

An electrochemical molecularly imprinted polymer sensor for rapid and selective food allergen detection

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ABSTRACT

Food allergies are a serious and rising public health concern. The potentially fatal consequence of food allergies makes managing them costly and anxiety-inducing. Rapid, on-site detection of allergenic ingredients in foods would greatly improve the health and quality of life of food allergy sufferers. This work demonstrates the feasibility of such a device using molecularly imprinted polymers (MIPs). The MIP sensor can detect allergenic soy markers at concentrations as low as 100 parts-per-billion, two orders of magnitude below clinically relevant thresholds, in both controlled and complex food samples. Sensor performance was qualitatively validated with commercially available soy allergen detection lateral flow devices (LFDs). The outcome of this application will address a long-standing analytical challenge to achieving fast, cost-effective, and scalable methods for direct detection of allergen tracers in food analysis.

1. Introduction

Food allergies pose a serious health risk, affecting approximately 32 million American adults (Gupta et al., 2019) and between 220 and 520 million people globally (Pawankar, Canonica, Holgate, & Lockey, 2011). Allergic reactions vary in severity, ranging from mild rashes to potentially fatal anaphylaxis (Bock, Muñoz-Furlong, & Sampson, 2007). The amount of allergen required to trigger an allergic reaction can be as low as 0.2 mg (Blom, Vlieg-Boerstra, Kruizinga, van der Heide, Houben, & Dubois, 2013), depending on the individual and food type. The risk of an allergic reaction is especially prevalent for foods consumed outside the home, which are responsible for over half of all fatal allergic reactions (Bock et al., 2007). The financial costs of allergic reactions are also substantial. In the United States, families bear an estimated \$25 billion in allergy-related costs each year (Asthma and Allergy Foundation of America, 2019). Inadvertent ingestion is also common: every three minutes a food allergy sends someone to the emergency room (Clark, Espinola, Rudders, Banerji, & Camargo Jr., 2011).

Currently, no effective preventative solution is available for food allergy sufferers other than strict avoidance of the allergen. Traditional allergen detection technologies such as immunochemical assays, DNA analysis, and mass spectrometry are time-consuming, require large and/

or costly equipment, and are ill-suited for consumer use (Sakamoto et al., 2018). A consumer device that allows allergy sufferers to detect the presence of allergens in food quickly, reliably, and cheaply could significantly improve quality of life, as well as greatly reduce the costs associated with allergy management.

To address this unmet consumer need, we investigate the feasibility of using molecularly imprinted polymers (MIPs) in electrochemical sensors to achieve rapid and selective determination of food allergens. MIPs are synthetic polymers with selective recognition sites for a target analyte or combination of analytes that mimic the behavior of natural receptor binding sites without the temperature sensitivity and high cost of natural systems (BelBruno, 2019). Imprinted polymers have long been known as durable, economical, and customizable materials for selective binding of target molecules (Cormack & Elorza, 2004). Applications of these cavities have been developed for chemical separation, drug delivery, sensors, and catalysis (Schirhagl, 2014). More recently, MIP recognition elements have been combined with a variety of reporting systems to create molecule-specific sensors for both gas- and solution-phase applications (Liu, Antwi-Boampong, BelBruno, Crane, & Tanski, 2013). While many methods for making and integrating MIPs into sensors exist, our approach uses electropolymerization for its superior adherence properties, high reproducibility, simplicity, preparation

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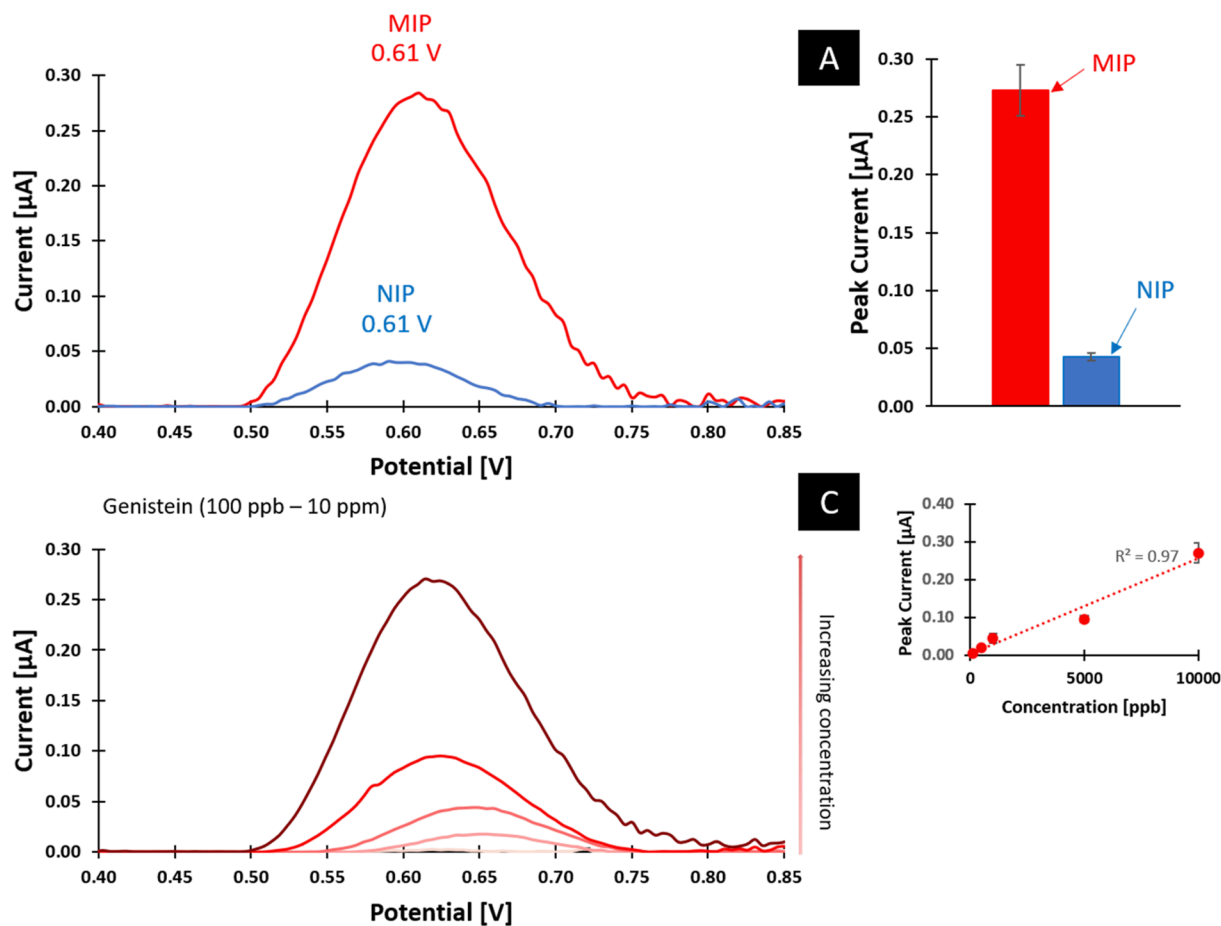


Fig. 1. (A) Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 10 ppm genistein in 10% ethanol and 90% 1X PBS solution (v/v, pH 7.4). (B) Average peak currents for MIP and NIP obtained from voltammetric measurements. (C) Differential pulse voltammetry recorded at varying concentrations of genistein. (D) The graph of the average peak currents for the MIPs at different concentrations.

speed, and the ability to control for film thickness and morphology (Sharma, Pietrzyk-Le, D'Souza, & Kutner, 2012).

