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Analytical Methods

Development and validation of a lateral flow assay for the detection of crustacean protein in processed foods

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A B S T R A C T

We developed and validated a novel lateral flow assay for the detection of crustacean protein in processed foods. This assay had high sensitivity; the visual detection limit for shrimp protein extract was 25 μg/L, equivalent to 1 μg/g protein in a food sample, and results could be obtained within 20 min without sophisticated procedures or expensive equipment. Concordance between our assay and another validated quantitative enzyme-linked immunosorbent assay was 97% for commercially processed foods. This assay is rapid, simple, reliable, and highly correlated with validated enzyme-linked immunosorbent assays and is thus suitable for monitoring of food products, especially in food-processing facilities.

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1. Introduction

In recent years, food allergies have become an important health problem in developed countries; it is estimated that more than 2–3% of people have some type of food allergy (Ebisawa, 2007; Rona et al., 2007). One of the most problematic aspects of food allergies is that effective therapies have not been established. Therefore, strict avoidance of the food allergen is currently the only means of preventing an adverse allergic reaction (Sampson, 2004; Wang & Sampson, 2011). However, various studies have shown that severe allergic reactions can be caused by accidental ingestion of food products containing allergenic materials (Furlong, DeSimone, & Sicherer, 2001; Yu et al., 2006), making it necessary to develop adequate and reliable information regarding potentially allergenic ingredients in processed foods.

Labelling system for allergenic ingredients in processed foods has been discussed by international organisations such as the Codex Alimentarius Commission of the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO). In 1999, the Joint FAO/WHO Codex Alimentary Commission Session agreed to recommend labelling of eight foods that contain ingredients known to be highly allergenic, including crustaceans (Hefle, Nordlee, & Taylor, 1996). In Japan, the Ministry of Health, Labour and Welfare (MLHW) has enforced a labelling system for allergenic food materials since April 2002 to provide information about these foods to allergic consumers. In this system, labelling is mandatory for seven food materials, including eggs, milk, wheat, buckwheat, peanut, shrimp, and crab, and labelling for an additional 18 food materials, such as soybean, is recommended (Akiyama, Imai, & Ebisawa, 2011).

An allergic reaction to crustacean products can be elicited by ingestion of trace amounts of crustacean protein, and anaphylaxis caused by exposure to such protein has been reported (Lopata, O'Hehir, & Lehrer, 2010; Musmand, Daul, & Lehrer, 1993). To protect allergic patients from exposure to hidden allergens, we developed a quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of crustacean protein (Seiki et al., 2007). The method was acceptably accurate and suitable for quantitative measurement of crustacean protein in processed foods; however, the ELISA method is relatively time-consuming and requires expensive instrumentation and well-trained people. In contrast, the lateral flow assay is simple, rapid, and can be applied to the monitoring of processed foods (Röder, Vieths, & Holzhauser, 2009).

In this study, we developed and validated a novel lateral flow assay for the detection of crustacean protein in commercially processed foods as a useful tool for monitoring food products.

2. Materials and methods

2.1. Food samples

Ingredients and commercially processed foods were purchased at local grocery stores in Tsukuba, Japan. We procured

Abbreviations: CV, coefficients of variation; mABS, milli absorbance; MS, mass spectrometry.

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commercially processed foods with and without crustacean labelling in the list of ingredients.

2.2. Preparation of shrimp protein extract and model processed foods

Shrimp protein extract was prepared from black tiger prawn (*Penaeus monodon*) (Seiki et al., 2007). Assay solutions were prepared by diluting the shrimp protein to 0.0, 12.5, 25.0, 50.0, 125.0, and 250.0 μg/L with extraction buffer containing PBS (8.1 mmol/L Na_2HPO_4, 1.47 mmol/L KH_2PO_4, 2.68 mmol/L KCl, and 137 mmol/L NaCl), 1% TritonX-100, and 0.009% NaN_3.

As a model processed food, tomato juice was prepared by adding freeze-dried black tiger prawn muscle powder at final concentrations of 0.0, 2.0, 5.0, or 10.0 μg/g; the liquid was thoroughly mixed for 60 min.

Fish-meat sausage and freeze-dried egg soup containing black tiger prawn muscle powder at final concentrations of 5.0 μg/g and 6.0 μg/g, respectively, were prepared as described (Seiki et al., 2007).

