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Detection of Mustard Traces in Food by an Official Food Safety Laboratory

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Abstract : Introdution: Food allergies occurs, in the Western World, 2% of adults and up to 8% of children. The protection of allergic consumers is guaranted, in Eurrope, by Regulation (EU) No 1169/2011 of the European Parliament which governs the consumer's right to information and identifies 14 food allergens to be mandatory indicated on the label. Among these, mustard is a popular spice added to enhance the flavour and taste of foods. It is frequently present as an ingredient in spice blends, marinades, salad dressings, sausages, and other products. Hypersensitivity to mustard is a public health problem since the ingestion of even low amounts can trigger severe allergic reactions. In order to protect the allergic consumer, high performance methods are required for the detection of allergenic ingredients. Food safety laboratories rely on validated methods that detect hidden allergens in food to ensure the safety and health of allergic consumers. Here we present the test results for the validation and accreditation of a Real time PCR assay (RT-PCR: SPECIALfinder MC Mustard, Generon), for the detection of mustard traces in food. Materials and Methods. The method was tested on five classes of food matrices: bakery and pastry products (chocolate cookies), meats (ragù), ready-to-eat (mixed salad), dairy products (yogurt), grains, and milling products (rice and barley flour). Blank samples were spiked starting with the mustard samples (Sinapis Alba), lyophilized and stored at -18 °C, at a concentration of 1000 ppm. Serial dilutions were then prepared to a final concentration of 0.5 ppm, using the DNA extracted by ION Force FAST (Generon) from the blank samples. The Real Time PCR reaction was performed by RT-PCR SPECIALfinder MC Mustard (Generon), using CFX96 System (BioRad). Results. Real Time PCR showed a limit of detection (LOD) of 0.5 ppm in grains and milling products, ready-to-eat, meats, bakery, pastry products, and dairy products (range Ct 25-34). To determine the exclusivity parameter of the method, the ragù matrix was contaminated with Prunus dulcis (almonds), peanut (Arachis hypogaea), Glycine max (soy), Apium graveolens (celery), Allium cepa (onion), Pisum sativum (peas), Daucus carota (carrots), and Theobroma cacao (cocoa) and no cross-reactions were observed. Discussion. In terms of sensitivity, the Real Time PCR confirmed, even in complex matrix, a LOD of 0.5 ppm in five classes of food matrices tested; these values are compatible with the current regulatory situation that does not consider, at international level, to establish a quantitative criterion for the allergen considered in this study. The Real Time PCR SPECIALfinder kit for the detection of mustard proved to be easy to use and particularly appreciated for the rapid response times considering that the amplification and detection phase has a duration of less than 50 minutes. Method accuracy was rated satisfactory for sensitivity (100%) and specificity (100%) and was fully validated and accreditated. It was found adequate for the needs of the laboratory as it met the purpose for which it was applied. This study was funded in part within a project of the Italian Ministry of Health (IZS PLV 02/19 RC).

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