FOOD COMPOSITION AND ADDITIVES

Detection of Peanut Allergen Ara h 6 in Commercially Processed Foods using a Single-Walled Carbon Nanotube–Based Biosensor

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***Background*: The peanut protein *Arachis hypogaea* (Ara h) 6 is one of the most serious food allergens that contributes to food-related, life-threatening problems worldwide. The extremely low allergic dose demands for more selective and rapid methods for detecting Ara h 6. *Objective*: The goal of this study was to develop a single-walled carbon nanotube (SWCNT)-based biosensor for the rapid detection of Ara h 6 in commercial food products. *Methods*: The detection principle of this biosensor was based on the binding of Ara h 6 to the anti-Ara h 6 antibody (pAb) through 1-pyrenibutanoic acid succinimidyl ester. The resistance difference (ΔR) was calculated via linear sweep voltammetry using a potentiostat. *Results*: The ΔR increased as the Ara h 6 concentrations increased above the range of 10^0–10^7 pg/L. A specificity analysis showed that the anti-Ara h 6 pAb selectively interacted with Ara h 6 molecules in the buffer solution (pH 7.4). *Conclusions*: This research proposes that an SWCNT-based biosensor in self-assembly with antibodies could be an effective tool for the rapid detection of allergen proteins in food. *Highlights*: The developed biosensor exhibited higher sensitivity and selectivity. Application studies resulted in precise Ara h 6 detection in peanut-containing processed food.**

Monitoring food safety is considered a crucial factor in the food industry. Although food regulatory agencies and manufacturers have adopted several methods to abate the risks in food, such as employing good manufacturing practices, hazard analyses, and critical control points (1), reducing food safety risks remains a challenge. Because of the rapid expansion of the global food supply chain with the production of minimally processed foods, the prevalence of food allergy outbreaks has increased and burdened our economy (2). The accidental ingestion of allergen-containing food that can trigger allergic reactions to immunologically hypersensitive people cannot be ignored (3), and the possible risks from these allergic reactions may cause the shrinkage of the food industries.

Peanut allergy is a global public health concern, and it has acquired special attention from food industries, food control agencies, and the scientific community (4). Allergy symptoms in normal and sensitive individuals range from mild urticaria to life-threatening anaphylaxis (5, 6). According to reports on food anaphylaxis fatalities, most anaphylactic deaths over the last 5–7 years were caused by food allergies (7). In recent studies, especially in the United States and Europe, the rate of allergic infection in children is alarmingly high, and the overall prevalence level reported is approximately 5% (8, 9). Additionally, the estimated proportion of allergy prevalence in school children has been found to be 20% (10, 11). Environmental and genetic factors have been reported to induce the prevalence of epidemiologic symptoms for peanut allergies (12). Although studies show that protein consumption of different peanut species is rather consistent around the world, the factors associated with harvesting and processing food containing peanuts have been found to induce allergenic properties in peanut food products (13). Most surveys have revealed that minimal amounts (1–3 mg) of peanut protein are enough to trigger an objective allergic reaction in patients (14, 15), and some studies provide evidence proving that only 200 μg of peanut protein can be sufficient to cause mild, subjective allergic symptoms in patients (16, 17). According to the International Union of Immunological Societies Nomenclature Subcommittee, peanuts containing eight distinct allergenic proteins are from *Arachis hypogaea* (Ara h) 1 to Ara h 8 (13, 18). Of these, Ara h 1 (vicillin-type, 7S globulin), Ara h 2 (2S albumin), and Ara h 3 (legumin-type, 11S globulin) are considered the major peanut allergens (9). However, more recently, Ara h 6 has been reported as a potent peanut allergen because it possesses a seroprevalence similar to that of Ara h 2 (19). It has a capacity equivalent to that of Ara h 2 to release and bind to histamines from basophils and can show more stability during peptic ingestion than Ara h 1 (19);

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in addition, the clinical reactivity of Ara h 6 has been reported in large patient populations (20). The molecular size of Ara h 6 is 14.5 kDa, and it belongs to the 2S albumin family, which is heat-resistant, immunogenic, and not digestible in the gut (20). Because of these properties, Ara h 6 can be considered a suitable biomarker agent in food and food-production lines.