In this study, we describe our efforts to develop MIP sensors for detecting soy allergenic markers in complex foods at clinically relevant levels. Soy is one of the eight major food allergens, affecting approximately 2 million Americans and is potentially life-threatening (Cordle, 2004). Soy allergies are primarily caused by a range of proteins, including soy hydrophobic protein, soy hull protein, soy profilin, and soy glycinin (Cordle, 2004). The lowest observed adverse effect level (LOAEL) of soy protein is approximately 88 mg (Yang, de Mejia, Zheng, & Lee, 2011). Soy is rich in isoflavones, including genistein and daidzein (Bhagwat & Haytowitz, 2015). These are the two major isoflavones found in soy, along with the minor product glycitein (Bhagwat & Haytowitz, 2015). Despite differing processing and preparation methods, soy food products have similar levels of genistein (~ 1.4 mg per 1 g of soy protein) (Koppelman, Lakemond, Vlooswijk, & Hefle, 2004), allowing these molecules to effectively act as markers for the presence of allergenic soy protein, while overcoming inherent challenges associated with protein detection, including limited stability to heat, fermentation, and storage, and high cost (Becktel & Schellman, 1987).

Through this work, we demonstrate that genistein can be successfully used as a marker for the detection of soy allergens in foods. The MIP-based sensors were able to both resolve genistein from other structurally analogous isoflavones and flavones, and achieve single analyte limits of detection of 100 ppb. We further tested soy in a range of solid and liquid foods, representing over 100 ingredients. Foods tested include store-bought products, home-baked goods, and restaurant dishes. In each case, our technology correctly identified the presence or

absence of soy in food samples subjected to the standard lateral flow devices (LFDs) allergen detection measurements, thus illustrating the potential of MIPs for rapid and accurate allergen detection applications.

2. Materials and methods

2.1. Chemicals and instruments

Genistein was purchased from BOC Sciences. Quercetin, daidzein, 7-hydroxyflavone, tyrosine, tryptophan, vitamin C, *ortho*-phenylenediamine (o-PD), potassium hexacyanoferrate (II) trihydrate, acetic acid, sodium acetate, and ethanol were purchased from Sigma-Aldrich. 10X phosphate-buffered saline (PBS) was purchased from Fischer Scientific. All reagents were of analytical grade and were used without further purification. 1X PBS solutions were prepared by performing a 1:10 dilution of 10X PBS in deionized (DI) water. Electrochemical experiments were conducted with a PalmSens4 potentiostat (Palm Instruments BV, Netherlands).

2.2. Food products

Food samples, including soy milk, chicken salad, egg salad, red pepper hummus, Italian salad dressing, and gingerbread were purchased from Whole Foods. Soy muffins and non-soy muffins were home baked. Samples from restaurant dishes, including cookies (soy and non-soy), caramel soy sauce, and ginger bok choy were received from Blue Dragon restaurant (Boston, MA). Confirmatory LFDs tests were carried out using a SensiStrip Soy LFD kit, purchased from Eurofins

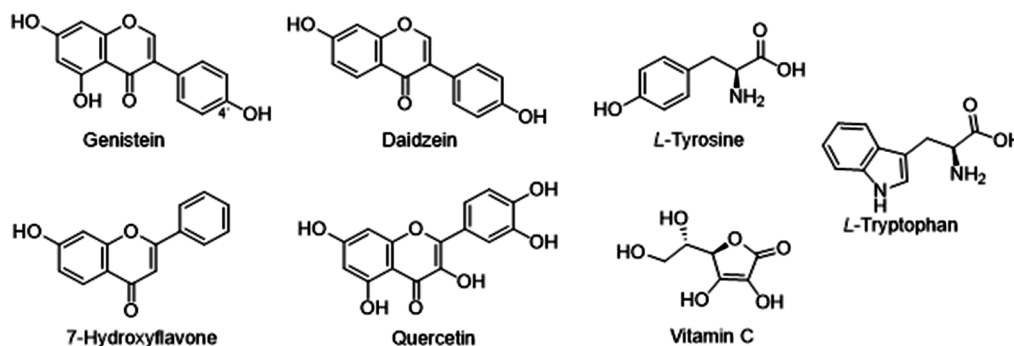


Fig. 2. Schematic representation of soy allergen tracer and potential cross-interferents used in electrochemical measurements. The hydroxyl group attached to the carbon labelled 4' on genistein is the site of electrochemical oxidation.

Technologies.

2.3. Preparation of imprinted polymers

Imprinted films were prepared using an optimized methodology outlined in the [Supplementary Information](#) (Figs. S1–S6). Specifically, 1.82 mg of genistein and 40.5 mg of o-PD were dissolved in 50 mL of a 10% ethanol, 90% 0.1 M acetic acid/sodium acetate buffer solution (v/v, pH 5.2) for one hour. The screen-printed electrodes (SPEs) (ET077-40, Zensor, Taiwan) were then placed in the solution, which was degassed under nitrogen for 15 min. Electropolymerization was performed for one minute at 1.2 V under nitrogen atmosphere. Genistein was extracted from the polymer film by washing in a solution of 0.1 M NaOH for one hour and left to dry at room temperature for 24 h. An identical procedure was used to form a non-imprinted polymer (NIP) in the absence of the template.

2.4. Characterization of MIP and NIP-coated electrodes

All electrochemical experiments were carried out at room temperature in a three-electrode configuration including a 3 mm carbon working electrode with electrodeposited MIP or NIP, an Ag/AgCl reference electrode, and a carbon auxiliary electrode.

The cyclic voltammetry (CV) responses were recorded in a 10% ethanol, 90% 1X PBS solution (v/v, pH 7.4) at room temperature, in a potential range from 0 V to 1 V, with a scan rate of 50 mV/s (Fig. S7) unless stated otherwise. Differential pulse voltammetry (DPV) experimental parameters: scan rate: 50 mV/s; pulse width: 50 ms; and amplitude: 50 mV. Each experiment was performed at least in triplicate.

Electrochemical impedance spectroscopy (EIS) responses for the bare SPE, MIP/SPE, MIP/genistein/SPE, and NIP/SPE were recorded in 0.01 M $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in a 10% ethanol, 90% 1X PBS solution (v/v, pH 7.4), using an excitation amplitude of 0.01 V within the frequency range spanning from 100 kHz to 0.1 Hz at room temperature (Fig. S8).

Attenuated Total Reflectance Infrared Spectroscopy (ATR-IR) was performed using a Thermo Scientific Nicolet iS5 FTIR spectrometer with an iD7 ATR accessory equipped with a diamond crystal. Infrared spectra were recorded under air by placing the MIP and NIP-coated electrodes directly on top of the crystal (Fig. S9). The pressure arm was then lowered to ensure sufficient contact between the electrode and the crystal.

2.5. Cross-reactivity and food testing measurements

To analyze the effect of potential interferent molecules, 0.5 mg of the interferent (quercetin, daidzein, 7-hydroxyflavone, tyrosine, tryptophan or vitamin C) was dissolved in 50 mL of a 10% ethanol, 90% 1X PBS solution (v/v, pH 7.4) for 15 min. For each food sampled, 5 g of food was homogenized using a mortar and pestle. The food was then incubated

and stirred in 50 mL of a buffer solution for 15 min. For both interferent and food tests, template-extracted MIP electrodes were inserted into a 4 mL aliquot of the test solution and allowed to equilibrate for 5 min, prior to the electrochemical measurements.

3. Results and discussion

3.1. Structural characterization of MIPs and observation of electrochemical response of MIP-coated electrodes towards soy allergenic marker

The electrochemical properties of the MIP- and NIP-coated electrodes were studied by cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy. Fig. 1 and Fig. S7 demonstrates voltammograms recorded for the oxidation of soy allergen tracer at MIP/SPE and NIP/SPEs after a 5-min incubation in 10% ethanol, 90% 1X PBS buffer solution (v/v, pH 7.4) containing 10 ppm genistein. The resulting sensors demonstrated a well-defined oxidation peak at 0.61 V (Fig. 1A), which corresponds to oxidation of the hydroxyl group in the 4' position (Fig. 2). This electrochemical response is consistent with the studies of [Popa and Diclescu \(2013\)](#). Specific differences in voltammetric response between different studies may be attributed to changes in experimental conditions, including type of background electrolyte, ionic strength of the solution, electrode configuration, and the presence of additives ([Gil & Couto, 2013](#)). The peak current observed for the MIP electrode was 6.6 times larger than that of the NIP electrode, indicating that the MIP successfully imprinted the genistein (Fig. 1B and Fig. S7). The reduced response of the NIP to genistein is due to the inhibition of electron transfer by the non-conductive polymeric matrix which covers the surface of the electrodes ([Menon, Jesny, & Kumar, 2018](#)). The weak voltammetric response observed for NIP-coated electrodes can be attributed to non-specific absorption of genistein to the host polymer ([BelBruno, 2019](#)). After extracting the template from the MIP- and NIP-coated electrodes with 0.1 M NaOH, a redox wave at 0.61 V disappeared (Fig. S7), confirming the effective extraction of the genistein molecules bound in the polymeric film.

