HALAL FOOD AUTHENTICATION
“Nanotechnology Perspective”

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Global Halalan Toyyiban Trade & Industries

Global H & Market, 2005** Percentage Estimate

100% = USD2.77 trillion

- Primary meat processing
  - Pharmaceutical: 23%
  - Nutraceutical: 6%
  - Confectionery: 5%
  - Cosmetics and personal care: 9%

- Processed food & beverages
  - Bakery products: 35%
  - 12%

Can we provide factual evidence to confirm that a PRODUCT is halal??
OUTLINE OF PRESENTATION

• CONCEPT on Halalan Toyibban
• Concern for Halal FOOD Origin a Serious Threat
• Detection methods
  – DNA via PCR
  – DNA via nanobiosensor

Halalan Toyibban Assurance: Functions & Operating Model

HTA & Halal Trading Hubs
### Halalan Toyyiban Assurance Service

#### Production

**PRODUCT VERIFICATION**
- Testing & Analysis of Products – Contamination Level Determination, Specification Verification
- Production Quality Verification – Process, Facilities and Raw Material
- Applicable Standards: ISO/IEC 17025, MS 1500, HACCP, GMP, MS 2400

#### Delivery

**DELIVERY ASSURANCE**
- Delivery Participants Standards Compliance Certification
- Change of Custody Process Verification
- Certification of Delivery based on Prescribed Standards
- Information Repository
- Applicable Standards: MS 2400, BRC Food Quality Standards, ISO 22000, MS 1500

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### Research Background

**Food Forgery**

- The most-concerning and most articulated socio-economic issue
- Has impact on health, religious rituals, food choices, economy and fair trades
- Food-borne Zoonotic threats:
  - transmission of HIV to human race from African Chimpanzee
  - transmission of BSE (bovine spongiform encephalopathy)
  - transmission of H5N1 virus and avian influenza
- Wild-life trafficking and endangered species
- Halal, kosher, vegetarian, GM, safe food certification
Rat Meat Scandal in China

Real mutton(A) and fake meat(rat)(B) and Mixed Meat foods(C)  Source Public safety blog

Ref: http://www.guardian.co.uk/world/2013/may/03/china-fake-meat-rat-mutton

Horse Meat Scandal in Europe

European supermarkets flooded with horse meat

Ref: http://www.guardian.co.uk/world/2013/may/03/china-fake-meat-rat-mutton
Research Background

Authentication Technique

- **Organoletic and Microscopic**
  - Cannot detect food components due to breakthrough in food processing and packing technologies
  - Modification of microscopic bio-markers

- **Spectroscopic and immunological testing**
  - Expensive and need expertise skills
  - Modification of maker proteins

- **PCR and DNA barcoding**
  - Cannot detect shorter DNA markers which are stable under extensive processing conditions
  - Expensive and time consuming

- **Biosensors and Microarray Techniques**
  - Can detect shorter DNA markers
  - Portable, Less expensive and fast detection
  - Detection of multiple species in a single assay platform

Current Authentication Methods:
**MOLECULAR ANALYSIS**

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Electrophoresis, Blotting, Sequencing, Sensors and Chips</th>
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<tbody>
<tr>
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<td>Chromatography, LC/GC-MS, MALDI-TOF MS, ELISA</td>
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| Lipids                 | FTIR, LC/GC-MS                                            |
|                        | Electronic nose coupled to MS                             |

| Nucleic Acids          | PCR, PCR-RFLP, RealTime PCR                               |
|                        | Biosensors and Chips                                      |

| Carbohydrate           | Cheap and usually plant origins                           |
|                        | Biosensors and Chips                                      |
**Methods for DNA detection**

- **Lipid Based Method**
  - Amount can be extensively modified
  - Cross reaction in closely related species

- **Protein Based Method**
  - Denaturation in heat, pressure and chemical shock

- **DNA Based Method**
  - Codon degeneracy
  - Detectable in every cell
  - More stable under heat condition
  - Identification is possible in complex background

**Not well suited for specific species detection**

- **Conventional PCR**
  - Require gel electrophoresis
  - Time consuming expose to hazardous reagent

- **PCR-RFLP**
  - Require gel electrophoresis
  - Restriction enzyme

- **RT-PCR**
  - Fast automated
  - Expensive instrumentation & reagent

**Can not detect short-length oligo targets**

**Biomarker testing - Species specificity**

- **Biomarker size**: 100 bp
- **Annealing Temperature**: 58°C
- **Specificity testing**: Dog and other animal and plant species DNA.
  - **Result**: 100% canine specific
DNA Stability Test