Despite detailed biological understanding and advanced technological progress, the existing detection methods have considerable limitations. For example, ELISAs have been reported as useful screening tools for the sensitive detection of food pathogens and peanut allergens (21). However, the main limitations of the ELISA method are that it is difficult to perform, is not reusable, requires trained personnel, is expensive, and consists of time-consuming steps (20). DNA amplification approaches are popular for offering accurate sensitivity, but they are laborious and expensive (21); magnet-based methods are usually used for complex food samples but consist of time-consuming sample preparation steps, require costly reagents, and show limited sensitivity (22). Of the various techniques, biosensors can effectively integrate molecular biology into information technology and have been proven as the alternative distinguished tool because of their portability and potential for rapid and sensitive detection (23). Recently, food industries have shown increasing interest in nano-based immunosensor assays, as nanomaterials confer the advantage of constructing catalytic devices and immobilization platforms or serve as optical labels that exhibit improved sensitivity, response time, and stability (24, 25).

Of all the nanomaterials studied, single-walled carbon nanotubes (SWCNT) have appeared as a building block for reforming biosensor platforms (26) because of their unusual mechanical, electrical, and structural properties (27). SWCNTs have been studied in broad spectrum to intensify the electrochemical reactivity for biomolecule detection (28, 29), as they can show high sensitivity toward minute variations in their surrounding environment (24). In addition, nanotubes can be used for constructing molecular junctions because of their ability to control the energy gap of electrons (30–32). Despite prospective applications, to the best of our knowledge, the development of the SWCNT-based biosensor for the rapid detection of Ara h 6 in food has not yet been reported. Therefore, we aimed to develop an SWCNT-based biosensor and evaluate the efficacy of the developed biosensor to detect food allergens. The specific objectives of this study were to (*1*) characterize the properties of the SWCNT-based biosensor and (*2*) apply the SWCNT-based biosensor to detect Ara h 6 in commercial food.

Materials and Methods

Materials

SWCNTs were supplied by Chengdu Organic Chemicals Co., Ltd (purity >95%, Chengdu, China). The naturally purified standard Ara h 6 prepared in 1% bovine serum albumin (BSA)/50% glycerol/phosphate buffered saline (PBS; pH 7.4, 1000 ng/mL), and polyclonal antibodies [1 mg/L, purified antibody (pAb)] were purchased from Indoor Biotechnologies, Inc. (Cambridge, United Kingdom). The solutions used in the experiments were prepared with deionized (DI) water. BSA (96–99%) and PBS (0.1 M, pH 7.4) were procured from Sigma-Aldrich (Seoul, South Korea). Ara h 6 was diluted using 10% PBS (v/v). Triple-distilled water was used throughout the experiments. The solutions were homogenized using a Vortex-Genie 2 (G560E; Scientific Industries, Bohemia, NY).

Preparation of SWCNTs, Linker, and pAbs

The SWCNT suspension was prepared by following the procedure developed previously in our laboratory (21). First, 0.1 mg SWCNTs were dispersed in 1 mL N,N-dimethylformamide (DMF; Daejung, Inc., Siheung, South Korea) and sonicated using an ultrasonic sonicator for 120 min. Then, 1-pyrenebutanoic acid, succinimidyl ester (PBASE) solution, as a linker, was prepared by adding 9.8 mg PBASE to 5 mL DMF (33). The pAb at a concentration of 1 mg/mL was diluted into carbonate–bicarbonate buffer at 12 000 rpm for 20 s prior to use.

Fabrication of SWCNT-Based Biosensor

The biosensor that was used as the base sensing platform contained gold microelectrodes for signal processing. The electrodes were prepared by first depositing a 40 nm thick gold layer on a chrome-deposited silicon wafer surface via an electron-beam evaporator (SRN-110-1505-R2; Sorona, Inc., Pyeongtaek, South Korea) under 4.0×10^{-6} Torr of high-vacuum circumstance. The conductivity of a chrome-deposited silicon wafer surface was tested before gold deposition to confirm the absence of electrical current on the biosensor surface. In addition, the conductivity of the gold-deposited platform was also tested using an electrical tester to ensure that the gold electrodes produced the appropriate electrical response during each measurement.

The biosensor was fabricated by following the procedure described in a previous study with minor modifications (27). The sensor alignment was briefly performed by dropping SWCNT suspension (15 μL) in microelectrode gaps and then annealed by incubating the aligned SWCNTs in a drying oven at 80°C for 15 min. For the noncovalent functionalization between the SWCNT network and the pAb receptor, PBASE was applied as a linker for 2 h at room temperature followed by rinsing with pure DMF and DI water to wash away any excess reagent. For the immobilization of pAb on the SWCNT surface using covalent bonding, each assembled SWCNT-based sensor plate was incubated with anti-Ara h 6 pAb overnight at 4°C, rinsed thoroughly with DI water and PBS (pH 7.4), and dried using N_2 gas. Then, 100 mM ethanolamine was added to the channel region of the SWCNT template and incubated for 30 min to block and deactivate the reactive groups remaining on the SWCNT surface. Finally, the modified SWCNT device was again rinsed with PBS (pH 7.4). The schematic diagram of the SWCNT-based biosensor for Ara h 6 detection has been presented in Figure 1.