The Nyquist plots of the EIS spectra (Fig. S8) were collected for four distinct devices comprising an unmodified carbon electrode (green), an MIP/SPE after both template removal and rebinding (red, gold, respectively), and an NIP/SPE system (blue). The unmodified carbon electrode exhibited two discrete features: (i) the presence of a partial semicircle in the high-to-intermediate frequency range, associated with the electrochemical processes related to electron transfer (charge transfer resistance); and (ii) Warburg impedance as evidenced by the presence of a line inclined at 45° in the low frequency range. In contrast, the EIS spectra recorded for both the MIP- and NIP-modified SPEs only exhibited charge transfer-related processes represented by the semicircle region. An increase in the impedance for the MIP- and NIP-coated

SPEs (from 7.2 k Ω for the bare electrode to 10 k Ω and 75 k Ω for the MIP and NIP, respectively) is due to the nonconductive nature of the o-PD polymeric matrix deposited during electropolymerization, inhibiting electron transfer (Waffo, Yesildag, Caserta, Katz, Zebger, Lensen, Woltenberger, Scheller, & Altintas, 2018). The MIP-coated electrodes demonstrated lower impedance than the NIP-modified electrodes, which can be attributed to the presence of cavities within the polymeric matrix that enable the diffusion of the redox probe through the polymer, making it easier for electron transfer to occur. The charge transfer resistance for the MIP-modified SPE increased after template rebinding, indicating that the redox marker was hampered from accessing the electrode surface in the presence of genistein.

The molecular structures of the MIP and NIP were characterized by ATR-IR spectroscopy (Fig. S9). The IR spectra of the unmodified commercial SPEs revealed the presence of numerous organic compounds originating from the carbon ink deposited on the electrode during manufacturing of SPE. As expected, MIP- and NIP-coated electrodes demonstrated several similar peaks, indicating their alike chemical composition. Specifically, a broad peak was observed at 473 cm⁻¹ which corresponds to the bending vibration of the N—H group in the o-PD based polymer while the peak at 784 cm⁻¹ can be attributed to the out-of-plane deformation of C—H of 1,2-disubstituted benzene. Fig. S9 also revealed the presence of distinctive signals at 3413, 3275, and 3157 cm⁻¹ caused by the asymmetric stretching vibration for the N—H group, thus successfully confirming the formation of the MIP and NIP polymers. These features are in agreement with literature-reported data on electropolymerization of *ortho*-phenylenediamine (Sayyah, Khaliel, Aboud, & Mohamed, 2014).

3.2. Evaluating detection sensitivity and stability of MIP-coated electrodes to allergenic soy markers

To quantify the limit of detection (LOD) of our sensors, we assessed the DPV response of the MIP electrodes for concentrations of genistein ranging from 100 ppb to 10 ppm (Fig. 1C-D). We used differential pulse voltammetry to measure the electrochemical response of MIP- and NIP-modified electrodes to genistein (10 ppm) in 10% ethanol, 90% 1X PBS (v/v, pH 7.4) (Fig. 1A-B) as DPV can offer significantly higher sensitivity than CV for measured analytes at low concentrations due to minimal contribution of capacitive current to the measured signal (Bănică, 2012).

To provide a realistic representation of the detection capability of fabricated MIP-based sensors, the reported detection limit was based on the lowest experimentally detectable concentration where the analyte signal is visually and statistically distinct from the baseline, with a signal-to-noise ratio greater than three. The DPV spectra recorded for genistein exhibited a redox wave at a characteristic potential and a peak current that increased linearly with the concentration of genistein from 100 ppb to 10 ppm ($R^2 = 0.97$) (Fig. 1D). The experimental LOD for genistein was estimated at 100 ppb, whereas the NIPs did not produce an electrochemical response to concentrations lower than 5 ppm (Fig. S10). These findings demonstrate that our MIP-based sensors are capable of detecting the soy allergen marker at concentrations known to produce adverse effect in patients (LOAELs).

The stability of the electrochemical sensors for the detection of allergen is an important criterion for device efficacy in practical applications. We evaluated sensor stability by storing MIP-coated electrodes for 10 days at 65 °C and subsequently examining their peak currents for 10 ppm genistein. The elevated temperature facilitated accelerate ageing, allowing for the long-term stability of the sensors at room temperature to be evaluated over a short period (Plota & Masek, 2020). Voltammograms recorded for the MIP/SPEs (Fig. S11) showed minimal change in current response when exposed to 65 °C for 10 days, indicating excellent stability and long-term performance of MIP-coated electrodes.

3.3. Detection of soy marker in the presence of interferents using voltammetry

A key feature of MIP sensor functionality is selective binding of the template molecule (BelBruno, 2019). To measure selectivity, we tested for cross-reactivity using interferent molecules structurally analogous to genistein. We selected three (7-hydroxyflavone, quercetin, and daidzein) members of the flavone and isoflavone family that have highly similar molecular structures and properties to genistein (Fig. 2). Vitamin C, and amino acids including tryptophan and tyrosine were also selected for cross-interference studies, due to their common presence in foods including soybeans, citrus fruits, meats, eggs, and dairy products (U.S. Department of Agriculture, 2016).

We then tested the electrochemical response of MIP and NIP-modified electrodes in 10% ethanol, 90% 1X PBS (v/v, pH 7.4) solutions containing 10 ppm of each of the flavone and isoflavone interferents. DPV measurements of MIP-coated electrodes showed distinct oxidation peaks for quercetin, daidzein, and 7-hydroxyflavone at 0.13 V, 0.60 V, and 0.89 V, respectively (Figs. S12–S14) which is consistent with the analyte-centered redox activity. The differences in redox potentials for quercetin and 7-hydroxyflavone (0.13 V, and 0.89 V) make it possible to distinguish these analytes electrochemically from genistein in the same sample (Figs. S15 and S16). While both daidzein and genistein demonstrated redox activity within the 0.60 – 0.65 V potential range, the peak current for genistein was twice as large as the peak current for daidzein. Additionally, the ratio of the MIP to NIP peak current responses for daidzein was approximately 1.8—much lower than the ratio of 6.6 for genistein, allowing for the successful detection of genistein when both genistein and daidzein were present in the sample (Fig. S17). The observed differences in electrocatalytic activity between genistein and its structural analogs (daidzein, quercetin, 7-hydroxyflavone) could be attributed to differences in chemical composition and structure including orientation of aromatic moieties or type and position of substituents on the molecule (Fig. 2).

The voltammetric responses of the MIP- and NIP-modified electrodes to 200 ppm of tyrosine, vitamin C, and tryptophan in 10% ethanol, 90% 1X PBS (v/v, pH 7.4) solution showed distinct oxidation waves at 0.45 V, 0.85 V, and 0.90 V, respectively (Figs. S18–S20). As the genistein peak occurred at 0.61 V, it is possible to readily distinguish between genistein and other molecular interferents typically present in food samples such as vitamin C, tyrosine and tryptophan (Figs. S21–S23). This high selectivity could be attributed to the complementary imprinted cavities within the MIP which match well with the unique structure of genistein in size, shape, and orientation of functionalities. Tryptophan, tyrosine and vitamin C in this case have very different structures (e.g. number of aromatic moieties, orientation of functional groups), resulting in poor fit within the polymeric binding pockets. As a result, these species could not establish a fully matched hydrogen-bond network with the conserved functionalities within the imprinted sites.

