

Development of a sandwich ELISA to detect *Anisakis simplex* antigens in fish products

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Anisakis simplex proteins have allergenic properties and are considered the most important hidden allergens in food. Allergies can also be caused by *A. simplex* proteins of live and dead larvae. These reactions may vary from urticaria to anaphylaxis. Since many *A. simplex* allergens are thermostable, cases of allergy may occur even after ingestion of canned fish. The aim of this study was to develop an efficient method to detect *A. simplex* antigens in fishery products. Proteins taken from the samples were extracted with PBS buffer at room temperature, and the solutions were analyzed by sandwich ELISA. The test was based on the use of polyclonal rabbit anti-*A. simplex* IgG antibodies against a crude extract of *A. simplex* third-stage larvae. These antibodies were used as the capture antibodies and biotinylated IgG as the detection antibodies. To reduce the matrix effect, PBS buffer containing 5% bovine serum albumin and 0.1% Tween 20 was used as blocking and incubation buffer. The limit of detection of sandwich ELISA was determined as 1 *Anisakis* larva in 200g of thermally-processed fish products. The laboratory sensitivity of the method seems sufficient to ensure consumer security against *Anisakis* allergens. No cross-reactions with samples of different species of fish muscles and related genera of parasites were found. The presented method allows for the simple and rapid detection of *A. simplex* proteins in food. An elaborated method will be introduced for examination of fishery products from the market.