Detection of Ara h 6 by the Fabricated Biosensor

The detection of Ara h 6 using the fabricated biosensor was performed according to Sobhan et al. (21). The biosensor was incubated with the diluted Ara h 6 solution at room temperature using a detection time of 30 min to allow the antigen–antibody reaction to occur. After the reaction, the resistant value of the SWCNT-based biosensor was measured using a potentiostat

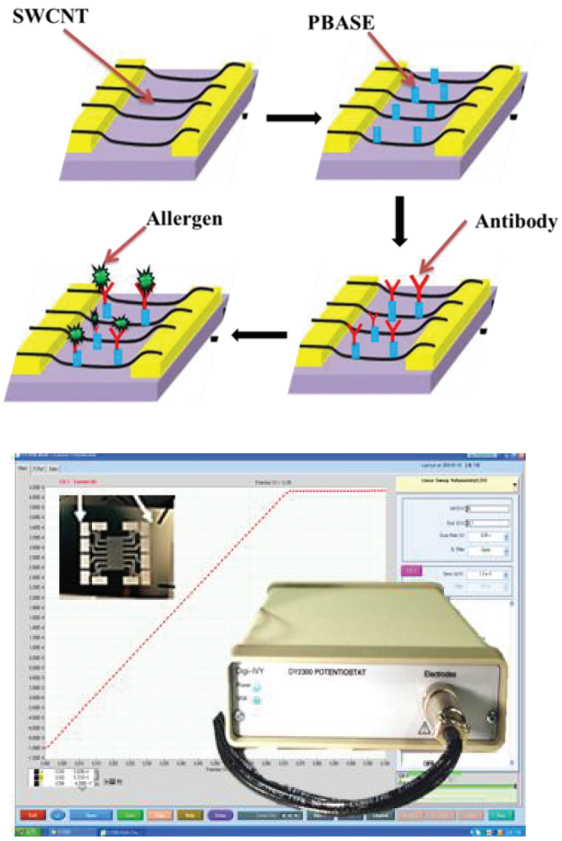

Figure 1. An overview of the fundamentals for the developed single-walled carbon nanotube (SWCNT)-based biosensor. (A) A schematic display of the manufacture of the biosensor device in which the SWCNTs are modified with 1-pyrenebutanoic acid, succinimidyl ester (PBASE) and antibody receptors; specific binding of allergens to the receptors on nanowire produced resistance values; (B) Setup for biosensor measurement using a potentiostat. Color images are available online at http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac.

(DY2013; EG Technology, Seoul, South Korea) via linear sweep voltammetry. For the control set, the Ara h 6 solution was substituted with PBS. The resistance difference (ΔR) was calculated using the following equation (27, 34):

$$\Delta R = (R_1 - R_0)/R_0, \quad (1)$$

where R_0 = the resistance of the biosensor measured with the linker and R_1 = the resistance of the biosensor measured with Ara h 6.

Indirect ELISA

Indirect ELISA is a well-established method to determine antibody specificity (35). As biosensors have recently gained attention for biomaterial analysis, it is essential to compare the results obtained using the newly developed assays with those of the standard assays. Therefore, the specificity of anti-Ara h 6 pAb against different peanut allergens including Ara h 1, Ara h 2, and Ara h 6 obtained using the biosensor and ELISA was compared to validate the biosensor as an innovative tool. The concentration of each peanut allergen was kept constant at 10^3 ng/mL. For the biosensor assay, the allergen suspensions were applied to an SWCNT-based biosensor surface one by one under the same conditions, and the ΔR of the biosensor electrode was measured against each allergen using potentiostats. However, for indirect ELISA, the procedure previously developed in our laboratory was followed (21). The obtained absorbance from the ELISA method was compared with the absorbance of the blank wells in which no allergen solution was coated. The background noise from the other reagents was ignored for the ELISA assay. The result was calculated using the following equation (36):

$$\text{Difference of absorbance} = \text{Absorbance at 405 nm after 30 min} - \text{Absorbance at 405 nm 0 min}$$