3.4. Detection of soy allergenic marker in food products

We qualitatively validated the performance of our detection technology by comparing the response for different food products with data obtained from measurements using portable and commercially available benchmark technology for soy allergen detection, such as lateral flow devices. The LFD kit had a stated LOD of 1 ppm allergenic soy protein and is required to be incubated in a targeted sample for at least 15 min (not including test preparation and incubation time). In this work, we tested 10 dishes and foods, representing restaurant meals, store purchased food products, and home baked goods. Each product was selected to validate in-field detection capability of MIPs in a wide range of foods having different textures (solids, liquids, and emulsions) and compositions including proteins, carbohydrates, fats, vitamins, and minerals. Together, these food products represent over 100 ingredients (Table S1). Positive detection was recorded when the peak current response of

Table 1

Detection responses recorded for MIP-coated electrodes and LFDs during food product measurements. A positive test result for both MIP and LFD kit detection confirms that soy allergen is present within a tested sample.

Food	# of Ingredients	Ingredients include soy	Genistein detection by MIP	LFD kit result
Soy Milk	11	Yes	Positive	Positive
Cookie with Caramel Soy Sauce	16	Yes	Positive	Positive
Cookie	10	No	Negative	Negative
Caramel Soy Sauce	6	Yes	Positive	Positive
Ginger Bok Choy	9	No	Negative	Negative
Red Pepper Hummus	9	No	Negative	Negative
Golden Italian Dressing	9	No	Negative	Negative
Egg Salad	11	No	Negative	Negative
Chicken Salad	10	No	Negative	Negative
Gingerbread	14	No	Negative	Negative
Soy Muffin	7	Yes	Positive	Positive
Non-soy Muffin	7	No	Negative	Negative

imprinted polymer film electrodes was at least two standard deviations above the mean current response of control electrodes (NIPs). In each case, the MIP-coated sensors correctly identified the presence or absence of targeted allergen at clinically relevant thresholds with 100% accuracy in all food samples subjected to immunoassay measurements (Table 1).

4. Conclusion

This manuscript describes the first application of MIPs to achieve voltammetric detection of soy in complex foods, through detecting a soy allergenic marker: genistein. The MIP-coated electrodes demonstrated parts-per-billion sensitivity (lower detection limits of 100 ppb) and were successful at distinguishing genistein from several structurally analogous isoflavone and flavone molecules. We also confirmed the applicability of MIP technology for detecting soy allergenic markers in complex foods through correctly identifying the presence or absence of targeted allergen in food samples subjected to both MIP and LFD measurements. The sensing results for soy allergen are comparable or superior to the performance of other commercially available portable allergen detection devices, including LFDs and ELISA (LODs: 1 ppm–10 ppm and 0.31 ppm, respectively) (Cellerino and Lopez, 2016; Wang et al., 2017). Although the performance of our device does not yet exceed the highest sensitivity reported for soy allergens obtained using sophisticated, expensive, and labor-intensive methods such as mass spectrometry and polymerase chain reaction (LODs: 0.48 ppb and 10 ppm, respectively) (Espíñera, Herrero, Vieites, & Santaclara, 2010; Jia, Zhou, Zhu, Shen, and He, 2018), the combination of our system's high sensitivity and selectivity, coupled with short detection times and low cost, offer significant advantages over leading detection technologies.

Through this work, we established the feasibility of developing cheap, selective, and sensitive electrochemical sensors for allergenic ingredients using MIP-based technology. As MIPs are broadly applicable and straightforward to synthesize, these sensors may be adapted to produce devices for a range of analytes, including other food allergens, toxins, and contaminants.

CRedit authorship contribution statement

Madanodaya Sundhoro: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. **Srikanth R. Agnihotra:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. **Brent Amberger:** Conceptualization, Methodology, Validation, Formal analysis, Investigation. **Keenan Augustus:** Conceptualization, Methodology, Validation,

Formal analysis, Investigation. **Nazir D. Khan:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. **Abigail Barnes:** Writing - review & editing, Resources, Supervision. **Joseph BelBruno:** Project administration, Conceptualization, Methodology, Supervision, Writing - review & editing. **Lukasz Mendecki:** Project administration, Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2020.128648>.

References

- Bănică, F. (2012). Chemical Sensors and Biosensors: Fundamentals and Applications. *John Wiley and Sons, Ltd.*. <https://doi.org/10.1002/9781118354162>.
- Becktel, W. J., & Schellman, J. A. (1987). Protein Stability Curves. *Biopolymers*, 26(11), 1859–1877. <https://doi.org/10.1002/bip.360261104>.
- BelBruno, J. J. (2019). Molecularly Imprinted Polymers. *Chemical Reviews*, 119(1), 94–119. <https://doi.org/10.1021/acs.chemrev.8b00171>.
- Bhagwat, S., & Haytowitz, D. B. (2015). USDA Database for the Isoflavone Content of Selected Foods, Release 2.1. U.S. Department of Agriculture. <https://doi.org/10.15482/USDA.ADC/1324538>.
- Blom, W. M., Vlieg-Boerstra, B. J., Kruizinga, A. G., van der Heide, S., Houben, G. F., & Dubois, A. E. (2013). Threshold dose distributions for 5 major allergenic foods in children. *Journal of Allergy and Clinical Immunology*, 131(1), 172–179. <https://doi.org/10.1016/j.jaci.2012.10.034>.
- Bock, S. A., Munoz-Furlong, A., & Sampson, H. A. (2007). Further fatalities caused by anaphylactic reactions to food. *Journal of Allergy and Clinical Immunology*, 119(4), 1016–1018. <https://doi.org/10.1016/j.jaci.2006.12.622>.
- Cellerino, K., & Lopez, L. B. (2016). Soy Protein Detection in Raw and Cooked Meat Products Using Different ELISA Kits. *Journal of Food and Nutrition Sciences*, 4(6), 170–174. <https://doi.org/10.11648/j.jfns.20160406.15>.
- Clark, S., Espinola, J., Rudders, S. A., Banerji, A., & Camargo, C. A., Jr. (2011). Frequency of US emergency department visits for food-related acute allergic reactions. *Journal of Allergy and Clinical Immunology*, 127(3), 682–683. <https://doi.org/10.1016/j.jaci.2010.10.040>.
- Cordle, C. T. (2004). Soy Protein Allergy: Incidence and Relative Severity. *Journal of Nutrition*, 134(5), 1213S–1219S. <https://doi.org/10.1093/jn/134.5.1213S>.
- Cormack, P. A., & Elorza, A. Z. (2004). Molecularly imprinted polymers: Synthesis and characterisation. *Journal of Chromatography B*, 804(1), 173–182. <https://doi.org/10.1016/j.jchromb.2004.02.013>.
- Espíñera, M., Herrero, B., Vieites, J. M., & Santaclara, F. J. (2010). Validation of end-point and real-time PCR methods for the rapid detection of soy allergen in processed products. *Food Additives & Contaminants: Part A*, 27(4), 426–432. <https://doi.org/10.1080/19440040903493777>.
- Gil, E. S., & Couto, R. O. (2013). Flavonoid electrochemistry: A review on the electroanalytical applications. *Revista Brasileira de Farmacognosia*, 23(3), 542–558. <https://doi.org/10.1590/s0102-695x2013005000031>.
- Gupta, R. S., Warren, C. M., Smith, B. M., Jiang, J., Blumenstock, J. A., Davis, M. M., Schleimer, R. P., & Nadeau, K. C. (2019). Prevalence and Severity of Food Allergies Among US Adults. *JAMA Network Open*, 2(1), Article e185630. <https://doi.org/10.1001/jamanetworkopen.2018.5630>.
- Jia, H., Zhou, T., Zhu, H., Shen, L., & He, P. (2018). Quantification of Gly m 5.0101 in Soybean and Soy Products by Liquid Chromatography-Tandem Mass Spectrometry. *Molecules*, 24(1), 68. <https://doi.org/10.3390/molecules24010068>.
- Koppelman, S., Lakemond, C., Vlooswijk, R., & Hefle, S. (2004). Detection of Soy Proteins in Processed Foods: Literature Overview and New Experimental Work. *Journal of AOAC International* (87), 1398–1407. <https://doi.org/10.1093/jaoac/87.6.1398>.
- Liu, Y., Antwi-Boampong, S., BelBruno, J. J., Crane, M. A., & Tanski, S. E. (2013). Detection of Secondhand Cigarette Smoke via Nicotine Using Conductive Polymer

