UNIVERSITI PUTRA MALAYSIA

QUALITATIVE AND QUANTITATIVE PCR METHODS FOR DETECTION OF FOODS CONTAINING GENETICALLY MODIFIED SOYBEAN AND CORN

TOSIAH ABDULLAH.

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By

TOSIAH BT ABDULLAH

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

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DEDICATION

I wish to dedicate this work to my beloved Husband Mr. Abd Razak Kadri, my beloved children's, Noor Aida Shazwani, Muhammad Hakimi, Mohammmad Sufi and Abdul Wafi, for their endless support to complete this study.

My beloved Son and My Father, Allahyarham Mohammad Iqbal and Allahyarham Hj. Abdullah b. Hj Bardan, ALFATIHAH,
You are always in my heart,

My Mother Nairah Mukhsan, who always pray for me,

My brothers and my sisters,

My respected teachers and lecturers.

My dearest friends.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Chairman: **Professor Son Radu, PhD**

Faculty: **Food Science and Technology**

Genetically modified organisms (GMOs) can be defined as organisms in which their genetic materials have been altered in the ways that does not occur naturally by mating or natural combination. Polymerase chain reaction (PCR) method is used to detect genetically modified events in foods. The specific objectives of this study are to establish a sensitive, robust and rapid method for the detection of genetically modified events by using PCR and to conduct a preliminary survey for distribution of foods derived from genetically modified events in Malaysia.

The two critical factors that were taken into account to achieve these objectives are the applicability of different DNA extraction methods for each kind of samples and PCR amplification conditions. Three different DNA extraction methods have been tested on soy, corn, potato and tofu (as a processed food).

The DNeasy method as in a widely used commercial kit, Wizard method (Hemmer, 1997) and Cetyl-trimethyl ammonium bromide (CTAB) method (Jankiewicz *et al.*, 1999) were evaluated in this study. The yield and purity of DNA were examined and compared. Quantification was accomplished by measuring UV absorbance at 260 nm and the suitability of DNA for PCR was tested. The results showed that there are significant differences between the methods used. CTAB, Wizard and DNeasy methods produced DNA with ratio of A_{260}/A_{280} range from 1.2 to 1.6, 1.9 to 2.2 and 1.7 to 1.9, respectively. However, the DNeasy method gave the optimum yield of DNA of high purity and was less time consuming. The primer pairs used for confirmation of the endogenous genes in the respective samples (*Lectin1 / Lectin6* for *lectin gene* in soya, *Zein n-3' / Zein n-5'* for *zein gene* in maize and *Pss01 n-5'/Pss01 n-3'* for *patatain gene* in potato) produced the expected size of 318, 157 and 216 base pair, respectively.

The results of this study showed that 18 out of 85 soy samples were contaminated by at least one of three introduced genetic elements consisting *35S promoter*, *Nopaline Synthase terminator* and the structural gene of *5-enolpyruvylshikimate-3-phpsphate-synthase*. Quantitative analysis of the 18 positive genetically modified soy samples showed that, seven samples contains 0.1 - 0.5% *Roundup Ready Soy*, four samples contains 0.5 - 1.0% *Roundup Ready Soy* and seven of them contains 1.0 - 2.0% *Roundup Ready Soy*.

In contrast, none of the 52 was positive with these assays. Therefore they were categorized as non-GM products.

These results revealed that PCR amplification method provides the key advantages of high sensitivity, robust and rapid operation whilst providing the requisites of careful experimental design that avoids both false-negative and/or false-positive results. Seven primer pairs (LEC1/LEC6; Zein n-3'/Zein n-5'; Pss01 n-5'/Pss01 n-3'; P35S 1-5'/P35S 2-3'; HA-NOS118-F/HA-NOS118-R, Cry1(A1)/Cry1(A2) and RRO1/RRO4) chosen in this study produced an expected size of 318, 157, 216, 101, 118, 107 and 356 base pair, respectively, fulfilling the product-size requirement and completed the whole detection procedure of GM events in food samples.

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

PENGESANAN KUALITATIF DAN KUANTITATIF MENGGUNAKAN KAEDAH TINDAKBALAS RANTAIAN POLIMER KEATAS MAKANAN BERASASKAN SOYA DAN JAGUNG YANG TERUBAHSUAI SECARA GENETIK

Oleh

TOSIAH BINTI ABDULLAH

Mac 2006

Pengerusi: **Professor Son Radu, PhD**

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Organisma terubahsuai genetik (GMO) boleh di definasikan sebagai organisma di mana pengubahsuaian kandungan genetiknya tidak berlaku secara kombinasi semulajadi. Kaedah tindakbalas rantaian polimerase (PCR) digunakan untuk mengecam GMOs dalam makanan. Objektif-objektif spesifik dalam kajian ini adalah untuk mengukuhkan kaedah operasi dalam pengecaman GMO dengan PCR yang sensitif, tegap and pantas serta mengendalikan pemeriksaan saringan terhadap pengagihan makanan terbitan dari GMOs di Malaysia.

Dua faktor kritikal yang diambilkira dalam mengecapi objektif-objektif tersebut adalah aplikasi kaedah ekstraksi DNA yang berlainan untuk setiap sampel dan keadaan amplifikasi PCR. Tiga kaedah ekstraksi DNA digunakan didalam kajian ini untuk melihat hasil dan kualiti daripada sampel soya,

jagung, kentang dan tauhu lembut iaitu kaedah Cetyl-trimethyl ammonium bromide (CTAB), Wizard dan DNeasy. Hasil and ketulenan DNA yang dihasilkan di analisa serta dibandingkan menggunakan pancaran Ultra violet pada jarak gelombang 260 nm dan menggunakan tindakbalas rantaian polymerase.