Sample Pretreatment

Several different processed foods (i.e., peanut butter, peanut pepper sauce, peanut chocolate, bean milk, and chocolate milk) manufactured by Matgouel Foods Co., Ltd (Sungnam, South Korea) were purchased from the local market. According to the product labels, predominant proportions of peanut protein were reported in peanut butter, peanut chocolate, and peanut pepper sauce at 89.9, 15, and 21.6%, respectively, along with sugar, vegetable fat, and phenolic compounds. The major ingredients of bean milk and chocolate milk, on the other hand, were lentils, chick peas, butter bean, and oatmeal with grain mixture. Therefore, the foods were pretreated to avoid the interference induced by phenolic compounds and nontargeted components present in these foods. Here, 1 g of each food sample was weighed and powdered into liquid N_2 gas. Then, 1 g skimmed milk powder (Sigma-Aldrich, St. Louis, MO) was added to bind to the phenolic compounds. Next, each sample mixture was poured into 20 mL Tris-HNO_3 (pH 8.2) buffer and heated at 60°C with continuous mixing for 20 min (37). The extracted food samples were kept at room temperature for a few minutes and centrifuged at 2500 × *g* at 4°C to produce a precipitate containing the bound phenolic compounds and a thick food sludge (37). The resulting supernatants were saved for biosensor and ELISA analysis.

Results and Discussion

Biosensor Manufacturing

The pAb concentration (dilutions of pAb in PBS, pH 7.8) is a crucial factor in maximizing the binding efficacy for target biomolecules during SWCNT-based biosensor development. To determine the optimum pAb amount for the biosensor, pAb solutions with a concentration range of 0.49–1.96 µg/mL were applied onto the biosensor. The ΔR of the biosensor device was measured for each pAb concentration. The ΔR initiated with the lowest pAb concentration was 0.49 µg/mL, as shown in Figure 2A.

ΔR increased with increasing pAb concentration; however, a considerable rise of ΔR was observed with a high slope between 0.49 and 0.99 µg/mL. This phenomenon of increasing resistance might be attributed to the interaction of the primary and secondary amine groups of the antibody with the ester groups of the PBASE linker (38). The interruption of electron transfer to *p*-type semiconducting SWCNTs because of negative charge adsorption by antibody molecules is also the reason for inducing biosensor resistance (27). Then, a plateau of ΔR was obtained at the concentrations of 0.99–1.76 µL/mL, implying the binding of

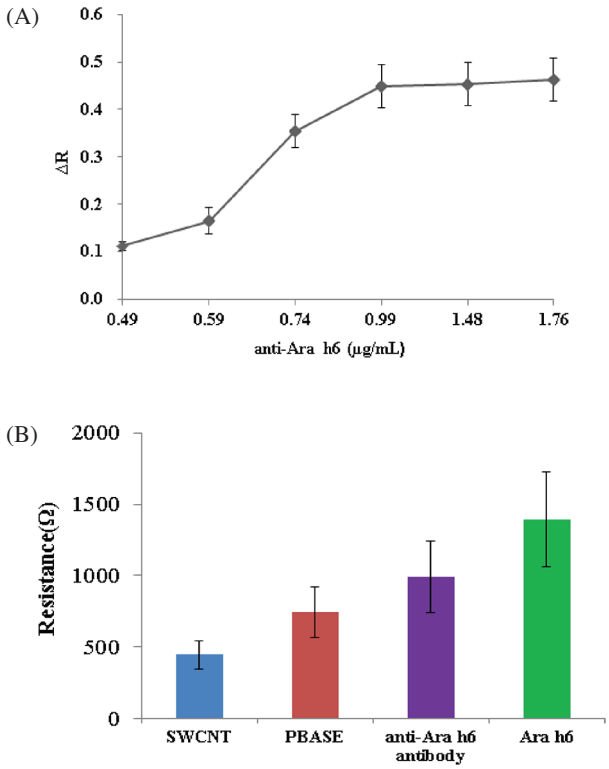

Figure 2. An optimal manufacturing process for single-walled carbon nanotube (SWCNT)-based biosensor. (A) Electrical response (ΔR) depending on the concentration of anti-Ara h 6 antibody (pAb); (B) Electrical resistance after immobilization of SWCNTs, PBASE, anti-Ara h 6 pAb, and Ara h 6. The experimental results are presented as average ± standard deviation (*n* = 3).

pAb on carbon nanotubes via covalent bonding with complete surface coverage. Therefore, the optimal pAb concentrations were expected to be between 0.99 and 1.76 μL/mL, and a minimal optimal concentration of 0.99 μg/mL was selected for biosensor development.

The biosensor was hybridized sequentially by the application of SWCNT, PBASE, and pAb as the receptor for detection of Ara h 6, and the electrochemical properties after each application were evaluated. As shown in Figure 2B, when SWCNTs were assembled onto the biosensor, a change in current was introduced. This indicates that SWCNTs assembled on the silicon wafer and were properly connected with the gold electrodes embedded in the biosensor template. After immobilizing the PBASE onto SWCNTs, the biosensor resistance values intensified. It was speculated that the hydrophobic ends of PBASE were irreversibly adsorbed onto the hydrophobic sidewall of SWCNTs through a π–π stacking interaction to inhibit the electric charge transfer at the existing Debye length to the configuration materials of the biosensor (27). After the biosensor was incubated with pAb overnight, the resistance value increased further because the succinimidyl ester groups on the other end of PBASE reacted with the primary and secondary amines of the antibody via nucleophilic substitution (21). When the pAb-immobilized sensor surface was exposed to the diluted Ara h 6 solution, a significant ΔR of values was observed. We assumed that the binding of pAb to the Ara h 6 molecule resulted in a decrease in the voltammetry current behavior. Therefore, an increased ΔR would be expected from the specific binding interaction between the antibody and the Ara h 6 molecule.

