UJI KINERJA *REAL TIME-POLYMERASE CHAIN REACTION* (RT-PCR) *PROBE* TaqMan GEN *CYT-B* DNA MITOKONDRIA SPESIFIK TIKUS HITAM (*Rattus rattus)* PADA PRODUK BAKSO

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INTISARI

Pengujian kinerja terhadap metode *Real Time-PCR* (RT-PCR) menggunakan *probe* TaqMan telah dilakukan untuk mendeteksi cemaran daging tikus hitam (*Rattus rattus*) dalam produk bakso. Penelitian ini dilakukan untuk menguji dan mengembangkan metode identifikasi DNA tikus pada produk bakso kemasan berbasis uji DNA sehingga dapat digunakan sebagai metode standar untuk uji kehalalan pangan.

Metode RT-PCR *Probe* TaqMan pada penelitian ini menggunakan primer spesifik gen sitokrom b (*Cyt-b*), yaitu *primer forward* (5'-GAC TTA CTA GGA GAC CCA GACA-3'), *primer reversed* (5'-TGT TAG GGA TGG AGC GT AGA-3'), dan *probe* (5'-6FAM/ACA CAC CTG/ZEN/CTA ACC CAC TAA ATA CCC/31ABkFQ -3'). Penelitian ini dilakukan dalam beberapa tahapan, yaitu uji kinerja metode RT-PCR *probe* TaqMan pada bakso (uji spesifitas, uji presisi, uji sensitivitas, dan uji batas deteksi), dan uji deteksi cemaran daging tikus pada 10 jenis produk bakso kemasan yang beredar di pasaran. Isolasi DNA bakso dilakukan dengan metode Fenol-KIAA, kemudian amplifikasi fragmen DNA dilakukan menggunakan RT-PCR dengan kondisi pra-denaturasi (95 ^{o}C selama 1 menit), denaturasi (95 ^{o}C selama 15 detik), *annealing* (52 ^{o}C selama 30 detik), dan fase ekstensi (60 ^{o}C selama 30 detik). Proses tahapan ini diulang sebanyak 35 siklus.

Hasil uji metode RT-PCR *probe* TaqMan menujukkan bahwa metode ini spesifik terhadap DNA tikus dalam sampel bakso, dengan tingkat presisi tinggi (nilai *Relative Standard Deviation*/RSD sebesar 5,3%), dapat mengamplifikasi DNA tikus hingga konsentrasi 0,005 ng, dan batas deteksi daging tikus hingga konsentrasi terendah 1%. Aplikasi metode telah dilakukan terhadap 10 sampel bakso kemasan yang dijual di pasaran, dan diketahui bahwa tidak ditemukan adanya cemaran daging tikus pada sampel-sampel tersebut.

Kata Kunci: Uji kehalalan pangan, *Rattus rattus, Real Time*-PCR, *probe* TaqMan

THE PERFORMANCE TEST OF REAL TIME-POLYMERASE CHAIN REACTION (RT-PCR) TaqMan *PROBE FROM CYT-B GENE MITOCHONDRIAL DNA SPECIFIC FOR BLACK RAT (Rattus rattus) IN MEATBALL PRODUCTS*

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ABSTRACT

The performance test of *Real Time*-PCR (RT-PCR) method using TaqMan *probe* has been tested for detecting rat (*Rattus rattus*) meat adulteration in meatball products. This research was intended to verify and develop the rat DNA-based identification in meatball products so that it could be applied as a standard method in halal analysis of food products.

This *Real Time*-PCR TaqMan *probe* method use rat-specific primers, including *primer forward* (5'-GAC TTA CTA GGA GAC CCA GACA-3'), *primer reversed* (5'-TGT TAG GGA TGG AGC GT AGA-3'), and *probe* (5'-6FAM/ACA CAC CTG/ZEN/CTA ACC CAC TAA ATA CCC/31ABkFQ -3'). This research was implemented in several steps, which is performance test of *Real Time*-PCR TaqMan *probe* method (including specifity test, precision test, sensitivity test and limit of detection test), and rat meat adulteration tests of 10 samples of commercially marketed meatball products. DNA isolation of meatball has been performed using Phenol-CIAA method, while amplification of DNA fragments has been performed using *Real Time*-PCR with condition of pre-denaturation (95 ^{o}C in a minute), denaturation (95 ^{o}C in 15 seconds), *annealing* (52 ^{o}C in 30 seconds), and extention phase (60 ^{o}C in 30 seconds). This process then was repeated up to 35 cycles.

The research has proved that *Real Time*-PCR TaqMan *probe* method was specific only for rat DNA in meatball samples, with a high precision level (Relative Standard Deviation/RSD value is 5.3%), could amplified rat DNA fragments up to 0.005 ng concentration, and limit detection of rat meat at the lowest of 1% concentration. This method has been applied on 10 samples of commercial meatball products. The final result was obtained that none of these samples have been adulterated by rat meat.

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Keywords: Halal analysis of food products, *Rattus rattus, Real Time*-PCR, TaqMan *probe*