Keputusan dari analisis perbandingan memaparkan bahawa terdapat perbezaan ketara bagi ketiga-tiga kaedah ekstraksi yang digunakan. Nisbah A_{260}/A_{280} bagi kaedah CTAB, Wizard dan DNeasy adalah antara 1.2 hingga 1.6, 1.9 hingga 2.2 dan 1.7 hingga 2.0, masing-masing. Walaubagaimanapun, kaedah DNeasy merupakan pilihan untuk kajian ini kerana kualiti DNAnya yang lebih baik dan masa analisa dapat dikurangkan. Tiga pasang primer khusus untuk pengesanan gen-gen kawalan bagi setiap sampel seperti gen lektin untuk soya, gen zein untuk jagung dan gen patatain untuk potato telah berjaya di amplifikasi dengan penghasilan amplikon bersaiz 318, 157 dan 216 pasangan bes masing-masing.

Keputusan tinjauan menunjukan bahawa 18 daripada 85 sampel soya mengandungi kandungan genetik terubahsuai terdiri daripada sekurang-kurang satu daripada tiga unsur-unsur genetik iaitu *'35S promoter'*, *'Nopaline Synthase terminator'* dan struktur gen *'5-enolpyruvylshikimate-3-phpsphate-synthase'*. Pengesanan secara kuantitatif menunjukkan bahawa daripada 18 sampel soya tersebut, tujuh sampel mengandungi peratusan *Roundup Ready*

0.1-0.5%, empat sampel mengandungi peratusan *Roundup Ready* 0.5 -1.0% dan tujuh yang lain mengandungi peratusan *Roundup Ready* antara 1.0- 2.0%. Sebaliknya, tiada sampel jagung (52 sampel) adalah positif dengan analisis tersebut. Oleh yang demikian, sampel tersebut boleh dikategorikan sebagai produk bukan GM.

Keputusan kajian menunjukkan bahawa kaedah amplifikasi PCR membekalkan kunci kelebihan dari segi sensitiviti, ketegapan dan operasi yang pantas sejurus membekalkan keperluan dalam rekabentuk eksperimen yang teliti untuk mengelakkan keputusan negatif-tiruan dan positif-tiruan. Tujuh pasang primer, khasnya LEC1/LEC6; Zein n-3'/Zein n-5'; Pss01 n-5'/Pss01 n-3'; P35S 1-5'/P35S 2-3'; HA-NOS118-F/HA-NOS118-R, Cry1(A1)/Cry1(A2) dan RRO1/RRO4 yang dipilih dalam kajian ini menghasilkan amplikons dengan pasangan bes sebanyak 318, 157, 216, 101, 118, 107 dan 356 masing-masing telah memenuhi syarat saiz-produk pengesanan genetik terubahsuai didalam sampel makanan.

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LIST OF ABBREVIATIONS

AIA	Advanced Informed Approval
CaMV	Cauliflower mosaic virus
CRM	Certified References Material
CP4-EPSPS	enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp.strainCP4)
CRD	Completed Randomize Design
Ct	Crossing Threshold
CTAB	Cetyl-trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
FRET	Flouresence Resonance energy Trasfer
E	Eficiency
EC	European Council
EDTA	Ethylene-diamine-tetra acetic acid
ELISA	Enzyme Linked Immunosorbant Assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
EtBr	Ethidium bromide
EU	European Union
FAO	Food and Agriculture Organisation
FDA	Food and Drug Association
GIPSA	Grain Inspection, Parkers and Stockyards Administration
GM	Genetically modified
GMAC	Genetically Modification Advisory Committee
GMF	Genetically modified food

GMO	Genetically modified organism
GMOs	Genetically modified organisms
HCL	Hydrogen Chloride
Mg	Magnesium
LFS	Lateral Flow Strip
$MgCl_2$	Magnesium Chloride
MM	Maximizer maize
NA	Nucleic Acid
NaCl	Natrium Chloride
NaOH	Natrium Hydroxide
NIRS	Near-infrared spectroscopy
NOS	Nopaline Synthase
P35S	35S promoter
PCR	Polymerase chain reaction
PEG	Polyethylene glocide
PVP	Polyvinylpyrolidone
QC-PCR	Qualitative Competitive PCR
RNA	Ribonucleic acid
RNase	Ribonuclease
ROSE	Rapid-One-Step-Extraction Solution
RRO	Roundup Ready Oligonucleotide
RRS	Roundup Ready soybean
SDS	Sodium dodecyl sulfate
TBE	Tris-Boric acid-EDTA buffer
UV	Ultraviolet
WHO	World Health Organization

LIST OF SYMBOLS AND UNITS

bp	Base pair
β	Beta
°C	Degree Celsius
U	Enzyme unit
kb	Kilo-base pair
µg	Microgram
µL	Microliter
mg	Milligram
mL	Milliliter
mM	Millimolar
M	Molar
ng	Nanogram
OD	Optical density
%	Percent
pmol	Picomole
rpm	Revolutions per minute
vol	Volume
v/v	Volume per volume
w/v	Weight per volume