- Raw
- Boiled
- Autoclaved

**Mixed meat matrix**
Dog X Chicken X Beef

Detection limit 0.1% of dog meat

**Meat-plant matrix**
Dog X Chicken X wheat flour
Detection limit 0.1% of dog meat
Frankfurter Analysis

**Frankfurter model:**
(Chicken and beef)
Detection limit
0.1% dog meat

**Detection Limit** 0.1%

**Commercial Frankfurter**
- 3 brand
- Positive control: 0.1% dog
- No positive

**PCR based DNA detection Methods**

- **Conventional PCR**
  - Require gel electrophoresis
  - Time consuming
  - Expose to hazardous reagent

- **PCR-RFLP**
  - Require gel electrophoresis
  - Restriction enzyme,

- **RT-PCR**
  - Fast automated
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**Can not detect short-length oligo targets**

**NANOTECHNOLOGY IS A SOLUTION**
Basic Principle Mechanism of SERS

Chemical: Charge transfer between the molecule and metal that modifies the response

Electromagnetic: Collective oscillations of electron in metal that induce near field at the molecule

Functionalization of SERS Active Nanomaterials

Modified DNA/ RNA

Raman label

Biomaterials immobilization

SERS active Nanostructure
Colorimetric Sensor for Pork Detection

(a) Pure pork (b) pork-beef (c) pork-chicken (d) chicken-beef (e) pure beef @ 1:1 w/w ratio

AuNPs aggregate in presence of pork targets in pure (a) and mixed forms (b, c). This could be detected by visual change of color and by UV-vis spectroscopy.

Fluorescence Sensor: Pork Detection

Fluorescence intensity is increasing when the number of mismatches are decreasing.

It can detect even a Single Nucleotides Polymorphism

It can detect MISMATCH of Single Nucleotides Polymorphism
Meatball analysis - Pork DNA Quantification

Fluorescence intensity is proportional to the concentration of pork DNA.

General Feature of SERS based Biosensing

- **Nanotechnology**
  - Noble Nanoparticle Synthesis

- **Biotechnology**
  - DNA Bioprobe Designing
  - DNA Immobilization
  - Nanobio hybrid

- **Raman Spectroscopy**
  - Raman Reporter molecules
  - Target Species DNA
  - Intensity
  - Raman Shift

Species | Sequence | MiMacch | GenBank | Meat
--- | --- | --- | --- | ---
Sus scrofa | CTA CGG TCA TCA CAA ATC TAT CAG | 0 | GU135837.1 | Pork
Bos taurus | CAA CAG TCA TCA CAA ACC TCT TAT CAG | 6 | EU907918.1 | Beef
Gallus gallus | CAA CAG TTA TCA CAA ACC TCT TAT CAG | 6 | EU894544.1 | Chicken
Capra hircus | CAA CAG TCA TCA CTA ATC TCT TAT CAG | 5 | EU130780.1 | Sheep
Ovis aries | CAA CAG TTA TCA CAA ACC TCT TAT CAG | 8 | EU565980.1 | Mutton
SERS Active DNA Detection

SERS Tool in DNA Sequencing and Single Molecule Detection


Z. Yi., et al., Biosensors and Bioelectronics 43 (2013) 308–314
SERS Based DNA Mismatch (SNP) Detection

(a) SERS spectra in the absence of target DNA
(b) SERS spectra in the presence of non-complementary DNA
(c) Double bases mismatched
(d) Single base mismatched
(e) Complementary DNA target

SERS intensity is increasing when the number of mismatches are decreasing

B. The corresponding histogram represented with the peak area at 1612 cm$^{-1}$

Z. Yi., et all., Biosensors and Bioelectronics 43 (2013) 308–314

SERS Based DNA Quantitative Analysis

SERS intensity is proportional to the concentration of target DNA

LOD -10 nM (nanomole)

Z. Yi., et all., Biosensors and Bioelectronics 43 (2013) 308–314
Summary

- Origin of Product (Meat) can be detected based on:
  - Lipid; protein; nucleic acid (DNA)

- DNA is stable; does not degrade under harsh treatment

- It can be analysed using PCR techniques, but less suitable when the DNA length is short

- Nanobiosensor can detect short DNA length:
  - Specific
  - Quantitative
  - Low detection limit
  - Can be Single Nucleotide Polymorph

Continuing Research Challenges

- Lack of Biomarkers
  - Shorter DNA markers with adequate species finger prints are not available at sufficient numbers and varieties

- Non-Multiplexed
  - Usually detect single species
  - Are not multiplexed and tested under complex matrices

- Poor sensitivity
  - Non-integration of biomarkers, Nanomaterials and spectroscopic detection
Halal Testing Group
Dr Mahfujur, Asing Rakhine, Raifana Abdul Rashid, Abdur Razzak, Muhammad Al Amin, Dr. Md. Eaqub Ali