INTISARI

Identifikasi spesies tertentu pada produk makanan penting karena dapat meminimalkan tingkat pemalsuan label pada suatu produk olahan daging. Beberapa metode telah dilaporkan untuk mengidentifikasi DNA spesies hewan yang diharamkan, salah satunya yaitu metode *Real-Time Polymerase Chain Reaction* (RT-PCR). Penelitian ini bertujuan untuk memvalidasi metode *Real-Time* PCR dan untuk deteksi DNA babi (*Sus scrofa domestica*) dan celeng (*Sus scrofa*) pada sosis ayam dan analisis sekuensing untuk mengetahui urutan basa DNA babi dan celeng pada amplikon target. Identifikasi DNA babi dan celeng dilakukan dengan menggunakan metode *Real-Time* PCR dengan primer hasil desain menggunakan *Software online* PrimerQuest dan NCBI yang spesifik babi dan celeng yaitu NK-ND1-Ssc1 (*Forward*: 5'-AAAGGACCCAACGTTGTAGG-3') dan (*Reverse*:5'-TAGTGCTAGGGATAAGGCTAGG-3') dengan target amplifikasi yakni mitokondria Sub-unit ND1. Uji spesifisitas primer dilakukan terhadap isolat DNA spesies lain (ayam, sapi, anjing, kambing dan kelinci) diperoleh suhu optimum penempelan primer yaitu 58,1^{o}C. Validasi metode *Real-Time* PCR meliputi uji sensitivitas, uji batas deteksi dan uji keterulangan. Uji sensitivitas dilakukan pada isolat DNA daging babi dan celeng segar serta pada sosis referensi babi dan celeng konsentrasi 100% dengan konsentrasi masing-masing secara berurutan 500 pg dan 50 pg didapatkan nilai Efisiensi 93,1% dan 94,8% serta 94,1% dan 91,3% secara berturut-turut. Uji batas deteksi yang masih dapat teramplifikasi pada sosis campuran babi-celeng-ayam pada konsentrasi 0,3% dengan nilai koefisien determinasi (R^2) dan efisiensi secara berturut-turut sebesar 0,926 dan 78,6%. Uji keterulangan daging babi dan celeng segar menghasilkan CV sebesar 0,29 dan 0,50% serta pada sosis babi dan celeng konsentrasi 1000pg/uL menghasilkan nilai CV sebesar 2,23 dan 0,21%. Hasil uji pada 12 sampel sosis pasaran menunjukkan adanya kandungan babi dan/celeng yang dibuktikan dengan naiknya kurva amplifikasi pada siklus 24.

Kata kunci: *Real-time Polymerase Chain Reaction*, Daging Babi (*Sus scrofa domestica*), Daging Celeng (*Sus scrofa*), Sosis Ayam, Sekuensing.

ABSTRACT

The identification of meat species in food products is important to minimize the adulteration practice. Due to its specificity, polymerase chain reaction (PCR)-based methods are the most reported method for detection forbidden species DNA of food adulteration. This study was aimed to evaluate real-time PCR using species-specific primer and sequencing analysis to identify two non-halal meats namely pork and wild boar meat (WBM) and sequencing to find out the difference sequence in chicken sausages. The primer was designed using online software PrimerQuest from NCBI (National centre for Biotechnology Information) which are specific to pork and wild boar. The annealing temperature of real-time PCR condition was optimized to get the highest response of relative fluorescence unit and the lowest cycles. Real-time PCR using primer of NK-ND1-Ssc1 (Forward: 5'-AAAGGACCCAACGTTGTAGG-3' dan Reverse: 5'-TAGTGCTAGGGATAAGGCTAGG-3') targeting on mitochondria subunit ND1 was validated by determining several parameters namely specificity, limit of detection for sensitivity, linearity and efficiency and repeatability. The optimum annealing temperature of NK-ND1-Ssc1 primer was 58.1^{o}C. The sensitivity evaluation revealed that limit of detection (LoD) of pork and WBM in reference sausage samples containing pork and WBM 100% was 500 pg and 50 pg corresponding to 0.3% meat in sausage products, respectively. The efficiency values of real-time PCR amplification were 93.1% and 94.8% for pork and WBM with coefficient variations of 0.29 and 0.50%, respectively. The validated real-time PCR method was further applied to analyse the commercial samples and among 12 sample evaluated, there was one sample positive to contain non halal meat (pork or WBM). Real-time PCR using specific primers is specific and sensitive, therefore, this method could be proposed as standard method for identification of non-halal meats in food products.

Keywords: *Real-time Polymerase Chain Reaction*, pork, wild boar, chicken sausage, Sequencing