UNIVERSITI PUTRA MALAYSIA

DETECTION OF RAW PORK TARGETING PORCINE-SPECIFIC MITOCHONDRIAL CYTOCHROME B GENE BY MOLECULAR BEACON PROBE AND REAL-TIME POLYMERASE CHAIN REACTION

MOHD HAZIM BIN MOHD YUSOP

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By

MOHD HAZIM BIN MOHD YUSOP

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

April 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement of the degree of Master of Science

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Chairman: Professor Shuhaimi bin Mustafa, PhD

Institute: Halal Products Research Institute

A real-time polymerase chain reaction (PCR) assay with molecular beacon probe was developed for the detection of pork in raw states. The method combined the swine-specific primers and molecular beacon probe to specifically amplify a 119-bp fragment of porcine mitochondrial cytochrome *b* (mt-cyt *b*) gene. Mitochondrial genes are present in multiple copies and thus ensure available targets even in degraded samples. A pair of 18-nucleotide swine-cytb-specific primers were designed using Primer 3 Plus software. On the other hand, a 34-nt molecular beacon probe was designed using Beacon Designer 4 software. The porcine specificity of the primers and probe were checked by basic local alignment search tools (BLAST) to avoid mismatches with other species. A specificity test with 10 ng DNA of nine common meat providing land and aquatic species yielded a Cq value of 18.70±0.12 to 19.08±0.06 only with the pork DNA in a 40 cycle PCR reaction, demonstrating the swine specificity of the primers and probe. The swine-specificity was further confirmed in a binary mixture of pork and beef. The method detected 0.1% pork in

binary pork-beef mixture with a Cq of 25.79±0.20. A sensitivity test with 10-fold serial dilution revealed that the assay can determine 0.0001 ng of porcine DNA with a PCR efficiency of 96% with a good reproducibility, precision and high correlation coefficient (r^2=0.9989). The shorter length target (119-bp) and strong sensitivity and specificity suggest the method can be used for the routine analysis of pork adulteration in raw meats.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN DAGING BABI MENTAH MENSASARKAN GEN SITOKROM B MENGGUNAKAN PROB PEMANCAR MOLEKUL MASA-NYATA TINDAKAN RANTAI POLIMERASE

Oleh

MOHD HAZIM BIN MOHD YUSOP

April 2012

Pengerusi: Professor Shuhaimi Mustafa, PhD

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Kaedah tindak balas berantai polymerase masa sebenar (PCR) dengan menggunakan prob pemancar molekul telah dibangunkan untuk mengesan daging babi dalam keadaan mentah. Kaedah ini telah menggabungkan sepasang pencetus khas-babi dan prob pemancar molekul untuk mengamplifikasi secara khusus 119 pasangan bes gen mitokondria sitokrom *b* babi. Gen mitokondria hadir dalam salinan yang banyak seterusnya memastikan sasarannya tersedia walaupun dalam keadaan sampel yang hancur. Sepasang pencetus telah direka dengan menggunakan perisian Primer3 Plus manakala satu prob pemancar molekul pula telah direka dengan menggunakan perisian Beacon Designer 4. Spesifikasi kesemua pencetus dan prob telah diperiksa menggunakan BLAST untuk mengelakkan kesalahan padanan dengan spesies-spesies lain. Ujian spesifikasi menggunakan 10 ng DNA daripada sembilan jenis daging haiwan darat dan laut yang biasa dimakan telah menghasilkan nilai Cq (18.70±0.12 hingga 19.08±0.06) khusus untuk DNA babi sahaja, dalam 40 kitaran PCR secara tidak langsung menunjukkan spesifikasi primer-primer dan prob yang

jelas. Ujian spesifikasi pada babi seterusnya dipastikan mengunakan dwi-campuran daging babi dan lembu. Ujian ini berjaya mengesahkan sehingga 0.1% babi dalam dwi-campuran daging babi dan lembu dengan nilai Cq 25.79±0.20. Ujian sensitiviti menggunakan kaedah pencairan sehingga sepuluh kali mendapati DNA babi berjaya dikesan sehingga 0.0001 ng dengan kecekapan PCR sebanyak 96% dan pekali kolerasi yang tinggi (r^2=0.9989). Panjang sasarannya yang pendek (119 pasangan bes), dan sensitiviti serta spesifikasi yang kuat mencadangkan kaedah ini dapat digunakan dalam analisis rutin pencemaran daging babi dalam produk daging mentah.

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I certify that an Examination Committee has met on (26042012) to conduct the final examination of Mohd Hazim bin Mohd Yusop on his thesis entitled "Detection of Raw Pork Targeting Porcine-Specific Mitochondrial Cytochrome *b* Gene by Molecular Beacon Probe and Real-Time Polymerase Chain Reaction" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other instituition.

MOHD HAZIM BIN MOHD YUSOP

Date: 26 April 2012

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