- Films. *Nicotine & Tobacco Research*, 15(9), 1511–1588. <https://doi.org/10.1093/ntr/ntt007>.
- Menon, S., Jesny, S., & Girish Kumar, K. (2018). A voltammetric sensor for acetaminophen based on electropolymerized-molecularly imprinted poly(o-aminophenol) modified gold electrode. *Talanta*, 179, 668–675. <https://doi.org/10.1016/j.talanta.2017.11.074>.
- Asthma and Allergy Foundation of America. (2019). My Life with Food Allergy: Parent Survey Report. Retrieved from <https://www.aafa.org/my-life-with-food-allergy-report.aspx>. Accessed September 11, 2019.
- Pawankar, R., Canonica, G. W., Holgate, S. T., & Lockey, R. F. (Eds.). (2011). WAO White Book on Allergy. World Allergy Organization.
- Plota, A., & Masek, A. (2020). Lifetime Prediction Methods for Degradable Polymeric Materials—A Short Review. *Materials*, 13(20), Article 4507. <https://doi.org/10.3390/ma13204507>.
- Popa, O. M., & Diclescu, V. C. (2013). Electrochemical Behaviour of Isoflavones Genistein and Biochanin A at a Glassy Carbon Electrode. *Electroanalysis*, 25(5), 1201–1208. <https://doi.org/10.1002/elan.201200657>.
- Sakamoto, S., Putalun, W., Vimolmangkang, S., Phoolcharoen, W., Shoyama, Y., Tanaka, H., & Morimoto, S. (2018). Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. *Journal of Natural Medicines*, 72(1), 32–42. <https://doi.org/10.1007/s11418-017-1144-z>.
- Sayyah, S. M., Khaliel, A. B., Aboud, A. A., & Mohamed, S. M. (2014). Chemical Polymerization Kinetics of Poly-O-Phenylenediamine and Characterization of the Obtained Polymer in Aqueous Hydrochloric Acid Solution Using K₂CrO₇ as Oxidizing Agent. *International Journal of Polymer Science*, 2014, Article 520910. <https://doi.org/10.1155/2014/520910>.
- Schirhagl, R. (2014). Bioapplications for Molecularly Imprinted Polymers. *Analytical Chemistry*, 86(1), 250–261. <https://doi.org/10.1021/ac401251j>.
- Sharma, P. S., Pietrzyk-Le, A., D'Souza, F., & Kutner, W. (2012). Electrochemically synthesized polymers in molecular imprinting for chemical sensing. *Analytical and Bioanalytical Chemistry*, 402(10), 3177–3204. <https://doi.org/10.1007/s00216-011-5696-6>.
- U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory (2016). USDA National Nutrient Database for Standard Reference, Release 28. Retrieved from <https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/methods-and-application-of-food-composition-laboratory/mafcl-site-pages/sr11-sr28>. Accessed January 2020.
- Waffo, A. F. T., Yesildag, C., Caserta, G., Katz, S., Zebger, I., Lensen, M. C., ... Altintas, Z. (2018). Fully electrochemical MIP sensor for artemisinin. *Sensors and Actuators B: Chemical*, 275, 163–173. <https://doi.org/10.1016/j.snb.2018.08.018>.
- Wang, Y., Li, Z., Pei, Y., Li, Q., Sun, Y., Yang, J., ... Hu, X. (2017). Establishment of a Lateral Flow Colloidal Gold Immunoassay Strip for the Rapid Detection of Soybean Allergen β -Conglycinin. *Food Analytical Methods*, 10, 2429–2435. <https://doi.org/10.1007/s12161-017-0800-y>.
- Yang, W. W., de Mejia, E. V., Zheng, H., & Lee, Y. (2011). Soybean Allergens: Presence, Detection and Methods for Mitigation. In H. El-Shemy (Ed.), *Soybean and Health* (pp. 433–464). InTech. <https://doi.org/10.5772/17858>.

Supplementary Information

An Electrochemical Molecularly Imprinted Polymer Sensor for Rapid and Selective Food Allergen Detection

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1. Optimization of Experimental Parameters

Sensor performance was optimized through varying different experimental parameters, including monomer and template concentrations, applied voltage, duration of electropolymerization and washing, and pH and ethanol solvent concentrations.

1.1 Effect of polymerization potential and time

The electrochemical properties and structure of an o-PD film depend on the applied potential during chronoamperometric polymerization (Monti, Bacciu, Arrigo, Marceddu, Migheli, Serra, & Rocchitta, 2020). Polymerization duration influences film thickness and cavity concentration, and was found to affect sensor performance (Monti et al., 2020). In order to study the influence of different synthetic conditions on MIP performance, we subjected it to a range of applied potential (at 0.8 V and 1.2 V) (**Figure S1A**) and times (from 10 seconds to 90 seconds) (**Figure S1B**). The current response (after rebinding in 10 ppm genistein) reached a maximum current when the MIP was polymerized for 10 seconds at 1.2 V. However, MIPs synthesized for shorter durations showed weaker stability, therefore further electropolymerization experiments were carried out for 60 seconds at 1.2 V.

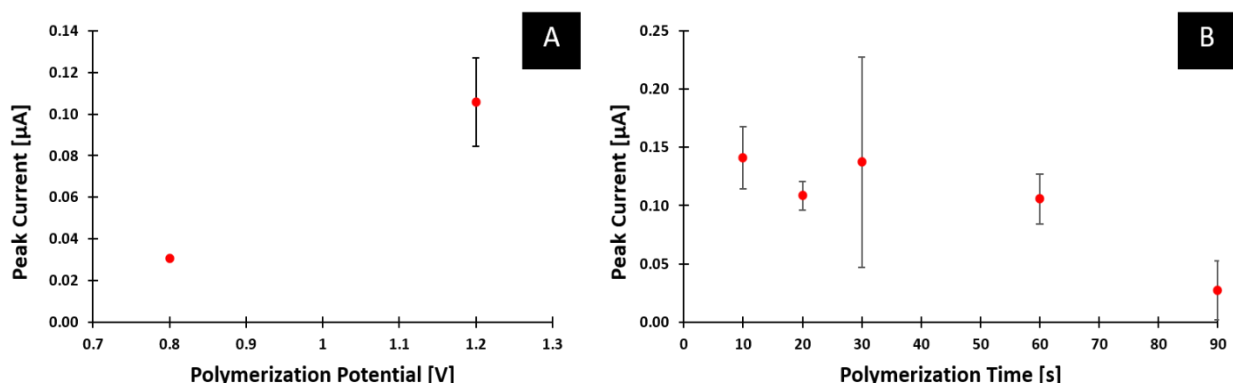


Figure S1: Peak current responses for MIPs to 10 ppm genistein after synthesis at (A) applied potentials of 0.8 V and 1.2 V; and (B) polymerization times between 10 and 90 seconds.

1.2 Effect of polymerization solution composition

Polymerization of o-PD is also known to depend on the pH of the solution (Losito, Palmisano, & Zambonin, 2003). The influence of pH and buffer solution on the analytical performance of MIP electrodes was studied in 10% ethanol and 90% of either an 0.1 M acetic acid/acetate buffer (pH 5.2) or a 1X PBS (pH 7.4) buffer solution (**Figure S2**). The higher peak current after rebinding in 10 ppm genistein was observed for 10% ethanol, 90% acetic acid/acetate

buffer combination, and therefore this solution was chosen as the supporting electrolyte during electropolymerization.

