, the Food Standard Agency of United Kingdom found 11 from 18 beef lasagna products contained 60-100% horsemeat. British meat industries also threatened by porcine and horse DNA finding in meat samples from three processing plants which two from Ireland and one from Britain. Since that, the scandal spread to 13 European countries includes a variety of findings in others meat products such as beef burgers, bolognese sauces and cottage pies.

In Indonesia, the biggest concerns of meat adulteration findings were in SMEs and traditional market sellers. Lack of capital and knowledge was main reason why adulteration occurs. The high price of meat and passiveness of consumer safety warranty further encourage the SMEs and traditional market sellers to substitute beef with other kind of meat such as pork in the making of meatballs. Pork substitutions in meatball productions were not a crime, however it could generate interest about non-halal foods especially in Country with most of the population are moslem such Indonesia. Identification of meat adulteration in processed foods as well as meatballs is difficult. The properties of protein used in species identification, often damaged by heat and meatball processing that generate denaturized proteins (Hoffman et al., 1996). Mixed meat or processed meat was complex substrates that need a sensitive assay method to identify the correct DNA target. A specific target sequence could amplify by an optimized PCR procedure, even in a complex genomic sequence (Tanabe et al., 2007). Real-time PCR used as a rapid quantitative detection method to identify pork adulteration in beef meatballs sold in Jatinangor Education Centre, Sumedang District, West Java, Indonesia. Aims of the study were to determine halal authentication of the beef meatballs so that safety warranty of the consumer can be achieved.

MATERIALS AND METHODS

Total 48 beef meatball samples taken from 21 SMEs merchants and 21 sellers at traditional

market around Jatinangor education centre. Samples stored at refrigerator for 12 hours then tested with RT-PCR.

DNA Isolation

Genomic DNA extracted from beef meatballs samples using Pure Link' Genomic DNA Kits. A total of 2 g sample entered into the eppendorf tube, add 20 µL Proteinase K, 20 µL of RNAse, vortex and incubate 2 min at room temperature. Add 200 µL GLBB, vortex and incubate at 55°C for 10 minutes. Add 200 µL of 95% ethanol and vortex it. Move into spin colums and centrifuged at 12500 rpm, 1 min, RT. Replace with the new collections tube, then add 500 µL of WB 1, centrifuged at 12500 rpm, 1 min, RT. Replace collections new tube add 500 mL WB 2, further centrifugation 12500 rpm, 3 min, RT. Replace with a new collections tube and add GEB 200 µL, RT incubation for 1 minute later centrifuged at 14000 rpm, the DNA genome can be stored at 4°C.

Table 1. Reaction Composition of RT-PCR

Material	Amount (µl)
KAPA"SYBR FAST	10
PCR water	9
Primer (forward)	0.4
Primer (reverse)	0.4
DNA template	0.5
Total volume	20

Table 2. Reaction Condition of RT-PCR

Step	Temp. (^{0}C)	Duration (Minutes)
Incubation	50	02:00
Polymerase Activation	95	10:00
PCR Cycling	95	00:10
PCR Cycling	60	00:30
Melt Curve	95	00:15
Melt Curve	55	00:15
Melt Curve	95	00:15

Equipment

Illumina Eco^{TM} Real Time PCR, KAPA "SYBR FAST, ddH_2O, ethanol 95%, primer with specific gen *cyt b* GCT GAT AGT AGA TTT GTG ATG ACC GTA (Matsunnaga).

RESULTS AND DISCUSSIONS

Mitochondrial gene such as cyt b DNA sequence that used could restrict the assay sensitivity. As shown in Figure 2, the assay could determine porcine DNA until 0.001% quantity with great reproducibility (Tanabe et al., 2007). As shown by the result, curve formed at Figure 1 was different with Figure 2 that mean no porcine DNA sequence amplified from 48 samples of meatball tested. Therefore, no pork added to meatball samples that taken from 21 SMEs merchants and 21 sellers at traditional market around Jatinangor education centre.

Presences of pork in meat and meat products were sensitive issues especially in country with moslem as the biggest population such as Indonesia. Pork is prohibited to consume, because it is not appropriate with halal clause and the consumption of halal foods was compulsory for moslems (Rohman et al., 2011).

Certainty of no beef meatballs adulterated with pork was a good result as base of moslems consumer safety warranty. The results can conclude those 21 SMEs merchants and 21 sellers at traditional market around Jatinangor education centre sold halal beef meatballs.

CONCLUSIONS

Identification of adulterated beef meatballs with pork using RT-PCR give a rapid quantitative result that shown no pork addition in all of the samples. Twenty-one SMEs merchants and 21 sellers at traditional market around Jatinangor education centre sold halal and safe beef meatballs especially for moslems consumer.

