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Application News

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Halal Authentication Analysis / MultiNA

Sensitive Detection of Pork DNA in Processed Meat products on PCR-MultiNA Platform

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Introduction

The authentication of species content in processed meat and food products is implemented in many countries for various reasons such as economic and cultural beliefs [1, 2]. Such identification is of importance in various religious communities where consumption of a particular species of meat is prohibited. Based on Islamic Shari'ah (law), the term Halal is often used in reference to food and drinks, where they are permissible for Muslims to consume. The most common example of non-halal (Haraam) food is pork. In this Application News, a highly sensitive method is described for detection of pig DNA in processed food such as sausages. It is based on DNA extraction and species-specific PCR amplification of pork specific, followed by microchip electrophoresis detection on MultiNA [3]. This PCR-MultiNA method is highly sensitive in detecting the extracted pork DNA fragment, with housekeeping fragments as confirmation.

Experimental

Reagents / Kits

- DNeasy mericon™ Food Kit (50) from QIAGEN
- PCR Mastermix Pod: Pork from NeoGen Corporation
- DNA-500 Reagent Kit for MultiNA from Shimadzu
- SYBR Gold nucleic acid gel stain from *life* Technologies
- 25 base-pair ladder from Invitrogen

Analytical Conditions for PCR Products

- Instrument : MCE-202 MultiNA
- Analysis Mode : DNA 500 On-Chip Mode

Procedure for detection of Pork DNA

The process workflow is described in Figure 1. It consists of three steps: (1) extraction of DNA from sausage sample, (2) species-specific PCR amplification of the extracted DNA and (3) detection of the pork DNA fragment by microchip electrophoresis on MultiNA.

DNA extraction from sausage sample was carried out using DNeasy mericon Food Kit from QIAGEN in accordance with the protocol of the kit [4]. The extracted DNA sample was amplified via PCR using BioKits PCR Mastermix Pod, pork specific obtained from Neogen Corporation [5]. DNA amplification was carried out in a 25 µL volume reaction mixture, which contained 19.9 µL of Pork PCR mastermix, 0.1 µL of NovaTaq™ Hot Start DNA polymerase from Novagen, 4 µL of TE buffer and 1 µL of the DNA extract. The amplification was run using the following program: 94°C for 10 minutes for activation of Hot Start Taq, followed by 30 cycles at 94°C for 15 seconds, 64°C for 15 seconds, and 72°C for 15 seconds, and ended by the extension step at 72°C for 3 minutes. The PCR products were held at 4°C until subjecting to microchip electrophoresis on MultiNA. The amplified PCR products were then analysed using Shimadzu MCE-202 MultiNA.

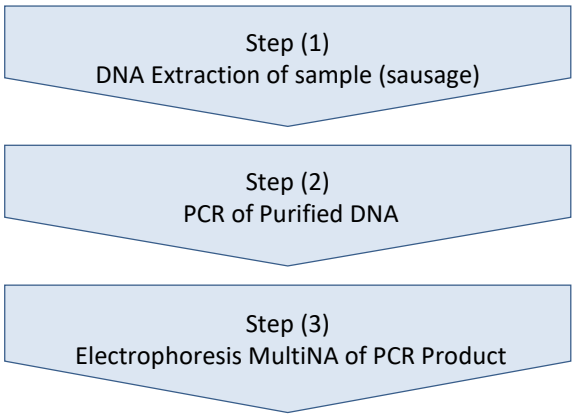

Figure 1: Experimental procedure for detection of Pork DNA on MultiNA

Results and Discussion

PCR-MultiNA procedure: The BioKits PCR Mastermix Pod is used normally with agarose gel electrophoresis for detection of the targeted species DNA [5]. Instead of the agarose gel electrophoresis, microchip electrophoresis (MCE) was used to detect the DNA fragment on MultiNA in this work [3]. The advantages of the MCE method are high detection sensitivity, easy quantitation, fast and fully-automated operation. The PCR tubes after PCR process of food samples can be loaded onto the MultiNA directly. The subsequent analysis for detection of targeted species DNA is carried out in a fully-automated manner from adding reagents, system checking, ladder calibration to batch sample measurement on the MultiNA. The results are displayed in electropherogram (Figure 2). The precise area of the

electropherogram peak of pork DNA (314 bp) can be obtained, which is proportional to the amount of the DNA molecules in the sample.

Figure 2 shows that the PCR Mastermix, pork specific, amplified the pork DNA fragment 313 bp (target size: 314 bp) and the housekeeping fragment 395 bp (target size: 380-420 bp) in pork sausage sample selectively. In the DNA extract of chicken sausage, pork DNA fragment was not detected. The only observed fragment was the housekeeping DNA at 418 bp. A negative control of PCR mixture without any template DNA was included as blank.

The accuracy and reproducibility of the PCR-MultiNA method are summarized in Table 1. The targeted pork DNA fragment is 314 bp. The measured size is at 314-324 bp, which is within the accuracy specification of MultiNA (±5%). The reproducibility of the method is at 0.2%~1.0%.