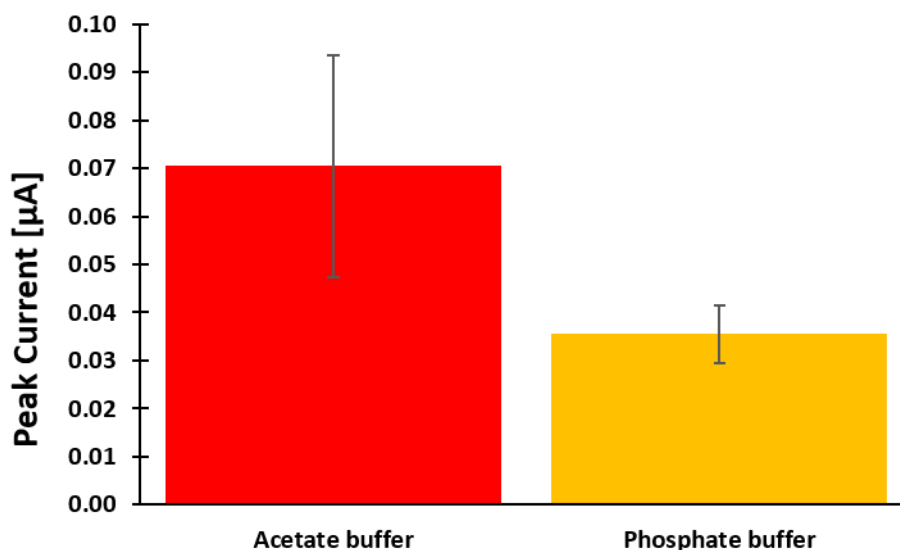


Figure S2: Peak current responses for MIPs to 10 ppm genistein synthesized in 10% ethanol, 90% 0.1 M acetate buffer (red); and 10% ethanol, 90% 1X PBS (gold).

1.3 Effect of monomer and template concentration

The template and monomer concentrations used during electropolymerization of MIPs influences the analytical behavior of the resulting sensor. To measure the effect of the monomer and template concentration on sensor performance, films were formed using a constant concentration of template while varying monomer concentration from 2.5 mM to 10 mM (**Figure S3**), and at a constant concentration of monomer while varying template concentration from 6 μM to 300 μM (**Figure S4**). The response of the MIP electrode to genistein was found to decrease with increasing monomer concentration from 2.5 mM to 10 mM. The peak current was considerably lower at concentrations below this concentration of ortho-phenylenediamine. Similarly, the effect of concentration of the template molecule on sensor response was also evaluated by changing template concentrations in the range of 6 μM to 300 μM. Maximum peak current was obtained at a template concentration of 135 μM. Despite a monomer concentration of 2.5 mM producing the largest current response, we selected 7.5 mM as the optimized value due to the significantly lower variance in its current response, indicating more consistent film formation. The peak currents with respect to different template concentrations showed similar consistencies, so the optimized value was chosen for the maximum peak current, at a template concentration of 135 μM.

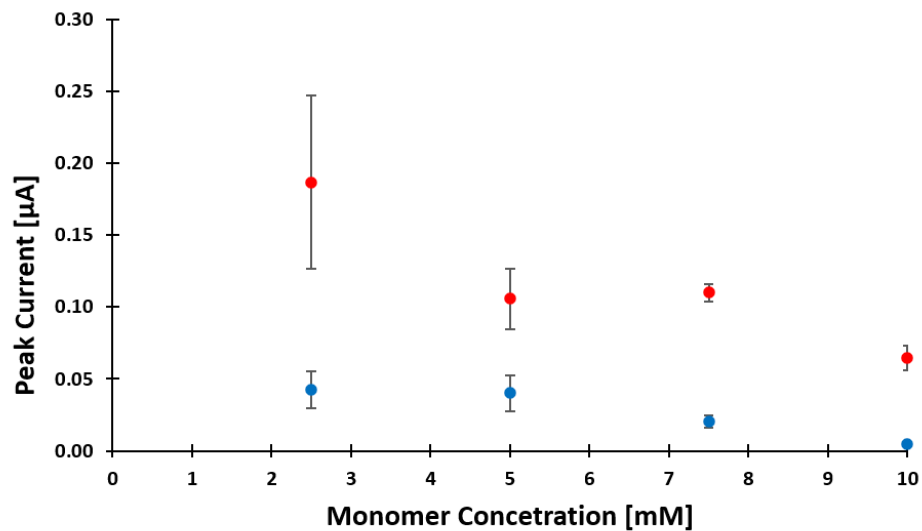


Figure S3: Peak current responses for MIPs (red) and NIPs (blue) synthesized with various monomer concentrations.

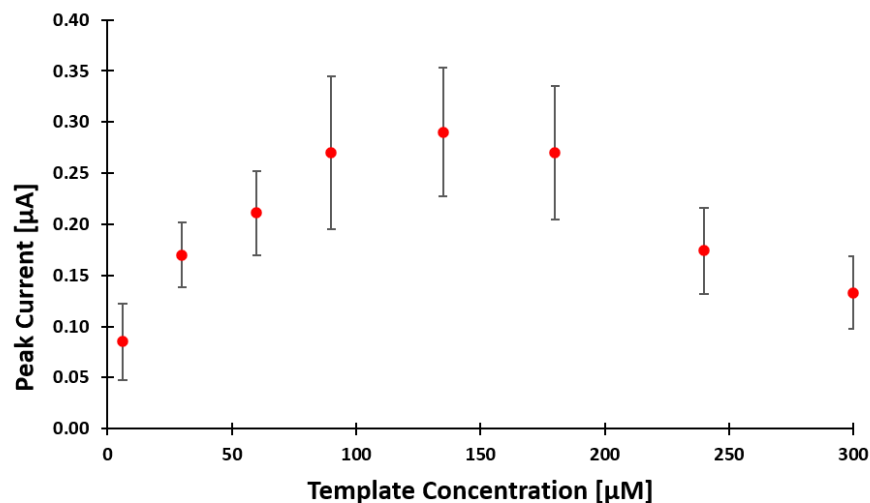


Figure S4: Peak current responses for MIPs synthesized with various template concentrations.

1.4 Effect of extraction solution composition and time

Multiple solvents were evaluated for their effectiveness in removing genistein from the polymeric matrix, including ethanol, tetrahydrofuran (THF), a sodium hydroxide (NaOH) solution, or some combination thereof (50/50 ethanol/NaOH, 10/90 ethanol/NaOH, 10/90 THF/NaOH v/v). 0.1M NaOH showed the best extraction capability from all tested solvents without damaging the film integrity (data not shown).

1.5 Effect of rebinding solution composition

The effect of pH of solution on the electrochemical response of genistein was probed in the pH range 4 to 12 in 10% ethanol and 90% 1X PBS solution (v/v) (**Figure S5**). A maximum peak current response was observed for pH in the range of 7.5 – 8.

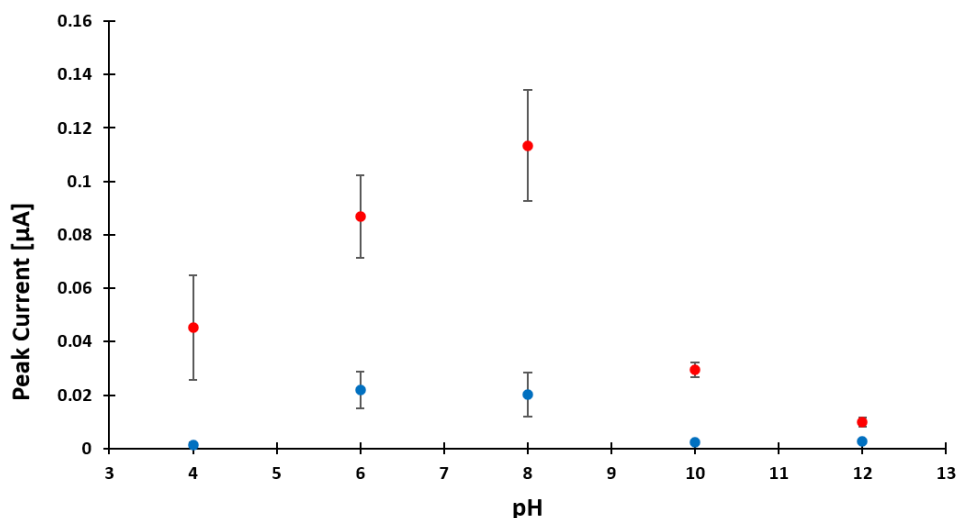


Figure S5: Peak current responses for MIPs (red) and NIPs (blue) in genistein solutions at pH values from 4 to 12.