Evaluation of the Sensitivity of the SWCNT-Based Biosensor

The performance of the biosensor in terms of sensitivity was tested by detecting Ara h 6 and comparing the obtained results with those from an indirect ELISA performed using the same antigen concentration.

The sensitivity of the SWCNT-based biosensor was evaluated at various concentrations of Ara h 6 (10^0–10^7 pg/L), i.e., biosensor resistance signals versus Ara h 6 concentrations. For each concentration, the device was characterized electrically by measuring the resistance. ΔR increased linearly with increasing Ara h 6 concentrations (Figure 3A). Although the underlying reason is not yet fully understood, this phenomenon might be attributed to antigen–antibody binding. As reported previously (25), antigen–antibody binding in a biosensor decreased the electrical current and intensified the resistance of the *p*-typed SWCNTs in the Debye length. A significant output of the biosensor response was found at concentrations of 10^3–10^7 pg/L, demonstrating that increased amounts of the Ara h 6 antigen in PBS were sufficient to react with the SWCNT-immobilized pAb. The results obtained imply that the biosensor cannot be used to determine the number of Ara h 6 molecules in PBS, but, owing to its low detection limit, it can certainly be used as a quantitative device for determining the amount. In addition, its performance in terms of sensitivity is better than most other sensing systems, which normally have sensitivities in the range of 1–10^3 ng/L of Ara h 6. For example, screen-printed immunosensors exhibited a sensitive response over a range of 10^6–10^9 pg/L (39), whereas the electrochemical impedance biosensor and the nanobead-enhanced surface plasmon resonance biosensor showed a detection response over a range of 2×10^7–24×10^7 pg/L and 10^8–10^9 pg/L of Ara h 1, respectively (37, 40). However, the SWCNT-based biosensor developed in this study showed sensitivity at Ara h 6 concentrations of 1–10^7 pg/L. In addition, there is no prior report on biosensor devices for detecting Ara h 6 in solutions, except for the voltammetric biosensor reported by Alves et al. (41). However, the LOD of this voltammetric biosensor was 10^4 pg/L for 60 min, whereas that of the SWCNT-based biosensor was 10 pg/L for Ara h 6 within a 30 min reaction time. In the ELISA test, similar Ara h 6 concentrations (10^0–10^7 pg/L) were assayed, and the absorbance results confirmed an excellent correlation between ELISA readings and Ara h 6 loadings (Figure 3B). The increased absorbance obtained was probably due to the antibodies that interacted with the epitope antigens located on the outer membrane of the Ara h 6 protein because epitope antigens comprise a large polymer of repeating oligosaccharides that can provide antigen specificity to the target antibody (42).

It is well known that cross-reactivity in antibodies is a major concern in immunoassays (43). To evaluate the specificity of anti-Ara h 6 pAb used in this study, a biosensor experiment was performed to distinguish Ara h 1, Ara h 2, and Ara h 6. The minimum resistance signal was recorded for nontarget peanut allergens, including Ara h 1 and Ara h 2, as well as PBS, as the control. As shown in Figure 3C, the maximum resistance signal was recorded for Ara h 6 that was target biomaterial, suggesting that cross-reaction occurred between Ara h 6 antigens and anti-Ara h 6 pAb.

