SHIMADZU

Application News

MALDI-TOF Mass Spectrometry No.B23

Detection of Allergenic Substances with MCE-202 MultiNA

Japan is the world's earliest adopter of a display system for foods containing allergens. In April, 2001 it become mandatory that food labels include likely allergens, and in June, 2008, two additional food articles, prawn and crab, were added to that list of likely allergens. The dissemination of allergen-related information to the consumer using this label display is meant to help avert the possibility of allergic responses and harm to health beforehand. Therefore, if specific substances are included or mixed in among the other ingredients, even at ultra small quantities, the label is required to inform the consumer to that fact. Among the allergenic substances required to be mentioned on the label, those that can be detected by qualitative PCR are wheat, buckwheat, peanuts, prawn and crab. Here we conducted amplification of allergen-related genes by PCR using extracted DNA as the template, according the method specified by The Japanese Ministry of Health, Labour and Welfare (Ministry of Health, Labour and Welfare Notification "Regarding the testing method for foods containing allergenic substances," No. 0724, Publication No. 1 issued by the Dept. of Food Safety, July 24, 2009). Here we introduce the detection of these substances using the MCE-202 MultiNA Microchip Electrophoresis System for DNA/RNA analysis.

■ Experimental Procedure

The DNA extraction and PCR were conducted according to the method and conditions specified in the above-mentioned Ministry of Health, Labour and Welfare Notification. We extracted the DNA from food products containing the wheat, buckwheat, peanuts, prawn and crab, respectively. Ion-exchange resins were used for carrying the extractions. The DNA purity verification and quantitation were conducted using the Shimadzu BioSpec-nano Life Sciences Spectrophotometer.

■ Reagents / Kits

DNA-500 Kit
(Shimadzu) P/N: 292-27910-91
SYBR® Gold nucleic acid gel stain
(Invitrogen) S-11494
25 bp DNA Ladder
(Invitrogen) 10597-011
QIAGEN Genomic-tip 20/G
(QIAGEN) 10223

■ Analytical Conditions for PCR Products

Instrument : MCE-202 MultiNA
Analysis Mode : DNA-500 on-chip mode

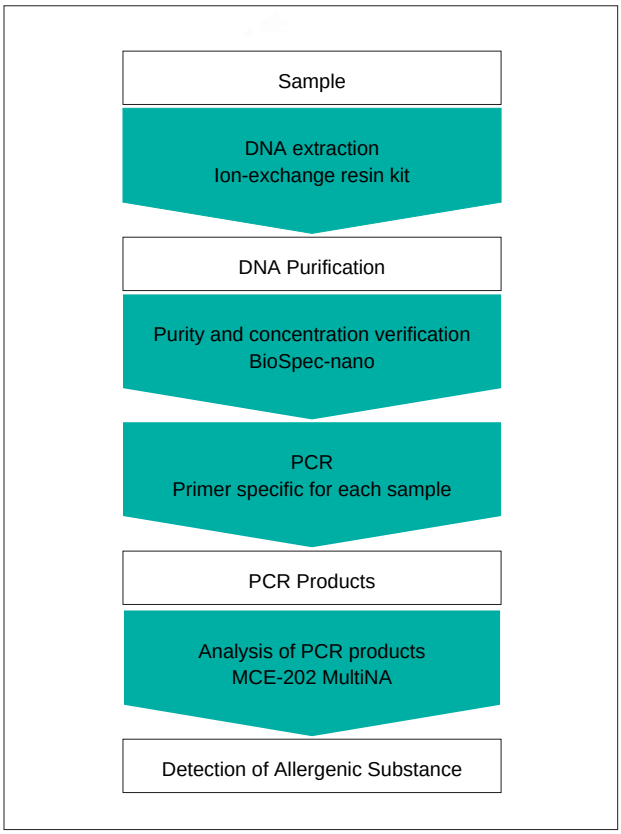


Fig. 1 Experimental Procedure for Detection of Allergenic Substances

■ Results

The results of analysis of the PCR amplification products of DNA derived from wheat, buckwheat, peanuts, prawn and crab, respectively, using the MultiNA are shown in Fig. 2. The PCR amplification products derived from the wheat, buckwheat, peanuts, prawn and crab substances were all clearly detected using the MultiNA. (The estimated sizes shown in the figure were obtained in this experiment. The sizes of PCR-amplified DNA indicated in The Ministry of Health, Labour and Welfare Notification are wheat: 141 bp, buckwheat: 127 bp, peanuts: 95 bp, prawn: 187 bp, and crab: 62 bp.)

