DEVELOPMENT OF A QUARTZ CRYSTAL MICROBALANCE (QCM) IMMUNOSENSOR TO DETECT POTENTIALLY ALLERGENIC SESAME IN FOOD

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Key words: food allergen, sesame, immunosensor, quartz crystal microbalance

INTRODUCTION

Food allergy is a hypersensitive immune response to the exposure of food allergens. Exact prevalence is unknown, but roughly estimated, 5 to 6% of children and about 2% of adults suffer from it. [1-3]. Since food allergy cannot be cured, total avoidance of the allergenic food is the only solution available. [2, 3]. For consumer protection, The European Commission has issued a directive for declaration of ingredients on food products. Sesame (*Sesamum indicum*) and its products are one of the 14 allergenic foods which must be labeled if present. [4] Formerly, sesame allergy was common in Eastern countries; recently its prevalence has increased in European countries. [5]. Sesame allergens are known to be extremely potent, causing severe reactions with a high risk of life threatening anaphylaxis [6].

Analytical methods for food allergen detection are of vital importance. Protein based methods, and among them enzyme linked immunosorbent assays (ELISAs), are most commonly employed. So far, immunosensors play a minor role in food allergen analysis. [7].

The present study aimed at the development of a quartz crystal microbalance (QCM) immunosensor for the detection of sesame in food. In QCM immunosensors the analyte is recognized by antibodies which are immobilized on a thin layer deposed on a crystal surface. The resulting mass change is then transformed into an electronically measurable

quantity. QCM sensors offer several advantages compared to ELISAs. They do not require labeled reagents and the analyte can be detected in real-time.

EXPERIMENTAL

Materials

Phosphate-buffered saline (PBS), pH 7.6, was prepared by dissolving 21.25 g of NaCl, 31.15 g of Na_2HPO_4 x 2 H_2O and 3.9 g of NaH_2PO_4 x 2 H_2O in 2.5 L of water. The sample extraction buffer was prepared by dissolving 6.06 g of Tris and 11.69 g of NaCl in 1 L of water, adjusting the pH to 8.2 with 1 M HCl.

Sesame extract preparation

Four g of white, peeled sesame were grinded to a homogenous paste; soxhlet extraction with n-hexane for 18 h was carried out for defatting. After drying overnight at room temperature, the defatted sesame was stirred with 30 mL of PBS buffer at room temperature for 2 h, and then centrifuged at 1500 *g* for 30 min. The protein concentration was determined with the Bradford assay using bovine serum albumin (BSA) as standard.

Polyclonal antibodies

Anti-sesame polyclonal antibodies (IgY) were produced by immunizing a hen with 1 mg protein/ mL PBS of sesame protein once in 30 days at the Department of Biochemistry and Cell Biology, University of Vienna, Austria. Antibodies were isolated from the egg laid seven days post fifth immunization by ammonium sulfate precipitation. The concentration of the isolated antibodies was determined with the Bradford assay using IgG as standard.

Sample extraction

All samples were purchased at local supermarkets and were extracted using the sample extraction buffer. To 10 g of the homogenized sample, 50 mL of the sample extraction buffer were added; the resulting mixture was then homogenized for 2 min and centrifuged

for 30 min at 1500 *g*. The supernatant was filtered and centrifuged for 5 min at 10000 rpm. The protein concentrations of the obtained extracts were measured using the Bradford assay.

Apparatus and preparation of the sensor

AT- cut quartz crystals of 10 MHz fundamental resonance frequency were used and particular electrode geometry was generated on the quartz via silk-screen printing of gold paste. The prepared quartz was then heated at 400 °C for 3 h. The prepared quartz was mounted in the measurement. After stabilizing the frequency signal with water the antibody solution was added in a concentration of 2 mg/mL and an incubation period of 15 min followed. After immobilization the surplus of antibodies was removed via solution outlet.

