INTISARI

Praktik pencampuran makanan menggunakan daging anjing telah banyak ditemukan. Hal ini disebabkan karena rendahnya harga daging anjing di pasaran. Tujuan penelitian ini adalah merancang *species-specific primer* (SSP) dan mengembangkan metode *real-time* PCR untuk analisis daging anjing (*Canis lupus familiaris*) dalam bakso ikan tenggiri guna autentikasi halal.

Perancangan primer spesies spesifik dilakukan secara *in silico* menggunakan situs NCBI-Primer BLAST dan IDT. Analisis spesifisitas primer dilakukan terhadap DNA anjing dan 8 hewan pembanding yaitu ikan tenggiri, ayam, babi, kambing, katak, sapi, tikus wirok, dan tupai menggunakan *real-time* PCR. Validasi metode ditentukan dengan uji sensitivitas, efisiensi amplifikasi, dan uji keterulangan.

Hasil penelitian menunjukkan bahwa primer Cytb (*forward*: CACTAATCT TCTCTCTGCCATCC, *reverse:* GAATCGTGTTAGGGTTGCTTTG) yang dirancang spesifik dapat mengamplifikasi DNA mitokondria anjing dengan suhu penempelan optimum 61,8°C. Uji sensitivitas yang dilakukan terhadap 8 seri pengenceran DNA 10 dilusi menghasilkan nilai batas deteksi (LoD) sebesar 1 pg/µL, dengan efisiensi amplifikasi (E) 96,2%, dan koefisien determinasi (R^2) sebesar 1. Uji keterulangan dari 6 replikasi isolat DNA daging segar menghasilkan nilai CV 0,72%. Analisis bakso referensi menunjukkan bahwa primer Cytb mampu mengamplifikasi campuran daging anjing hingga konsentrasi 10%. Dapat disimpulkan bahwa, primer Cytb yang telah dirancang dan metode *real-time* PCR yang telah dikembangkan dapat digunakan untuk analisis daging anjing dalam bakso ikan tenggiri guna autentikasi halal.

Kata Kunci: anjing, autentikasi halal, *species-specific primer* (SSP), *real-time* PCR

ABSTRACT

The practice of mixing food using canine meat has been widely found. This practice is motivated by the low price of canine meat in the market. The purpose of this research was to design a species-specific primer (SSP) and develop a real-time PCR method for analyzing canine meat (*Canis lupus familiaris*) in mackerel meatballs intended for halal authentication.

The design of SSP was carried out in silico using NCBI-Primer BLAST and IDT sites. The primer specificity analysis was performed on DNA canine and 8 animals namely mackerel, chicken, pig, goat, frog, cow, rat, and tree shrew using real-time PCR. Method validation is determined by sensitivity test, amplification efficiency, and repeatability test.

The results showed that the Cytb primer (forward: CACTAATCTTCTCTCT GCCATCC, reverse: GAATCGTGTTAGGGTTGCTTTG) which is specifically designed to amplify canine mitochondrial DNA with an optimum annealing temperature of 61.8°C. A sensitivity test was performed on 8 DNA dilution series with 10 dilutions resulting the limit of detection (LoD) value of 1 pg/µL, an amplification efficiency (E) of 96.2%, and a coefficient of determination (R^2) of 1. The repeatability test of 6 replicate DNA isolates of fresh meat resulted in a CV value of 0.72%. Analysis of the reference meatballs showed that Cytb primer could amplify canine meat mixtures up to 10% concentration. Therefore, the Cytb primer that has been designed and the real-time PCR method that has been developed can be used for the analysis of canine meat (*Canis lupus familiaris*) in mackerel meatballs for halal authentication.

Keywords: canine, halal authentication, species-specific primer (SSP), real-time PCR