SHIMADZU
Excellence in Science

Microchip Electrophoresis System MultiNA™ II MCE-301

Application News

Analysis of Allergen Genes in Food Using a Microchip Electrophoresis System

Hitomi Tsujihata, Nozomi Maeshima, and Yuji Sogabe

User Benefits

- Allergen testing in food is available efficiently.
- The DNA fingerprinting analysis function automatically determines the presence or absence of bands compared to the reference.
- MultiNA II can fully automate electrophoresis gel preparation, sample injection, electrophoresis, and detection.

■ Introduction

In 1963, the Food and Agriculture Organization and the World Health Organization established the Codex Alimentarius Commission (CAC) to protect the health of consumers and ensure fair practices in international food trade. The CAC develops international food standards and guidelines, including food labeling, and many countries use these standards as a reference for their own food labeling systems. The CAC standard for the labeling of pre-packaged food (CODEX STAN 1-1985) includes requirements for allergen labeling. Since food allergies cause a wide range from mild, localized symptoms to serious, systemic symptoms, many countries have developed labeling systems and testing methods for allergens.

Based on fact-finding studies and symptom severity, Japan has designated the following eight food allergens as priority ingredients: egg, milk, wheat, buckwheat, peanut, walnut, shrimp, and crab[1].

This article describes an analysis of food allergens that was performed based on the Japan Consumer Affairs Agency (CAA) notification: "Inspection methods for foods containing allergenic substances." The analysis results of PCR products for wheat, buckwheat, peanuts, shrimp, crab, and walnuts are presented.

■ Testing Methods for Foods Containing Allergen

Enzyme-linked immunosorbent assay (ELISA) is used for quantitative testing of food allergens, and Western blotting and polymerase chain reaction (PCR) are used for qualitative testing. While ELISA is simple, it may mistakenly detect similar proteins, producing false-positive results. Therefore, when ELISA cannot reliably identify an allergen, PCR and Western blotting are used.

PCR targets genes that are specific to allergenic ingredient, which allows them to differentiate between closely related species such as wheat and barley. In addition, DNA is more stable than protein during processes such as heating and fermentation, allergen genes are more likely to be detected in processed foods. In contrast, PCR is difficult to identify foods including same genes such as egg and milk. In this case, these allergenic ingredients can be identified using Western blotting, which uses electrophoresis and an antigen-antibody reaction to identify specific proteins and their molecular weights.

■ Sample Preparation and Analysis Conditions

The workflow from sample preparation to analysis is shown in Fig. 1. Each process is described below.

1. DNA Extraction

Wheat, buckwheat, peanut, walnut, shrimp, and crab were used as positive controls, and confectioneries were used as samples. DNA extraction was performed using the GM quicker 3 (Nippon Gene, P/N: 311-07241). Following the protocol for the DNA extraction kit, DNA was extracted from 1 g of each homogenized sample.

2. PCR

AmpliTaq Gold (Applied Biosystems, P/N: 43-118-06) includes DNA polymerase, PCR buffer, magnesium chloride, and deoxynucleotide triphosphate for the PCR. PCR amplification was performed using extracted DNA and primers specific to wheat, buckwheat, peanut, shrimp, and crab DNA based on the CAA notification method.[2]

The Allergen Checker [Walnut] (Oriental Yeast, P/N: 49567000) was used as primer of walnut, and PCR amplification of walnut was performed based on the included conditions.

3. Electrophoresis Using MultiNA II

Electrophoresis was performed using the DNA-500 reagent kit and SYBR Gold fluorescent dye. The MultiNA II was used to perform electrophoresis on PCR products.

4. Fingerprinting Analysis

Base pair lengths specific to each allergen were used to confirm the presence or absence of each allergen gene in the confectionery.

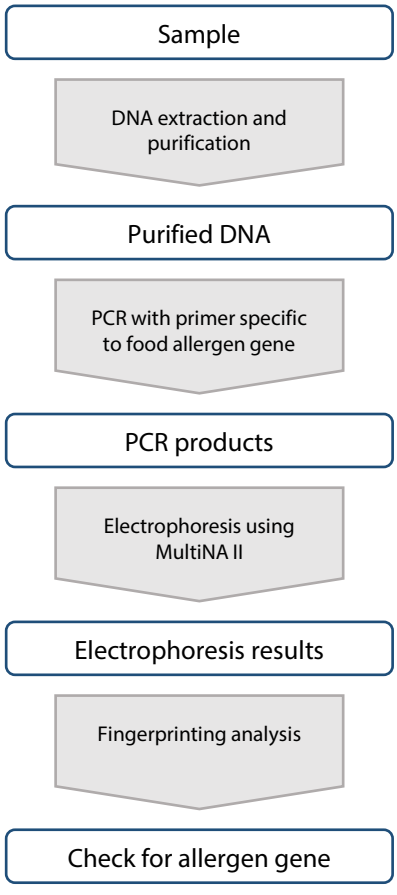


Fig. 1 Workflow from Sample Preparation to Analysis

■ Electrophoresis Results

Fig. 2 shows the electrophoresis results of the PCR products for shrimp, wheat, buckwheat, walnut, peanut, crab, and the confectionery. According to the CAA notification, PCR amplification fragment lengths unique to shrimp, wheat, buckwheat, peanut, and crab are defined as 187, 141, 127, 95, and 62 base pairs, respectively. Each of these amplification fragments was clearly detected by electrophoresis. Walnut was detected as a 120 base pair length amplification fragment, as specified by the Allergen Checker [Walnut]. Electrophoresis of the confectionery after PCR amplification with a wheat primer (A6) and the confectionery after PCR amplification with a walnut primer (E6) detected amplification fragments specific to both wheat and walnut, respectively. Fingerprinting analysis was also performed on each of these amplification fragments.