Measurement procedure

The sample was injected in a volume of 180 µL. The antibody-antigen bond was dissociated through elution by 2 M guanidine hydrochloride solution. After rinsing with water, the next sample was injected. For measurements dealing with cross reactivity tests and testing commercial food samples a washing step was included to remove interfering matrix compounds. This was done by injecting 180 µL of the sample and incubating it for 5-7 min, removing it via solution outlet and adding 180 µL of deionized water and carrying out the measurement. To regenerate the antibodies, in order to reuse the sensor for another measurement the same day or the next one, PBS was added followed by an incubation period of at least 15 min.

RESULTS AND DISCUSSION

Immobilization of the antibodies and formation of the antibody-antigen complex

The results shown in Figure 1 represent the immobilization of antibodies. The figure indicates that there was a signal response when the antibody solution (2 mg/mL) was added. Injection of the sesame extract in a concentration of 1 mg/mL resulted in a further

distinct change in frequency, indicating a mass change due to the formation of the antigen-antibody complex.

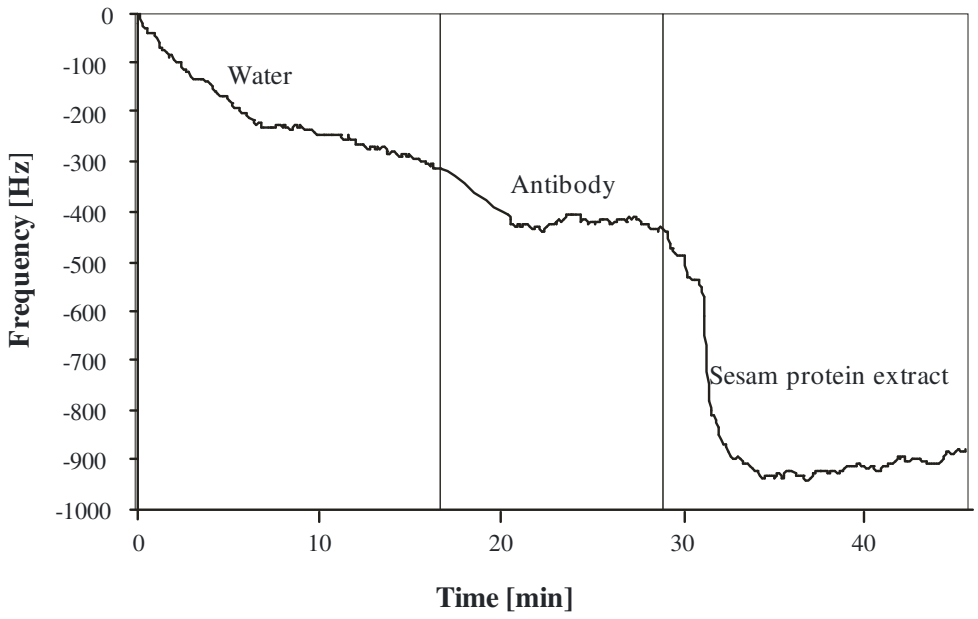

Figure 1: Sensor response to the antibody solution and to the sesame protein extract (concentration 1 mg/mL)

Cross reactivity studies

Fourteen foods and food ingredients were tested for cross reactivity. These included almond, brazil nut, peanut, hazelnut, walnut, sunflower seeds, poppy, rice, oat, chocolate, rye, wheat, honey and soybean. Protein extracts of the above mentioned foods were injected into the flow cell in a concentration of 1 mg/mL. Figure 2 shows some of the results obtained.

The figure not only illustrates the cross reactivity tests done for chocolate and walnut but also the signal dependency on the concentration of sesame added. When sesame was initially injected in a concentration of 0.88 mg/ mL, a sensor response was obtained, whereas there was no sensor response when measurements were carried out after injecting

the chocolate and walnut extracts. The sensor gave, however, a response when chocolate and walnut extracts spiked with sesame were injected in the measurement cell. For the spiking, the walnut and chocolates extracts were diluted with the sesame extract in such a way that the protein concentration of walnut and chocolate extracts was 1 mg/mL and the sesame protein concentration was 0.88 mg/mL in chocolate and 0.44 mg/mL in walnut extract. It is evident from the figure that the magnitude of the signal response depended on the sesame protein concentration in the extracts.