Table 1: Accuracy and reproducibility of PCR-MultiNA procedure for detection of pork DNA using Neogen Biokits PCR Mastermix

Pork Content	Repeat No. (n)	Measured Ave (bp)	Accuracy, Ave (%)	RSD (%)
100%	4	317.7	101.2	0.74
3% & 5%	3	316.3	100.7	1.02
1%	3	316.7	100.8	0.18
0.5%	4	320.3	102.0	0.95
0.1%	5	322.0	102.5	0.54

Sensitivity: Pork sausage was spiked into halal certified chicken sausage to prepare spiked samples with pork content in 0.1%~5%(wt) for testing the detection sensitivity and reliability of the method. The results (Figure 3, Table 1) show that the PCR-MultiNA method established is able to detect pork DNA fragment (314 bp) in chicken sausage spiked with pork sausage at level as low as 0.1 % firmly. Furthermore, the peak areas of five measurements of 0.1% pork-spiked chicken sausage samples prepared in different days are considerably consistent with a CV (Coefficient of Variation) of 40.6%. For 0.5% pork-spiked chicken samples, the CV of four measurements are 20.7%. A correlation between peak area and content of pork in the sample is plotted in Figure 4, which reveals that the peak area increases proportionally with the pork content at a low range (0.1%~1%). Based on this finding, it is possible to establish at least a semi-quantitation method to detect the presence and content level of pork in processed food.

Conclusions

Using Neogen Biokits PCR Mastermix Pod, pork specific (314 bp), a highly sensitive procedure for detection of pork in processed meat and food products was established on a PCR-MultiNA platform. The microchip electropherosis and fluorescence detection of MultiNA exhibits the advantages of high sensitivity, reliability and easiness in operation. The method can detect as low as 0.1% (wt) of pork sausage spiked in halal certified chicken sausage sample.

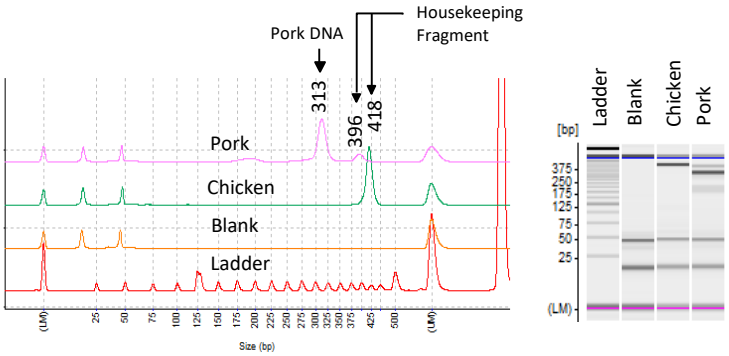

Figure 2: Electropherograms (EC) of PCR product of food samples (left), Detected peaks are pork DNA fragment (~313bp), housekeeping fragments (pork~395bp, chicken~418bp) and DNA ladders . A gel imaging display converted from EC (right).

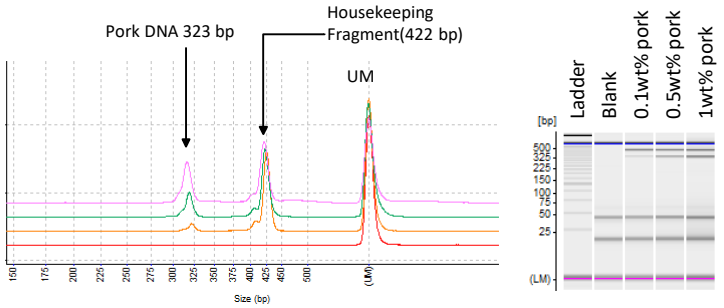

Figure 3: Detection of pig DNA fragment (~313bp) in chicken sausage samples spiked with 0% (blank), 0.1%, 0.5% and 1% of pork sausage (from bottom to top).

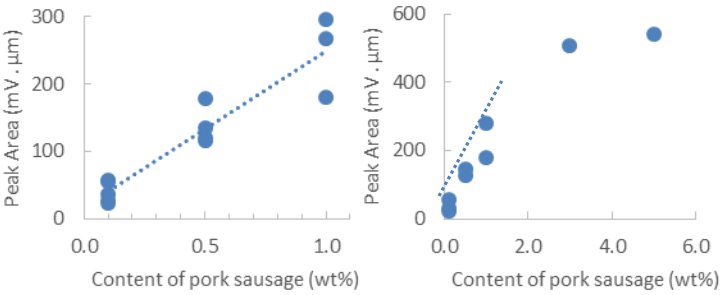

Figure 4: Correlation of area of electropherogram peak with content of pork sausage spiked in chicken sausage sample on PCR-MultiNA using Biokits PCR Mastermix Pod.

References:

1. S. Edris, M.H.Z. Mutwakil, O.A., Abuzinadah, H.E. Mohammed, A. Ramadan, N.O. Hadalla, A.M. Shokry, S.M.Hassan, R.M. Shoaib, F.M. El-D0omyati and A. Bahieldin, Life Sci. Journal, 2012; 9(4), 5831-5837.
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3. Application Notes 11, Rapid Identification of Meat Species with MCE-202 "MultiNA", Shimadzu.
4. DNeasy® *mericon*™ Food Handbook, QIAGEN Sample & Assay Technologies. 2010.
5. Biokits Animal Speciation Selection Module, Neogen® Corporation 2013, 8 – 10.

Note: The Application and Shimadzu Instruments are for research use only, not for use in diagnostic procedures.

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