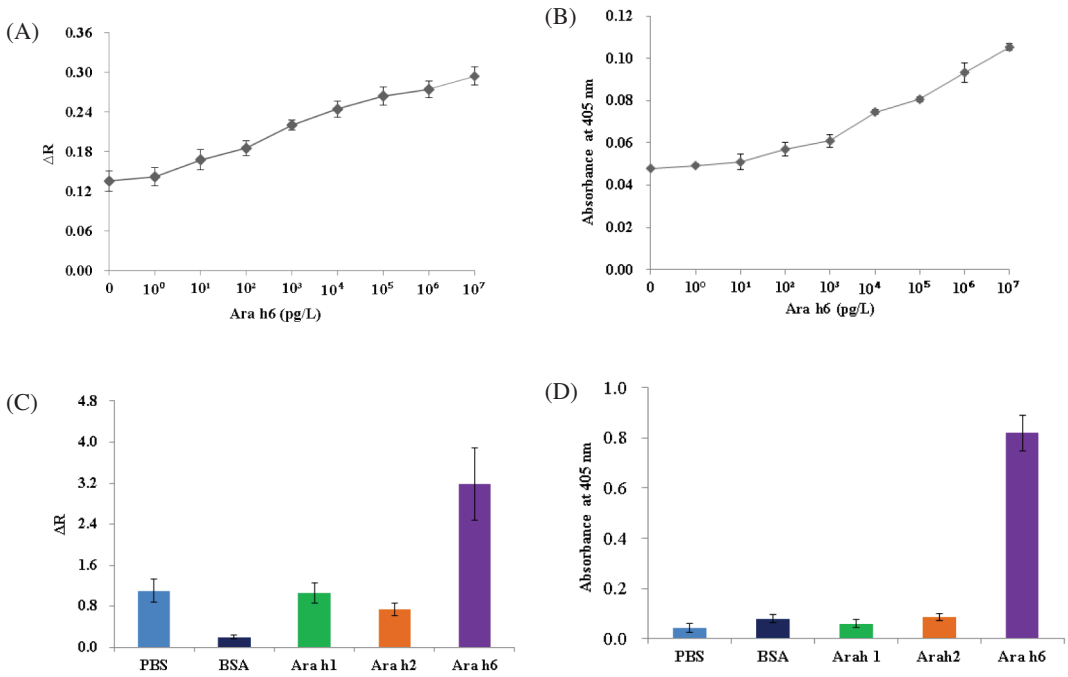

Figure 3. The comparison of the sensitivities of the single-walled carbon nanotube (SWCNT)-based biosensor and ELISA. (A) Sensitivity of the SWCNT-based biosensor; (B) Sensitivity of the corresponding ELISA; (C) Specificity of the SWCNT-based biosensor; (D) Specificity of the corresponding ELISA test. The experimental results are presented as mean ± standard deviation (*n* = 3).

In addition, the *P* value calculated based on the signal for Ara h 6 was significantly greater than 0.05, indicating that the specificity results obtained with the SWCNT-based biosensor using commercial anti-Ara h 6 pAbs were quite satisfactory.

Indirect ELISA is used extensively for confirming the specific reactions of antibodies toward a desired antigen (44). The ELISA performed in this study revealed that only Ara h 6 reacted to the anti-Ara h 6 pAb with an absorbance value of 0.88 (Figure 3D). The other peanut allergens, including Ara h 1 and Ara h 2, were not specific enough, as is evident from the absorbance values less than the cutoff value of 0.079 (mean of negative control + 0.009). In addition, compared with Ara h 1 and Ara h 2, Ara h 6 showed higher binding specificity and lower background signal. Based on these antibody specificity tests, the anti-Ara h 6 pAb was considered a suitable antibody for the development of a biosensor to detect Ara h 6 in food products.

Although ELISA is widely used to determine pAb specificity, its use is limited by the high cost of the measurement instrument, time-consuming procedure (6–7 h), and lack of portability. In this study, ELISA was considered a reference method for validating the biosensor assay, which is a sensitive, rapid, and label-free assay for detecting biomolecules. Therefore, this SWCNT-based biosensor could be a useful alternative detection method to detect peanut allergens due to its short detection time (30 min), portability for measurement in the field, and low costs.

Detection of Peanut Allergens in Real Food Samples

The presence of Ara h 6 in real food was detected using the SWCNT-based biosensor and by indirect ELISA, and the performance of these assays was compared. Two sets of peanut and nonpeanut food samples were used: one set with the spiked Ara h 6 solution and one without; the spiked Ara h 6 concentration in food samples was 10^3 pg/mL. Additionally, the PBS experiment was run as a control with each set of testing used to nullify the effects of any food particle antibody conjugates loosely bound to the biosensor surface. As food samples consist of hard cell matrixes expected to reduce antibody sensitivity, samples were pretreated for allergen recovery, as described previously (37), to eliminate nonspecific surface binding of food particles during the biosensor assay. The obtained food extracts were assayed using the SWCNT-based biosensor, and the possible interference of a food matrix was verified. The results obtained were compared with those from the indirect ELISA performed using the same immune reagents (Figure 4). It is important to state that the same sample dilution step was needed when the suggested protocol was employed for Ara h 6 allergen testing in food products.