2.3. Preparation of assay solution

The samples were homogenised in an Ace AM-4 homogenizer (Nissei, Tokyo, Japan) three times for 30 s each. Extraction buffer (39 mL) was added to 1 g homogenised sample, which was then shaken using an Ace AM-4 homogenizer or VORTEX-GENIE2 (Scientific Industries Inc., New York, USA) three times for 30 s at room temperature. After extraction, the sample was centrifuged at 3,000×*g* for 20 min at 4 °C, and the supernatant was filtered through 5AB paper (Advantec, Tokyo, Japan).

2.4. Preparation of test strips

Test strips were prepared as described (Millipore Corporation). The monoclonal and polyclonal antibodies against black tiger prawn protein described in our previous ELISA study (Seiki et al., 2007) were used here. Monoclonal antibodies diluted in PBS (0.5 g/L) were applied to a nitrocellulose membrane in a test line, and anti-rabbit IgG antibodies diluted in PBS (2.5 g/L) were applied in a control line. The membrane was soaked in blocking solution containing 5 g/L casein and dried. Gold colloid solution (10 mL) was adjusted to pH 8.15 and 0.8 mL of 0.1 g/L polyclonal antibody was added. After 30-min incubation at room temperature, 1.2 mL of 100 g/L BSA was added. The mixture was centrifuged and the precipitate resuspended in 0.5 mL distilled water. Next, 190 μL distilled water was added to 10 μL polyclonal antibody–gold conjugate and 800 μL dilute solution; a glass fiber filter was soaked in the mixture and dried. The lateral flow devices were assembled with a mount, nitrocellulose membrane, glass fiber filter, filter paper, non-woven fabric, and cover (Fig. 1).

2.5. Evaluation of the lateral flow assay

Sample solution (2 mL) was placed in a plastic tube, and then a test strip was added for 3 s. The test strip was removed and placed on an aluminum sheet with the test line facing up for 20 min. The signal intensity of the test line was estimated visually; when at least 1 of 3 observers considered the results positive, it was judged positive and quantitated using an Immunochromato Reader C10066 system (Hamamatsu Photonics K.K., Shizuoka, Japan). To compare the reaction intensity, sample solutions of black tiger prawn, red king crab, pollicipes, skeleton shrimp, opossum shrimp, and euphausia were diluted 1:10,000 with extraction buffer and retested. Since black tiger prawn is comprised of approximately 18.4% protein (Ministry of Education, Culture, Sports, Science and Technology, 2010), the concentration of protein contained in diluted black tiger prawn solution was equivalent to approximately 20 μg/g in a food sample. ELISA was performed as described (Seiki et al., 2007).

2.6. Evaluation of assay variation

Intra- and inter-assay precision were measured as described (Anfossi et al., 2011). Model processed foods containing black tiger prawn protein at 0.0, 2.0, 5.0, 6.0 and 10.0 μg/g were used. To determine intra-assay precision, the mean coefficients of variation (CVs) were calculated from five replicates performed on the same day. Inter-assay variances were calculated from experiments performed on five different days.

3. Results

3.1. Optimisation of the lateral flow assay

Our first approach was to select for antibody combinations showing high immunoreactivity for shrimp protein. Shrimp protein extract at 100 μg/L was not detectable with the combination of gold-conjugated monoclonal antibodies and polyclonal antibodies in the test line; in contrast, sufficient reactivity was observed for 100 μg/L shrimp protein extract with a combination of gold-conjugated polyclonal antibodies and monoclonal antibodies in the test line.

We next examined the effect of reaction time on assay sensitivity. When the reaction time was less than 15 min, detection intensity for 25 μg/L shrimp protein was very low. However, reaction times over 20 min yielded sufficient reaction intensity with 25 μg/L shrimp protein extract for visual detection.

In lateral flow assays, a false-negative reaction known as the prozone phenomenon often occurs when a sample solution contains excessive amounts of the target protein (Butch, 2000). In this study, the prozone phenomenon was observed on the control line with the concentrated crab extract (data not shown). To solve this problem, we optimised the amount of antibody in the control line and found that the concentrated crab extract does not cause the prozone phenomenon when the antibody concentration is ⩾2.5 g/L.

3.2. Limit of detection for shrimp protein extract

The limit of detection (LOD) was determined for the shrimp protein extract. As shown in Table 1, reactivity was detected in samples at 12.5, 25.0, 50.0, 125.0, and 250.0 μg/L, and non-specific

Fig. 1. The lateral flow device. When test and control lines appear, the sample is positive. If test line does not appear, the sample is negative.

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