The results of analysis by agarose gel electrophoresis of the same samples as those analyzed with the MultiNA are shown in [Reference]. The sizes of the PCR amplification products are imprecise due to lack of objectivity in interpreting the gel electrophoresis results. However, the results obtained using the MultiNA consist of an electropherogram in addition to a gel image, ensuring a high level of accuracy. Particularly noteworthy is that the amplification products of wheat and buckwheat are very near each other. Compared to agarose gel electrophoresis, the MultiNA's excellent resolution and sensitivity allow these to be clearly detected.

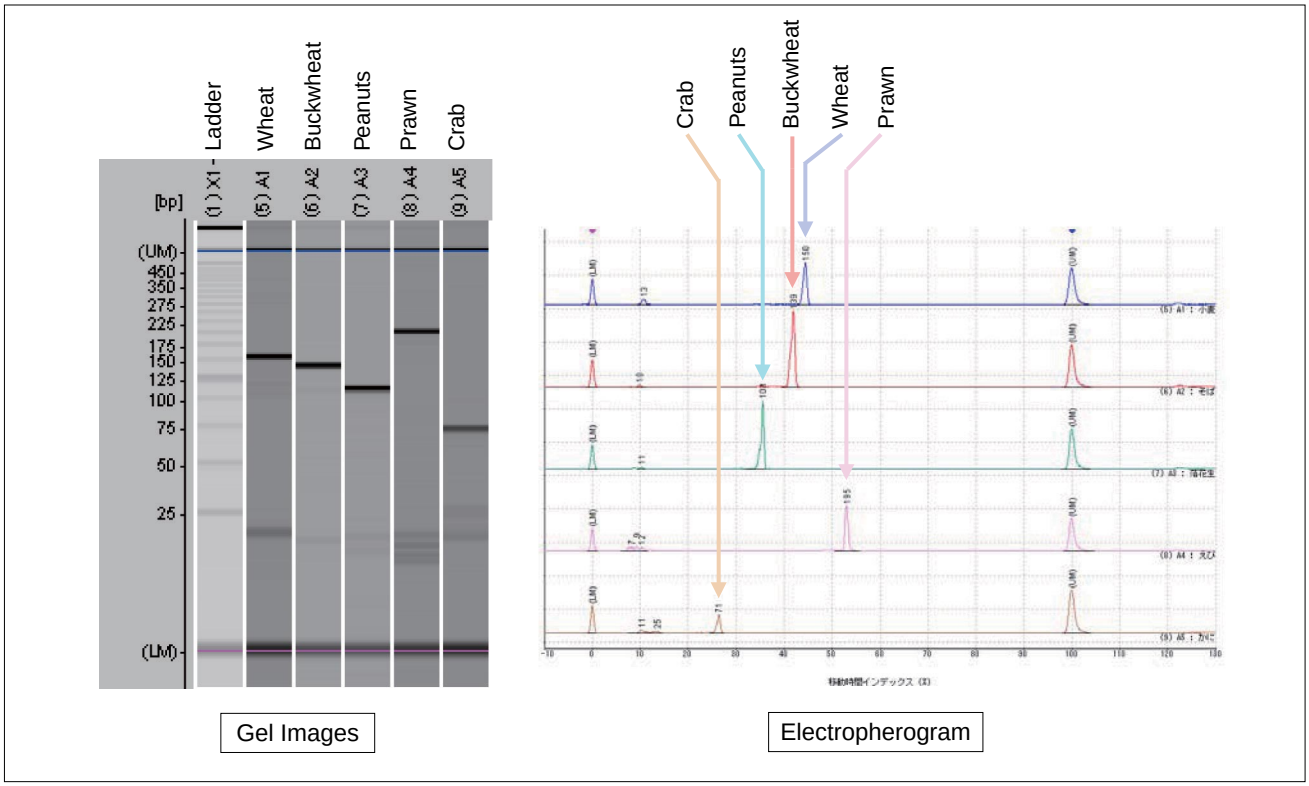
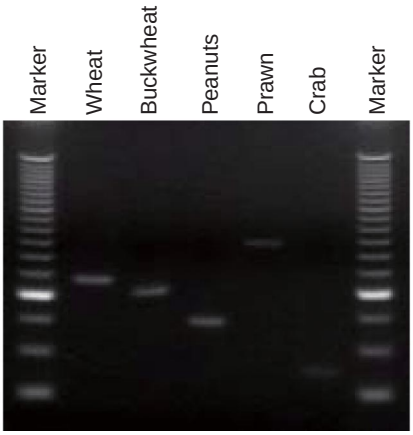


Fig. 2 Analytical Results for PCR Products from Allergenic Substances



[Reference] Agarose Gel Electrophoresis of PCR Products from Allergenic Substances

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