As observed in Figure 4A, the biosensor responded significantly to the Ara h 6-spiked food samples with clear evidence of ΔR, whereas nonspiked food extracts prepared from the same samples exhibited less system response at similar conditions. It is assumed that samples spiked with a standard Ara h 6 solution successfully reacted to the biosensor pAb, decreased the current, and increased the biosensor resistance values. In addition, ΔR values obtained for processed food containing peanut ingredients (i.e., peanut butter, peanut chocolate, and peanut pepper sauce) were significant compared with those for nonpeanut samples (i.e., chocolate milk and bean milk). These results confirmed that endogenous Ara h 6 was extracted from the samples obtained after pretreatment, as peanut food contained 5–9% of Ara h 6 of the total peanut allergens (20). Although the presence of food particles in the sample extracts was a major concern for interfering biosensor signals, this result could be a clue to clarify that electrochemical responses could not be affected by the existence of other

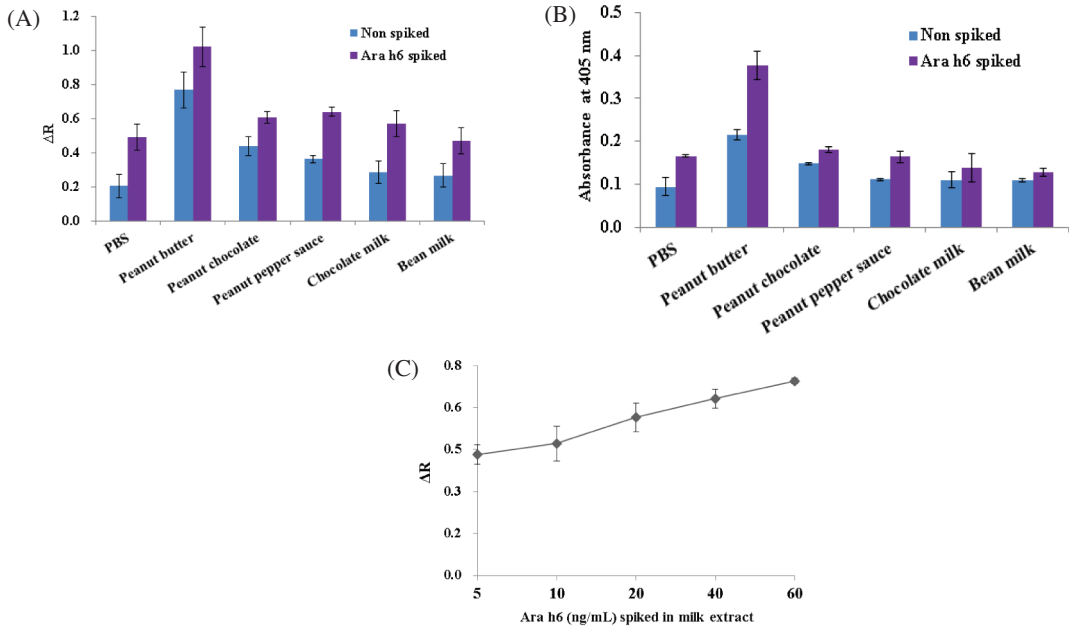

Figure 4. Analysis of real food samples using immunoassay. (A) Ara h 6 detection using the single-walled carbon nanotube (SWCNT)-based biosensor; (B) Ara h 6 detection using ELISA; (C) Dose response curve of SWCNT-based biosensor based on Ara h 6 detection in spiked milk extracts. The experimental results are presented as average ± standard deviation (*n* = 3).

competing food particles. In the ELISA test, a similar response was obtained for spiked food sample extracts (Figure 4B), thus confirming that pAb could detect increased Ara h 6 molecules effectively in spiked samples. This result was in agreement with those obtained from the biosensor assay, in which more Ara h 6 was detected in processed foods that contained peanuts. While the results from ELISA were in agreement with the results obtained from the biosensor, the detection time for ELISA (about 6–7 h) was higher than that of the developed biosensor (about 30 min).

To validate the performance of the SWCNT-based biosensor, a recovery analysis of Ara h 6 in actual food extracts was performed. For this analysis, additions of Ara h 6 concentrations in chocolate milk extract were tested using the fabricated biosensor and the response of the sensor electrode as ΔR was subsequently assessed. The obtained ΔR value was then interpolated and compared with pure Ara h 6 as the standard curve (R^2 = 0.97) and recovered amounts were calculated from linear parts of the standard curve. Analytical results obtained from the recovery analysis have been described in Table 1, in which the biosensor method confirmed the detection of Ara h 6 in food extracts despite having possible food matrix interferences. The range of recovery of Ara h 6 in chocolate milk extract was laid from 82.6 to 144.6%, assuming some indigenous Ara h 6 allergens in the food sample had been extracted. In addition, to verify the possible interference of food matrixes, dose response experiments were performed. The biosensor performance was consistent with Ara h 6 detection in spiked food extracts (Figure 4C), i.e., ΔR increased as Ara h 6 concentrations increased over 5–60 ng/mL, confirming that the biosensor can specifically detect Ara h 6 in food.

