

Triplex Detection of Pistacia vera, Arachis hypogaea and Pisum sativum in Processed Food Products Using Probe Based PCR

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Abstract : In recent years, food allergies which cause serious health problems affect to public health around the world. Foodstuffs which contain allergens are either intentionally used as ingredients or are encased as contaminant in food products. The prevalence of clinical allergy to peanuts and nuts is estimated at about 0.4%-1.1% of the adult population, representing the allergy to pistachio the 7% of the cases of tree nut causing allergic reactions. In order to protect public health and enforce the legislation, methods for sensitive analysis of pistachio and peanut contents in food are required. Pea, pistachio and peanut are used together, to reduce the cost in food production such as baklava, snack foods. DNA technology-based methods in food analysis are well-established and well-rounded tools for species differentiation, allergen detection. Especially, the probe-based TaqMan real-time PCR assay can amplify target DNA with efficiency, specificity, and sensitivity. In this study, pistachio, peanut and pea were finely ground and three separate series of triplet mixtures containing 0.1, 1, 10, 100, 1000, 10,000 and 100,000 mg kg⁻¹ of each sample were prepared for each series, to a final weight of 100 g. DNA from reference samples and industrial products was successfully extracted with the GIDAGEN® Multi-Fast DNA Isolation Kit. TaqMan probes were designed for triplex determination of ITS, Ara h 3 and pea lectin genes which are specific regions for identification pistachio, peanut and pea, respectively. The real-time PCR as quantitative detected pistachio, peanut and pea in these mixtures down to the lowest investigated level of 0.1, 0.1 and 1 mg kg⁻¹, respectively. Also, the methods reported here are capable of detecting of as little as 0.001% level of peanut DNA, 0,000001% level of pistachio DNA and 0.000001% level of pea DNA. We accomplish that the quantitative triplex real-time PCR method developed in this study can be applied to detect pistachio, peanut and peatraces for three allergens at once in commercial food products.

Keywords : allergens, DNA, real-time PCR, TaqMan probe

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