UJI KINERJA *REAL-TIME POLYMERASE CHAIN REACTION* (RT-PCR) *PROBE* TaqMan SPESIFIK GEN ATPase6 UNTUK DETEKSI CEMARAN DAGING BABI PADA BAKSO

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INTISARI

Telah dilakukan uji kinerja *real-time polymerase chain reaction* (RT-PCR) dengan primer dan *probe* TaqMan spesifik gen ATPase6 untuk deteksi cemaran daging babi pada bakso. Penelitian ini bertujuan untuk menentukan kinerja primer dan *probe* TaqMan spesifik gen ATPase6 yang bersifat spesifik terhadap spesies babi sehingga dapat mendeteksi adanya kontaminasi daging babi di dalam bakso berbasis uji DNA.

Penelitian ini terdiri dari dua tahap yang meliputi uji kinerja metode dan pengujian kontaminasi daging babi di dalam bakso komersial. Uji kinerja metode yang dilakukan berupa uji spesifitas menggunakan DNA bakso babi 100% sebagai kontrol positif dan DNA bakso babi 0% sebagai kontrol negatif, uji presisi dilakukan dengan pengulangan amplifikasi kontrol positif sebanyak 10 kali, uji sensitivitas dilakukan dengan pengenceran DNA kontrol positif dari 2,5 ng/µL sampai 0,25 pg/µL, dan penentuan batas deteksi menggunakan bakso dengan variasi campuran daging babi 1, 5, 10, 20, 50, dan 100 % (b/b). Pengujian kontaminasi daging babi di dalam bakso komersial digunakan 10 bakso yang dijual di Yogyakarta.

Primer *forward* ATPase6 (5'-CAC ACC CAC CAC ACA ACT AT-3'), primer *reverse* ATPase6 (5'-GGT AGA AAG TGG GCT AGT GAT-3') dan *probe* TaqMan (5'-/FAM/TCA GCA ACC/ZEN/GTA TTC ACA GGA TTC CG/3IABkFQ/-3') yang digunakan bersifat spesifik membedakan bakso yang terkontaminasi daging babi. Uji kinerja metode membuktikan bahwa metode ini menunjukkan presisi dengan nilai *relative standard deviation* (RSD) = 5,07% dan dapat mendeteksi kontaminasi daging babi di dalam bakso sampai konsentrasi 0,25 pg/µL DNA *template* dan konsentrasi 1% (b/b) daging babi di dalam campuran bakso. Dua dari 10 sampel bakso komersil yang dianalisis diduga adanya kontaminasi daging babi.

Kata kunci: babi, gen ATPase6, RT-PCR, *probe* TaqMan, bakso

REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR) PERFORMANCE TEST OF TaqMan PROBE SPECIFIC ATPase6 GENE TO DETECT CONTAMINATION OF PORK IN MEATBALLS

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ABSTRACT

A real-time polymerase chain reaction (RT-PCR) performance test of primer and TaqMan probe specific ATPase6 gene to detect contamination of pork in meatballs has been conducted. The aim of this research was determining the performance test of ATPase6 gene primer and TaqMan probe specific to pork as well as the capability to detect contamination of pork in meatballs based DNA test.

This study consists of two steps which include performance test of the method and detect contamination of pork in commercial meatballs. Method performance test was separated into specificity test which was performed using a 100% pork meatballs DNA as the positive control and a 0% pork meatballs DNA as the negative control, precision test was performed by amplifying the positive control 10 times repeatly, and limit of detection determination included sensitivity test was performed by amplifying a DNA positive control which is diluted from 52,5 ng/µL to 0,25 ng/µL and contaminant test was performed using meatballs mixture with variation of pork contamination of 1, 5, 10, 20, 50, and 100% (w/w). In other hand, 10 samples were collected to detect contamination of pork in commercial meatballs.

Forward primer of ATPase6 gene (5'-CAC ACC CAC CAC ACA ACT AT-3'), reverse primer of ATPase6 gene (5'-GGT AGA AAG TGG GCT AGT GAT-3'), and TaqMan probe (5'-/FAM/TCA GCA ACC/ZEN/GTA TTC ACA GGA TTC CG/3IABkFQ/-3') was specifically distinguish contamination of pork in meatballs. The method has a good precision with relative standard deviation (RSD) value 5.07% and able to detect the contamination of pork in meatballs up to 0.25 pg/µL of DNA template and 1% of pork contamination in meatballs mixture (w/w). Two of the 10 meatballs samples were found to contain of pork.

Keywords: pork, ATPase6 gene, RT-PCR, TaqMan probe, meatballs