Table 1. Recovery analysis of Ara h 6 from chocolate milk extract by an SWCNT-based biosensor

Concn of added target, ng/mL	ΔR	The detected concn of target, ng/mL	Rec., %
5	0.529	6.9 ± 2.4	138
10	0.558	14.4 ± 6.8	144
20	0.565	16.5 ± 3.3	82.5
40	0.632	34.4 ± 7.1	86
60	0.694	51.3 ± 7.3	85.5

Analytical Performance of Carbon Nanomaterial-Based Sensor Assay

Diverse carbon nanomaterial–modified electrodes for recognizing analysts (e.g., pathogens, heavy metals, and proteins along with their LOD and analytical characteristics) are presented in Table 2. Most of these carbon-based electrochemical assays have been widely applied in pure bacterial cultures rather than food samples and used for detecting heavy molecular weight metals persisting in the environment, e.g., CNT-based chemiresistor for *Escherichia coli* O157:H7 (27), graphene-based sensor for *E. coli* K12 (45), CNT-modified glossy carbon electrode (GCE) for *Salmonella typhi* (46), carboxylated CNT-modified GCE for Cu^{+2} and Pb^{+2}, (47) and CNT-modified biosensor for As^{+3} (48). Except for one biosensor described by Sobhan et al. (21), for peanut allergen Ara h 1 (molecular weight 150 kDa), no CNT-based electrode has yet been reported for the detection of proteins with molecular weights less than 150 kDa. Generally, CNT-modified electrode methodology is popular because of higher electron transfer kinetics, improved sensitivity, and potential control over detected biomolecules (21). In addition, as the incorporation of carbon nanomaterial onto the biosensor confers technological feasibility for surface modification, this assay was the first attempted to detect Ara h 6 (14.5 kDa) molecules in food samples. Based on the data, future research may be recommended on the applicability of SWCNT-based biosensors for the detection of biomolecules with molecular weights below 17 kDa.

Table 2. Reports on different carbon nonmaterial–based electrochemical biosensors for the detection of pathogens, heavy metals, and peanut allergens

Types of analyte	Electrode type	Detected analyte	Analytical characteristics	Ref.
Microorganism	CNT-based biosensor	*E. coli* O157:H7; bacteriophage T7	ΔR; LOD bacteria: 10^3 CFU/mL; LOD bacteriophage: 10^3 PFU/mL	(27)
	Graphene-based FET[a]	*E. coli* K12	Real-time current; LOD: 10 CFU/mL	(45)
	Carboxylated CNT-modified GCE[b]	*S. typhi*	Real-time potential; LOD: 0.2 CFU/mL	(46)
Pollutants	CNT-modified GCE	As (III)	LSV[c]; LOD: 0.05 l g/L	(48)
	Carboxylated CNT-modified GCE	Cu (II); Pb (II)	CV[d]; LOD Cu^{2+}: 15 ppb; LOD Pb^{2+}: 1 ppb	(47)
	CNT-based chemiresistor	Hg (II)	ΔR; linear range: 100 nm–11 m	(49)
Proteins	CNT-based FET	IgE[e]	LOD 250 pm; linear range: 250 pm–20 nm	(50)
	CNT-based biosensor	Ara h1	ΔR; LOD: 1 ng/mL	(21)
	Au nanoparticle-modified carbon electrode	Ara h 6	CV; LOD: 0.27 ng/mL	(41)
	CNT-based biosensor	Ara h 6	ΔR; LOD: 10 pg/mL	This work

[a] FET = Field effect transistor.

[b] GCE = Glossy carbon electrode.

[c] LSV = Linear sweep voltammetry.

[d] CV = Cylindric voltammetry.

[e] IgE = Immunoglobulin E.

Conclusions

A functionalized SWCNT-based biosensor was developed for the label-free, rapid, and sensitive detection of the peanut allergen Ara h 6 because of its miniaturized structure, high performance, and sensitive response as compared with the conventional methods. Experimental evidence exhibited that the immobilization of different concentrations of anti-Ara h 6 pAbs onto the sensing platform optimized the sensor's signal responses. Indirect ELISA confirmed the specificity of anti-Ara h 6 pAb to Ara h 6 antigens. In addition, biosensor selectivity toward Ara h 6 was confirmed ($P < 0.05$), whereas a negligible biosensor response was obtained against Ara h 1 and Ara h 2. The SWCNT-based biosensor developed was also used to detect Ara h 6 in pretreated food extracts. Future studies are required to explore methodologies for the application of SWCNT-based biosensors without the need of sample treatment.

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