

HALAL AUTHENTICATION OF GELATIN CAPSULES USING
POLYMERASE CHAIN REACTION, FOURIER TRANSFORM
INFRARED SPECTROSCOPY AND
CHEMOMETRIC METHODS

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ABSTRACT

Gelatin, a high molecular weight polypeptide obtained from protein, is an important material for capsules of health supplements. A reliable method for *halal* authentication of gelatin is needed because some supplement products are capsulated using gelatin from non-*halal* source. In this study a real-time polymerase chain reaction (PCR) assay using SYBR green fluorescent dye was done to detect porcine in gelatin capsules supplements. The method combined the porcine and bovine specific primers and SYBR green dye to specifically amplify a 120-bp fragment of bovine and 131-bp fragment of porcine mitochondrial *Cytochrome b* (CYT b) gene. Mitochondrial genes were present in multiple copies and thus ensure available targets even in degraded samples. Using basic local alignment search tools (BLAST) with 1 ng DNA standard, bovine and porcine species yielded a melt temperature at $\pm 80.00^{\circ}\text{C}$ only with one specific peak in the melt curve reaction, demonstrating the porcine and bovine specificity of the primers. A sensitivity test with 5-fold serial dilution revealed that the assay can determine 10^{-1} ng/ μl of porcine and bovine DNA with efficiencies of 97.4% and 90.6% with good repeatabilities and high correlation coefficients ($R^2 = 0.995$ bovine; $R^2 = 0.962$ porcine). From a total of 20 samples of supplements in capsules with *halal* certifications from various countries, eight (8) samples were confirmed with adulteration of porcine DNA while four samples were confirmed to contain bovine DNA. FTIR technique was then used to confirm the results of real-time PCR in terms of chemical representation. The FTIR results were later confirmed using principal component analysis (PCA). Eight wavelengths chosen from the twelve samples were 694, 1249, 1342, 1404, 1450, 1558, 1635, and 3271 cm^{-1} .

ABSTRAK

Gelatin, polipeptida berat molekul tinggi yang diperoleh daripada protein, adalah bahan penting untuk kapsul suplemen kesihatan. Kaedah yang boleh dipercayai untuk pengesanan *halal* daripada gelatin diperlukan kerana sesetengah produk tambahan dikumpulkan menggunakan gelatin dari sumber bukan *halal*. Dalam kajian ini reaksi rantai polimerase (PCR) masa nyata menggunakan SYBR hijau pewarna berpendar dilakukan untuk mengesan babi dalam suplemen kapsul gelatin. Kaedah ini menggabungkan sepasang pencetus khas babi dan lembu dan menggunakan pewarna hijau SYBR untuk menguatkan secara khas potongan 120 bp pada lembu dan potongan 131 bp pada babi daripada *Cytochrome b* (CYT b) gen mitokondrial. Gen mitokondria hadir dalam pelbagai salinan dan dengan itu memastikan sasaran yang ada walaupun dalam sampel yang terdegradasi. Menggunakan alat carian penjajaran tempatan asas dengan 1 ng standard DNA, spesies lembu dan babi menghasilkan nilai suhu lebur pada ± 80.00 °C hanya dengan satu puncak spesifik dalam tindak balas lengkung cair, menunjukkan spesifikasi pada susunan basa lembu dan babi. Ujian kepekaan dengan pencairan bersiri 5 kali ganda menunjukkan bahawa ujian dapat menentukan 10^{-1} ng / μ l pada DNA babi dan lembu dengan kecekapan pada 97.4% dan 90.6% dengan penguraian yang baik dan pekali korelasi yang tinggi ($R^2 = 0.995$ lembu; $R^2 = 0.962$ babi). Dari sejumlah 20 sampel suplemen dalam kapsul dengan pensijilan halal dari pelbagai negara, lapan (8) sampel telah disahkan dengan pencampuran DNA babi manakala empat sampel disahkan mengandungi DNA lembu. Teknik FTIR kemudiannya telah digunakan untuk mengesahkan keputusan PCR masa nyata dari segi perwakilan kimia. Keputusan FTIR kemudiannya disahkan menggunakan analisis komponen utama (PCA). Lapan panjang gelombang yang dipilih daripada dua belas sampel adalah 694, 1249, 1342, 1404, 1450, 1558, 1635, dan 3271 cm^{-1} .