1.6 Effect of equilibration time

The equilibration time of genistein absorption was determined by evaluating the current response of the MIPs to 1 ppm genistein in 10% ethanol, 90% 1X PBS (v/v) solution. We observed that the peak current remained unchanged after incubating for 60 seconds (**Figure S6**).

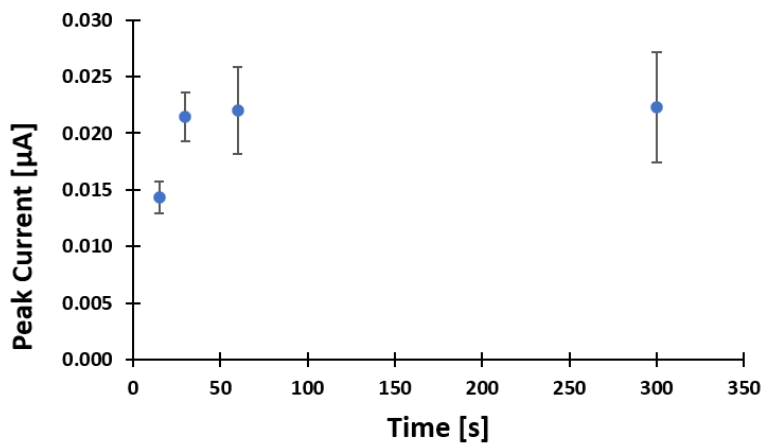


Figure S6: Peak currents of MIP sensors after equilibration for 15 seconds to 5 minutes of time in a 1 ppm genistein in 10% ethanol, 90% 1X PBS (v/v) solution.

2. Characterization of MIP- and NIP-coated electrodes

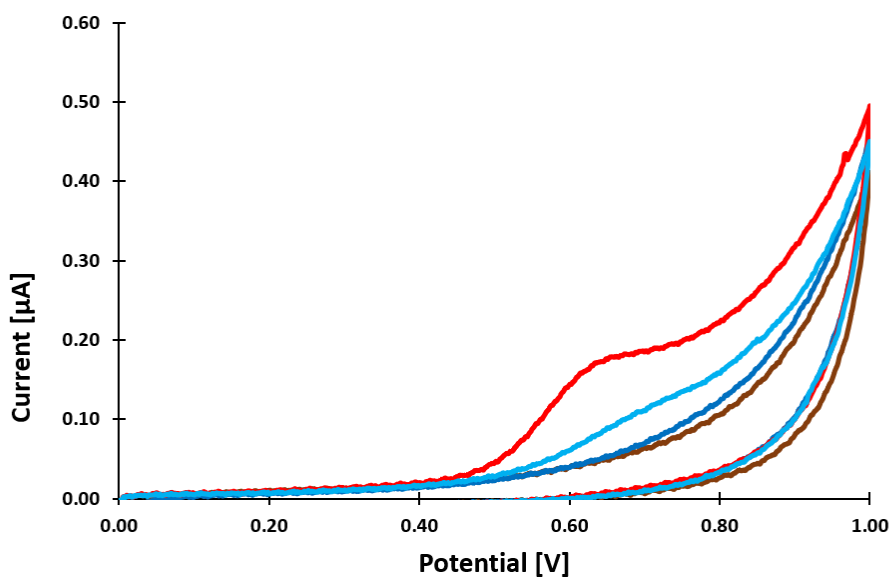


Figure S7: CV response in 10% ethanol, 90% 1X PBS solution (v/v) of MIP after genistein removal (dark red); MIP after incubation in 10 ppm genistein solution (red); NIP after genistein removal (dark blue); and NIP after incubation in 10 ppm genistein solution (light blue).

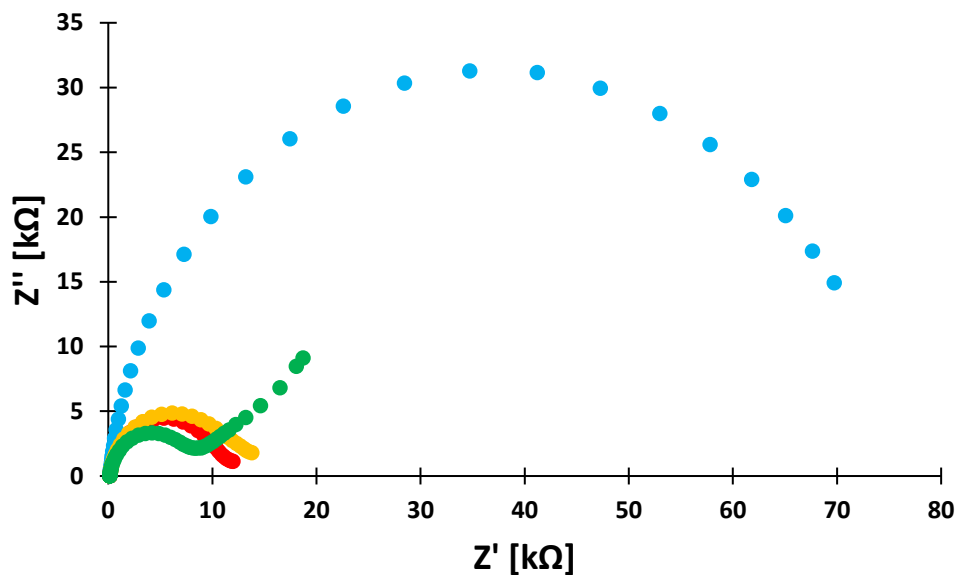


Figure S8: Nyquist plots of modified electrodes in 10 mM $[\text{Fe}(\text{CN})_6]^{4-}$ in 10% EtOH, 90% 1X PBS (v/v) of: bare SCE (green); MIP after genistein extraction (red); MIP after rebinding in 10 ppm genistein (gold); NIP after genistein extraction (blue).

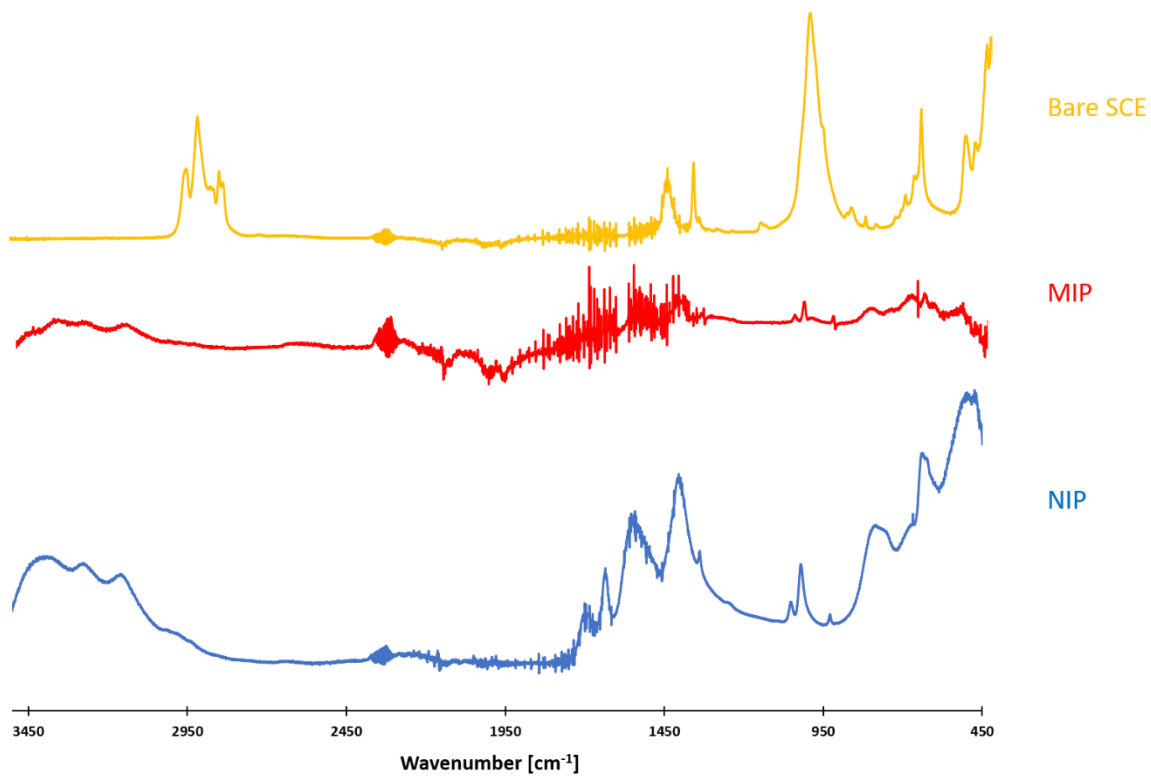


Figure S9: FTIR spectrum of the bare SCE (gold); MIP (red); and NIP (blue).