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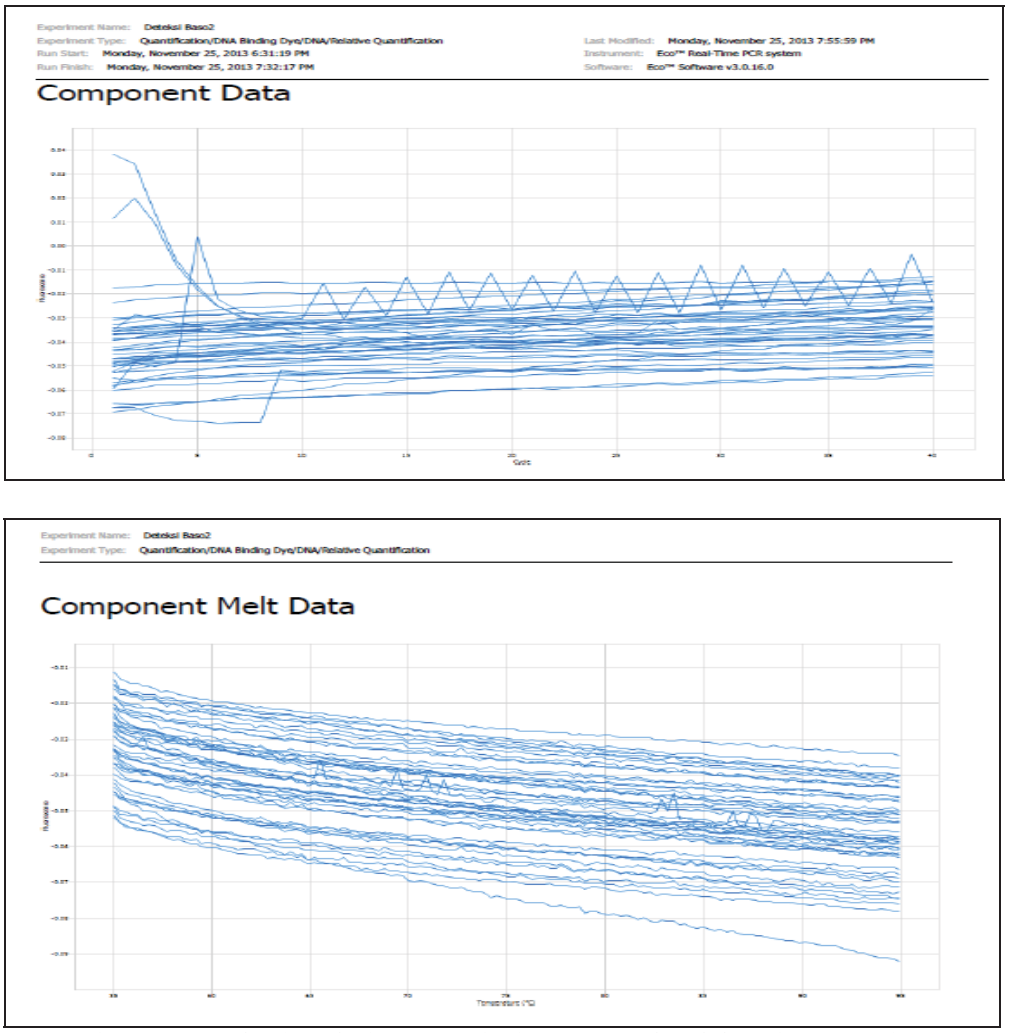

Figure 1. Result of Real Time Quantitative PCR Pork Adulteration Detection

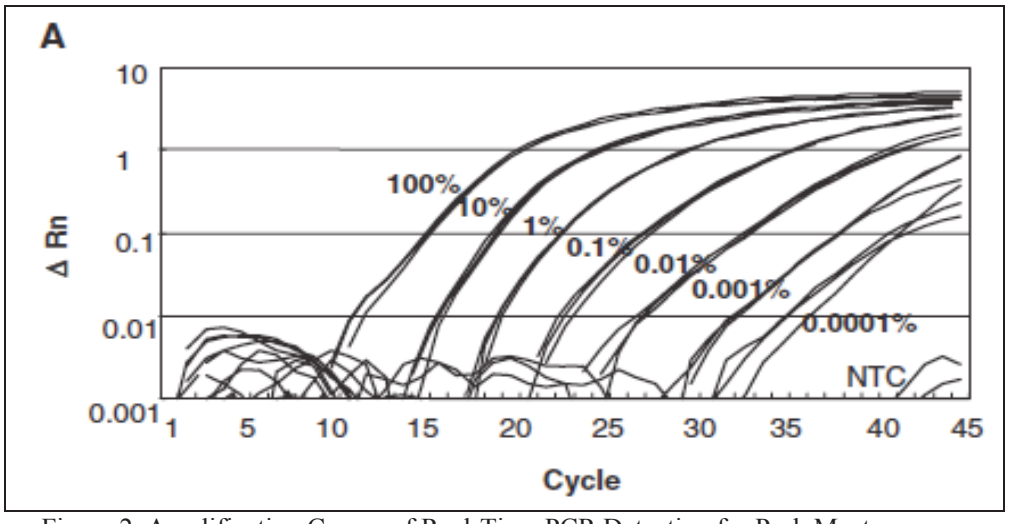

Figure 2. Amplification Curves of Real-Time PCR Detection for Pork Meat (Tanabe et al., 2007)