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A method for the detection of shrimp/prawn and crab DNAs to identify allergens in dried seaweed products

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Abstract

Crustacean protein (tropomyosin) has frequently been detected in processed foods containing seaweed. In Japanese regulations for the labeling of allergenic food ingredients, the PCR method for detecting extracted shrimp/prawn and crab DNA is stipulated to discriminate shrimp/prawn and crab in processed foods. It has, however, been difficult to extract shrimp/prawn and crab DNA in processed foods including seaweed. We modified the DNA extraction protocol of the DNeasy *mericon* Food kit, and compared the yield and purity of DNA extracted from dried seaweed powder containing 1, 5, 10, 100, or 10,000 μg/g of freeze-dried edible shrimp/prawn or crab using various commercially available DNA extraction kits. The improved DNA extraction method provided sufficient yield and purity of extracted DNA suitable for the detection of specific DNA using the PCR method. To directly evaluate the applicability of the DNA extraction method, we employed PCR amplification with primers (PyrbcL01–5'/PyrbcL01–3') designed for the detection of the *Pyropia yezoensis rbcL* gene. The primer pair could generate amplicons from several commercial nori food products and dried seaweed powder containing shrimp/prawn or crab. The limit of detection for shrimp/prawn or crab DNA extracted by the improved DNA extraction method is 1 μg per g dried seaweed powder. In conclusion, we showed that the improved method is simple, rapid and highly sensitive, and can be used to detect shrimp/prawn and crab DNA in dried seaweed food products.

Keywords : allergen, crustacean, DNA extraction method, dried seaweed product, PCR

I Introduction

The Japanese Ministry of Health, Labour, and Welfare (MHLW) stipulated the allergen labeling system by amending the Food Sanitation Law in April 2001. In particular, the labeling of egg, milk, wheat, buckwheat, and peanut ingredients in any commercial processed food became mandatory in April 2002 in response to individuals with food allergies. Therefore, the MHLW has prescribed official Japanese methods for determining allergens to validate the labeling of food products.

The labeling of shrimp/prawn and crab became mandatory in June 2008, and the enzyme-linked immunosorbent assay (ELISA) methods for quantitative screening and PCR for qualitative confirmation were announced as the official methods for the detection of shrimp/prawn and crab[1]. Two commercially available ELISA kits for screening were validated according to international validation protocols[2] and were used to validate the labeling for shrimp/prawn and crab[3]. However, because of the high amino acid sequence homology between shrimp/prawn and crab[4], these ELISA kits failed to distinguish between shrimp/prawn and crab tropomyosin. Furthermore, these ELISA kits can detect tropomyosin derived from other crustaceans and insects not encompassed by the food labeling regulation. In addition, PCR is commonly used to identify either shrimp/prawn or crab contamination and to exclude false positives.

Crustacean protein was frequently detected in a recent survey of processed food products primarily containing

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