BOOK OF ABSTRACTS

5th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

November 1–4, 2011
Prague, Czech Republic

Jana Pulkrabová and Monika Tomaniová
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COMPARISON OF DNA EXTRACTION METHODS TO DETECT TRACE AMOUNTS OF TREE NUT ALLERGENS IN CHOCOLATES

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Food-induced allergies represent an emerging problem of food safety. Thus, to safeguard the health of sensitised consumers, food ingredients that may cause allergic reactions should be properly labelled and possible cross-contamination should be avoided. Among food allergies, abnormal immunological responses towards tree nuts are pointed as a frequent source of serious atypical reactions, in which the hypersensitivities associated to almond and hazelnut ingestion are considered dangerous due to their incidence and severity [1,2]. Although immunological methods have been used for the direct detection of the almond and hazelnut allergens with high sensitivity, these assays are susceptible to cross-reactivity with other tree nuts. More recently, alternative approaches based on polymerase chain reaction (PCR) have been developed for the detection of almond and hazelnut allergens in foods [3-5]. However, highly processed food matrices such as chocolate, are very rich in polyphenols, carbohydrates and aromatic compounds that can interfere and inhibit DNA amplification. Since molecular methods are extremely dependent on the DNA extraction and purification procedures, adequate recovery of nucleic acids and removal of PCR inhibitors are required. Presently, several extraction methods are commercially available for the isolation of DNA from foods although only a few can provide extracts with suitable quality and purity for PCR amplifications. In these work we intend to compare different DNA extraction protocols (CTAB, Wizard, Wizard Magnetic and Nucleospin methods) with and without the addition of polyvinylpyrrolidone (PVP) and/or the presence of RNAse. For this purpose, model chocolates containing known amounts of almond or hazelnut (10-0.01%) were prepared and the DNA isolated with the selected protocols. DNA amplification was tested by qualitative PCR and real-time PCR with the use of specific fluorescent probes for almond and hazelnut. From the tested protocols, Nucleospin Food Kit evidenced the best results for almond and hazelnut isolation and amplification. This method showed the highest reproducibility of PCR fragments for almond and hazelnut chocolates until a relative limit of detection (LOD) of 0.01%, which was confirmed by real-time PCR. CTAB-PVP and Wizard methods exhibited the second best results for almond and hazelnut extraction, respectively. However, both methods were less reproducible since the DNA extracts presented major variations in PCR amplifications. To our knowledge this was the first attempt to compare different extraction methods for the specific detection of nut allergens in chocolates.

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Keywords: Food allergens, almond detection, hazelnut detection, PCR, chocolate

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