CORPUS PUBLISHERS

World Journal of Food and Nutrition (WJFN)

ISSN: 2833-3535

Volume 3, Issue 1, 2023

Article Information

Received date : January 23, 2023

Published date: February 03, 2023

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DOI: 10.54026/WJFN/1014

Key Words

Food allergens; Allergenic potency; ELISA; PCR

Research Article

Heat Treatment Effects on Food Allergens and Laboratory Test Sensitivity

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Abstract

Food allergy is a global health concern. Most food allergens are proteins which, even in small quantities, can cause an allergic reaction in sensitized persons. For the provision of food information to consumers, food products containing any of 14 food allergens must be labelled according to Regulation (EU) no. 1169/2011 of the European Parliament. Food preparation often employs high temperatures to improve conservation. Prolonged heat treatment, however, can alter food molecules. Food manufacturers and food agencies carry out tests to determine the absence of undeclared allergenic substances in food products. Laboratory tests are based on enzyme immunoassay (ELISA-based) or molecular biology (PCR-based) techniques in which proteins are the target molecules, and a portion of DNA is a marker of the allergenic ingredient, respectively. With this study we wanted to determine the effect of heat treatment on almond and hazelnut allergens. To do this, we compared the ability of the two techniques to detect allergenic proteins at different temperatures and duration of heating. The allergenic potential of almond and hazelnut ingredients after heating is crucial to consumer health and safety.

Introduction

Food allergens can induce an immunological response in sensitized individuals. Adverse reactions caused by food-induced allergy can range from mild allergic effects (e.g., gastrointestinal symptoms, hives, dermatitis) to anaphylactic reaction. Intake of even tiny amounts of food allergens, most often proteins, can trigger adverse health reactions in allergic individuals [1,2], the severity of which is unrelated to the amount of allergen ingested but rather is variable from person to person [3]. For the provision of food information to allergic consumers, any of 14 food allergens must be labelled on food products according to Regulation (EU) no. 1169/2011 of the European Parliament [4]. Food manufacturers and food safety agencies regularly test for the absence of undeclared allergenic substances in food products by means of enzyme immunoassay (ELISA-based) or molecular biology (PCR-based) techniques. For the detection of trace allergens in food, ELISA uses proteins as target molecules, while PCR detects a portion of DNA as a marker of the allergenic ingredient [5]. Tree nuts are a common food allergen [6,7]. Hazelnuts and almonds are frequent ingredients in bakery products.

To improve their microbiological, organoleptic, and nutritional properties, they are heated during food processing by cooking, roasting, and baking, for example. Prolonged heating processes can alter food protein structure and chemistry, however, resulting in denaturation, unfolding, hydrolysis of peptide bonds, and aggregation by non-covalent and disulfide bonds [8-10]. A consequence of heat-induced change in protein structure is increased allergen potency [7,9,11,12], which can also alter the effectiveness of allergen detection testing [13]. The effect of heat processing on allergenic potential is a critical issue in consumer health and safety. The performance of allergen detection testing methods on heat-treated foods should be determined by food safety agencies and by food manufacturers to ensure that the tests are sufficiently sensitive and specific. With this study we wanted to compare the effect of heat treatment at different temperature and duration on the ability (sensitivity) of ELISA and PCR to detect almond and hazelnut allergens.

Methods and Materials

Soft wheat flour, almond and hazelnut flours were purchased at a local market. The soft wheat flour was tested to establish the absence of target allergens and then used as negative control and blank sample for ELISA and PCR analysis. Almond and hazelnut flours were treated at 140° C for 5, 15, 30, and 60 min and at 180° C for 5, 15, 22, and 30 min. Spiked samples were prepared using soft wheat flour, which contained no hazelnut or almond, and the two target flours at a final concentration of 1000 ppm (mg/kg). All samples were treated and prepared at our laboratory using either disposable equipment (trays, containers, test tubes) or washed and autoclaved instruments (studs and blades, steel cutlery, beakers, other glassware). The samples were transferred to sterile test tubes and stored at -18° C until analysis by ELISA and PCR.

PCR assay

DNA was extracted using an ION Force DNA Extractor FAST commercial kit (Generon) according to the manufacturer's instructions. DNA extracted from the spiked samples was diluted and DNA extracted from the blank samples to a final concentration of 10 ppm of target allergen. Amplification was performed using a real-time (RT)-PCR SPECIALfinder Almond MC (Generon) and a SPECIALfinder MC Hazelnut kit (Generon). The master mix was prepared under a laminar flow hood to prevent contamination with foreign DNA. The extracted DNA for each sample and for the positive and negative controls was dispensed onto microplates with the master mix. Amplification was then carried out on a CFX real-time system (Bio-Rad) using the following detectors: RT-PCR kit SPECIALfinder Almond MC: Almond (FAM), IPC (HEX); RT-PCR kit SPECIALfinder Hazelnut MC: Hazelnut (FAM), IPC (HEX).