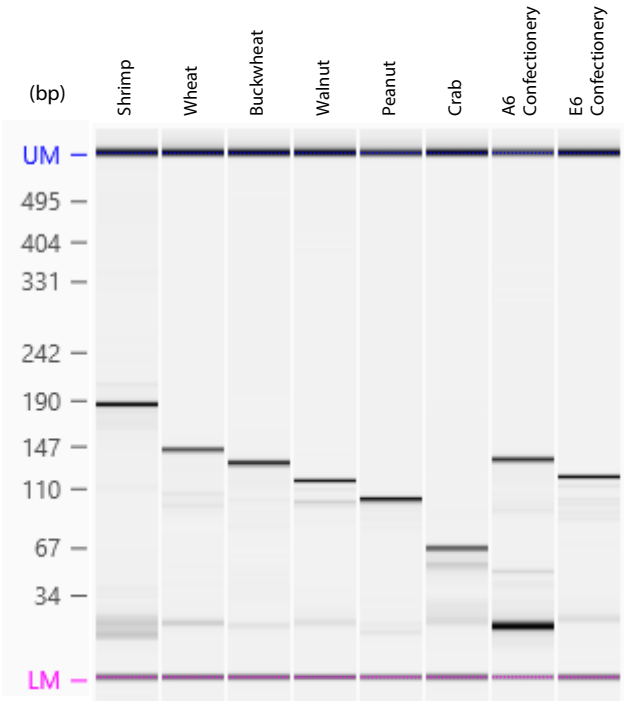
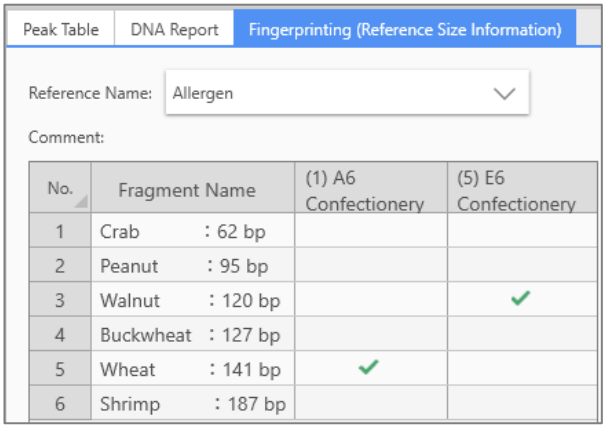


Fig. 2 Electrophoresis Results

■ Fingerprinting Analysis

The fingerprinting analysis function automatically determines whether the same DNA fragment is present or absent in the sample using empirical data or a specific length of base pairs as a positive control.

For this analysis, the base pair lengths for shrimp, wheat, buckwheat, peanut, and crab noted in the CAA notification method and the base pair length for walnut noted in the Allergen Checker [Walnut] were used as positive controls to check for allergen DNA in the confectionery. The allowable tolerance for base pair length was set to 5 % in the judgment criteria. As a result, the A6 confectionery was determined to contain wheat gene, the E6 confectionery was determined to contain walnuts gene (Fig. 3). The allergen DNA detected in the confectionery matched the ingredients listed on the confectionery packaging.



No.	Fragment Name	(1) A6 Confectionery	(5) E6 Confectionery
1	Crab : 62 bp		
2	Peanut : 95 bp		
3	Walnut : 120 bp		✓
4	Buckwheat : 127 bp		
5	Wheat : 141 bp	✓	
6	Shrimp : 187 bp		

Fig. 3 Fingerprinting Analysis of a Confectionery

■ Conclusion

The allergen genes for wheat, buckwheat, peanut, walnut, shrimp, and crab were detected using MultiNA II. The fingerprinting analysis also automatically determined wheat and walnut allergens in a confectionery.

The MultiNA II fully automates electrophoresis gel preparation, sample injection, electrophoresis, and detection, and it also offers superior separation and reproducibility compared to agarose gel electrophoresis. The tests performed in this article demonstrate that MultiNA II can effectively performs allergens testing in food using PCR amplification fragment.

According to CAA notification methods, real-time PCR or PCR-DNA chromatography can be used to detect walnut DNA. The results for walnut DNA obtained by the method described in this article are shown as screening results.

References

1) Handbook on food allergy labeling of processed foods, Consumer Affairs Agency, March 2023 Edition.
2) Appendix: Inspection methods for foods containing allergenic substances, Consumer Affairs Agency, Notification concerning food labeling standards

01-01110-EN First Edition: Mar. 2026

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

› Please fill out the survey

Related Products Some products may be updated to newer models.

› MultiNA II MCE-301
Microchip Electrophoresis System

Related Solutions

› Food and Beverages

› Food Dietary Restrictions

› Price Inquiry

› Product Inquiry

› Technical Service / Support Inquiry

› Other Inquiry