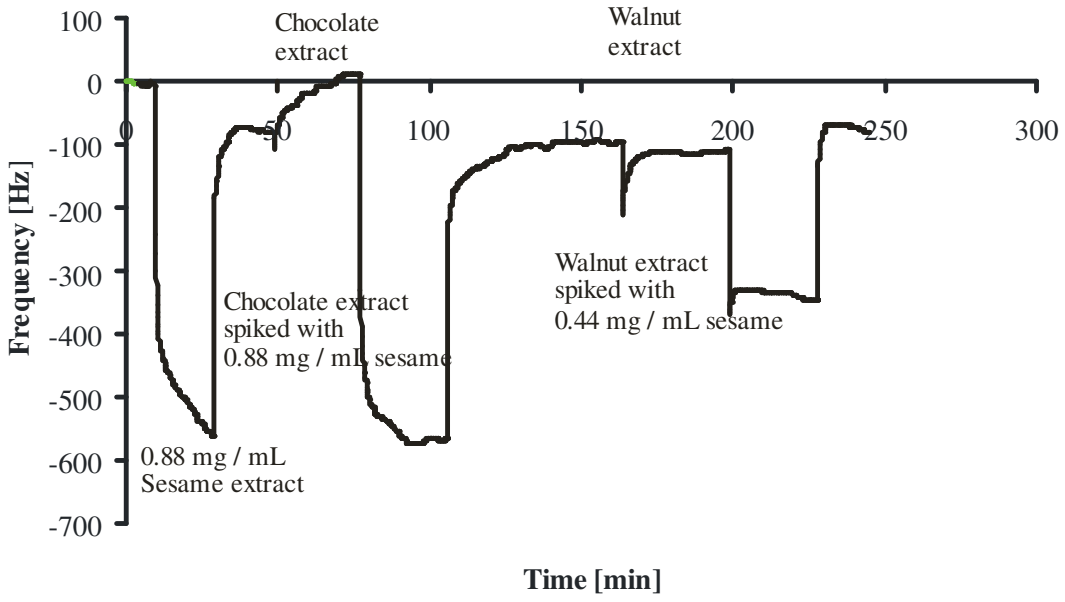

Figure 2: Results obtained in cross reactivity tests; sensor response to sesame, chocolate and walnut

As shown in the figure the signal response is almost half in the case of walnut with sesame to that of chocolate with sesame, since the spike level of the walnut extract is also half in comparison to that of the chocolate extract.

REFERENCES

1. BURKS, W.; BALLMER-WEBER, B. K. Food allergy. Mol. Nutr. Food Res. **2006**, 50, 595-603.
2. HOFFMANN-SOMMERGRUBER, K.; MILLS, E. N. C.; VIETHS, S.. Coordinated and standardized production, purification and characterization of natural and recombinant food allergens to establish a food allergen library. Mol. Nutr. Food Res. **2008**, 52, S159-S165.
3. KIRSCH, S.; FOURDRILIS, S.; DOBSON, R.; SCIPPO, M.-L.; MAGHUIN-ROGISTER, G.; DE PAUW, E. Quantitative methods for food allergens: a review. Anal. Bioanal. Chem., **2009**, 395, 57-67.
4. DIRECTIVE 2006/142/EC AMENDING ANNEX IIIA OF DIRECTIVE 2000/13/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL. Official Journal of the European Union L 368/110, 23.12.2006, p 19.
5. KÄGI, M. K.; WÜTHRICH, B. Falafel-burger anaphylaxis due to sesame seed allergy. Lancet, **1991**, *338*, 582.
6. DALAL, I.; BINSON, I.; REIFEN, R.; AMITAI, Z.; SHOHAT, T.; RAHMANI, S.; LEVINE, A.; BALLIN, A.; SOMEKH, E. Food allergy is a matter of geography after all: sesame as a major cause of severe IgE-mediated food allergic reactions among infants and young children in Israel, Allergy, **2002**, 57, 362–365.
7. MOHAMMED, I.; MULLETT, W. M.; LAI, E. P. C.; YEUNG, J. M. Is biosensor a viable method for food allergen detection? Anal. Chim. Acta, **2001**, 444, 97–102.