ELISA

Hazelnut detection testing was performed using a Ridascreen®Fast Hazelnut kit according to the manufacturer's instructions. Almond detection testing was performed using a SENSISpec Almond ELISA kit (Eurofins Technologies) according to the manufacturer's instructions. The spike samples were diluted with extraction buffer to a final concentration of 10 ppm of target before application to the microplates. For hazelnut detection by ELISA, spectrophotometric reading at 450 nm was performed using a Ridascreen®Fast Hazelnut kit, while for almond detection spectrophotometric reading at 450 nm (reference wavelength 620 nm) was performed using a SENSISpec Almond ELISA kit (Eurofins Technologies).

Results

Almond was detected by RT-PCR in 87.5% (7 out of 8) samples after each heat treatment; samples treated at 180° C for 30 min resulted negative (Ct 31.46). Hazelnut was detected by RT-PCR in 62.5% (5 out of 8) of samples after each heat treatment; samples treated at 140° C for 60 min and at 180° C for 22 and 30 min resulted negative (Ct 35). Almond was found by ELISA in 75% (6 out of 8) of samples after each heat treatment; samples treated at 140° C for 60 min and at 180° C for 30 min resulted negative (limit of detection, LOD <0.2 ppm). Hazelnut was found by ELISA in 37.5% (3 out of 8) of samples after each heat treatment; samples treated at 140° C for 30 and 60 min and at 180° C for 15, 22, and 30 min resulted negative (LOD <2.5 ppm) (Table 1).

Table 1: Detection by ELISA and PCR of hazelnut and almond in samples contaminated with 10 ppm of allergen at different heat temperature and durations.

	ALMOND 10 ppm		HAZELNUT 10 ppm	
Heating Protocol	Ct	Results (ppm)	Ct	Results (ppm)
140° C for 5 min	21.39	6.96	23.73	4.34
140° C for 15 min	21.55	3.96	25.09	8.37
140° C for 30 min	25.3	0.26	33.48	< 2.5
140° C for 60 min	28.63	< 0.2	>35	< 2.5
180° C for 5 min	21.46	2.68	23.92	6.63
180° C for 15 min	23.18	2.26	28.51	< 2.5
180° C for 22 min	29.98	0.22	>35	< 2.5
180° C for 30 min	>31.46	< 0.2	>35	< 2.5

Discussion

All analytical sessions were carried out correctly and the four commercial methods were easy to use and optimized for the food matrix. No interference was observed, and standard curve and internal controls were satisfactory in each test session. ELISA performance was less efficient than PCR owing to the difference in target molecules and target protein the tests employ. Heat treatment is known to alter molecule structure in food: protein structures start to undergo partial denaturation at 55° C (unfolding and loss of secondary structure), considerable modification at 70-90° C (intra-intermolecular interactions and disulfide bond rearrangements), and aggregation at 100° C. Chemical interactions with constituents of food matrix (covalent bonds) occur at temperatures above 100° C [10,14]. DNA is partially denatured at 90-95% [15]. This difference in denaturation temperature can explain the difference in test performance: ELISA was able to detect almond proteins after 30 min but not after 60 min at 140° C, while it was unable to detect hazelnut 140° C after 30 and 60 min. These results refer to a total protein content of 19% in almond and 14% in hazelnut [16].

Unlike proteins, DNA can resist high temperatures, as shown by the positive PCR results for almond at 180° C for 5, 15, and 22 min, and for hazelnut at 180° C for 5- and 15-min. High temperature and exposure time impact on denatured protein structure. We observed a progressive loss of sensitivity and specificity of PCR target allergen detection, as demonstrated by the progressive increase in Ct in samples undergoing prolonged treatment (30 min) at 140° C and 180° C. Previous studies reported the effect of roasting on the allergenic potential of hazelnut and almond. Hansen et al. found a marked decrease in allergenic potential when hazelnuts were roasted at 140° C for 40 min [12]. Verhoeckx et al. (2015) reported decreased hazelnut allergenicity after roasting [2]. Other studies on the effect of heat on almond demonstrated that its allergenic potential is reduced after thermal treatment [8,17].

Conclusions

Overall, our results show that heat treatment at high temperature (140° C and 180° C for 5-15 min) had no effect on the performance of RT-PCR for almond and hazelnut detection, whereas prolonged heat treatment at high temperature led to partial or total denaturation of constituent food molecules, with loss of specificity and sensitivity of ELISA. Food processing methods can alter the structure of allergenic proteins as well as their allergenicity. Official food safety laboratories carry out highly sensitive analytical methods to detect food allergens. Lab testing results should inform allergen control plans and actions to protect the safety of consumers with a food allergy. Food producers should follow recommendations by competent agencies to provide correct food label information. Food allergen information is essential to the safety of sensitized individuals. The effect of heat treatment on the allergenic potency of food ingredients is a critical issue in consumer protection. It is also important in differentiating the performance of detection methods by official laboratories, food producers, and food safety agencies when drawing up their analytical control plans.

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This study was funded by the Italian Ministry of Health IZSPLV02/19RC.