الملخص

لقد تم بناء طريقة ردود الفعل باستخدام تلوين الإضاءة الخضراء إس واي بي آر للكشف عن المواد الخنزيرية الكائنة بالملفوفات الجيلاتينية. وتقوم هذه الطريقة بدمج زوج المؤثرات للمواد الخنزيرية و الملون الأخضر إس واي بي آر للقيام بإشباع 120 و 131 زوج من خلايا سيتوكروم بي الكائنة بالبقر و الخنزير. ثم تتكاثر جينات هذه الخلايا للتأكد من وجودها ولو على شكل دقيق. ويرجع الفضل في اكتشاف هذه المؤثرات إلى تنابي و زملاؤه. وقد تم تحديد مواصفات جميع المؤثرات باستخدام بي أل أس تي وتم اختبار انحاء الانصهار تجنباً من الوقوع في خطأ الاشتباه بأنواع أخرى. و نتج من الاختبار التدقيقي باستخدام 1 أن جي دي أن أ من نوع البقر و الخنزير قيمة درجة الانصهار 80 درجة حرارية و بنقطة قمة التدقيق بمنحنى الانصهار للدلالة على التدقيق الحقيقي. وقد أجري هذا الاختبار 5 مرات و تمكن من الحصول على دي أن أ الخنزير و البقر بنسبة 10 أن جي دي أل و بدقة في سي آر بقدر 97.4 % و 90.6 % و بعدد التغيرات العالية $0.995 = \text{مربع}$ للبقر، و $0.962 = \text{مربع}$ للخنزير. و بحسب طول الاتجاه القصير و درجة الحساسية و الدقة المتناهية لهذه الطريقة، تقترح الباحثة استخدامها في عملية التحليل و الكشف عن ملفوفات الجيلاتينات المخزرة بدلا من استخدام في سي آر التقليدية. وقد تم إجراء الاختبار على 8 عينات بها مواد خنزيرية إيجابية ، و 4 عينات بها مواد بقرية وذلك باستخدام طريقة إف تي آر أي للتأكد من صحة نتيجة في سي آر من الناحية الهيكل الكيميائي، مع القيام بتحليل المحتوى العام في سي آر. وقد تم اختيار الأمواج الثمانية من بين 12 عينة وهي: 694، 1249، 1342، 1404، 1450، 1558، 1635 و 3271 سي أم مربع

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LIST OF ABBREVIATIONS

ATR	Attenuated Total Reflectance
Bp	Base pairs
BLAST	Basic Local Alignment Search Tool
Ct	Cycle Threshold
CTAB	Cetyl Trimethylammonium Bromide
CYT b	<i>Cytochrome b</i>
dATP	Deoxyadenosine Triphosphate
dCTP	Deoxycytidine Triphosphate
dGTP	Deoxyguanosine Triphosphate
dTTP	Deoxythymidine Triphosphate
dNTPs	Deoxyribonucleosides Triphosphate
DNA	Deoxyribonucleic Acid
dsDNA	Double Stranded Deoxyribonucleic Acid
EB	Elution Buffer
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
FTIR	Fourier Transform Infrared
GC	Guanine Cytosine
gDNA	Genomic Deoxyribonucleic Acid
GMIA	Gelatin Manufactures Institute of America
HPLC	High Performance Liquid Chromatography
HRM	High Resolution Melt
ICH	International Conference on Harmonization
ISO	International Organization of Standardization
LoD	Limit of Detection
LoQ	Limit of Quantification
mRNA	Messenger Ribonucleic Acids
MS	Mass Spectrometric
mtDNA	Mitochondria Deoxyribonucleic Acid
NCBI	National Center for Biotechnology Information

NTC	No Template Control
PB	Phosphate Buffer
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
qPCR	Quantitative Polymerase Chain Reaction
RNase	Ribonuclease
RSD	Relative Standard Deviation
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SNPs	Single Nucleotide Polymorphisms
STR	Short Tandem Repeats
TAE	Tris Acetate EDTA
TE	Tris-EDTA
T _m	Melting Temperature
T _a	Annealing Temperature
WHO	World Health Organization

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