3. NIP Concentration Profile

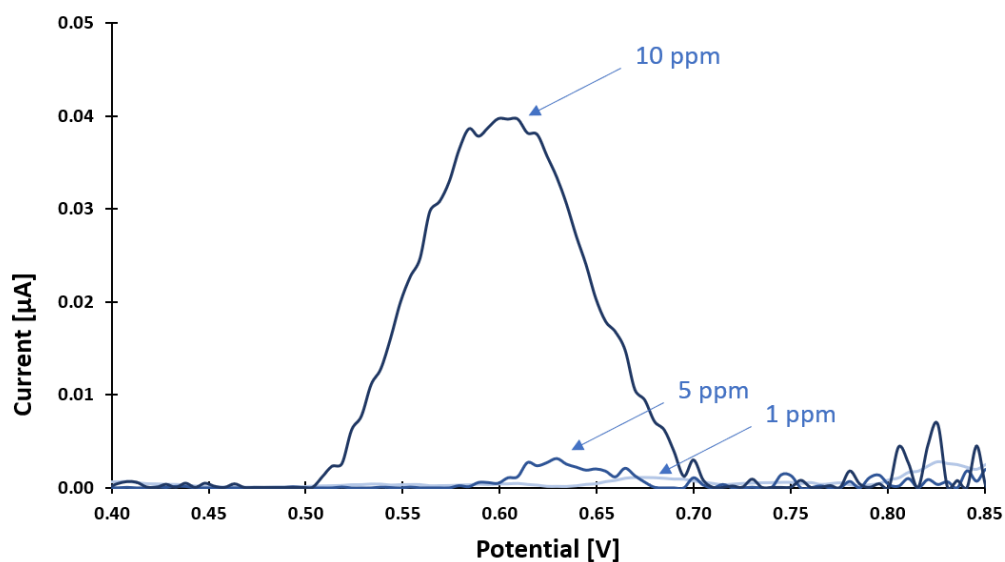


Figure S10: Differential pulse voltammetry recorded at varying concentrations of genistein and the corresponding concentration dependence graph recorded for genistein in 10% ethanol and 90% 1X PBS solution (v/v, pH 7.4) using NIP-coated screen-printed electrodes.

4. Sensor Stability

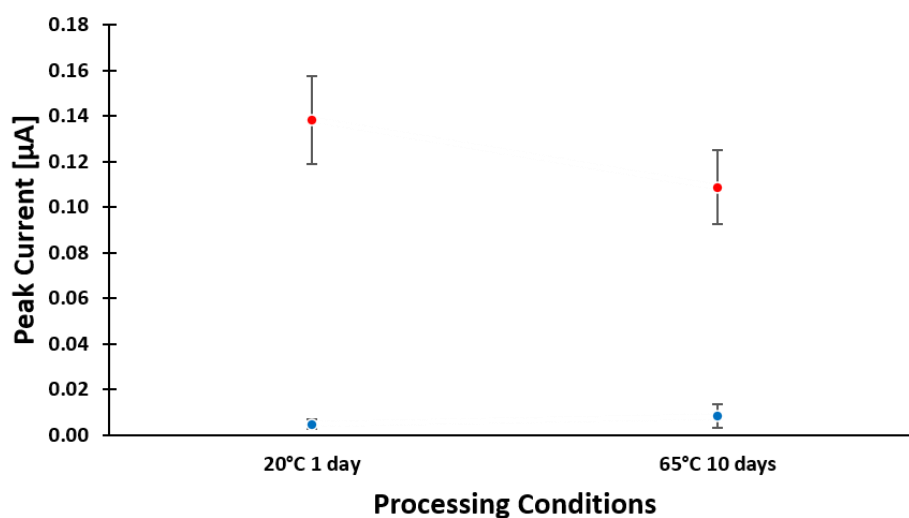


Figure S11: Comparison of the peak current responses for the MIP before and after being stored at 65 °C for 10 days.

5. Interferent Experiments

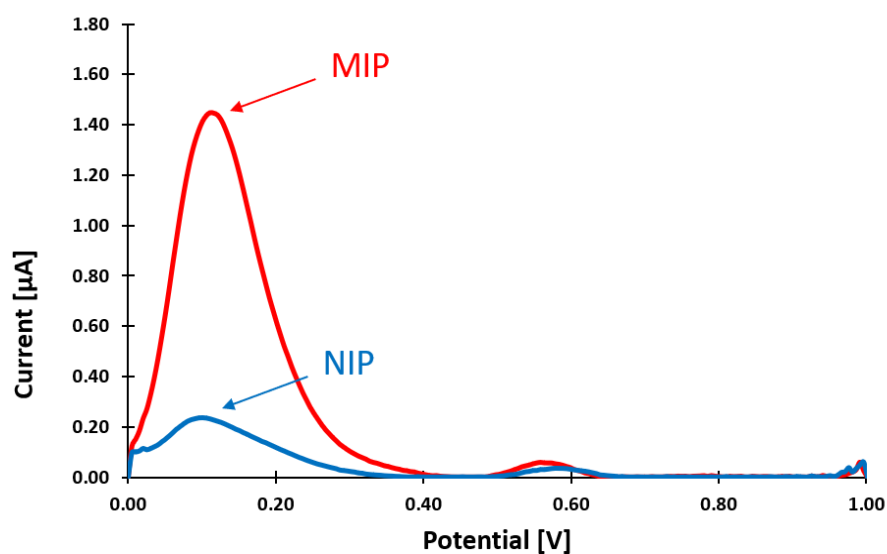


Figure S12. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 10 ppm of quercetin in 10% ethanol and 90% 1X PBS solution.

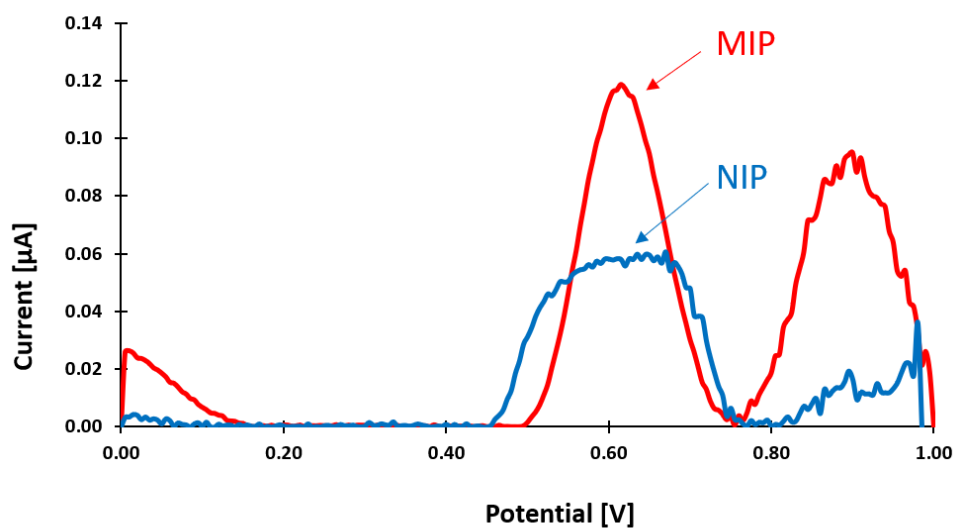


Figure S13. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 10 ppm of daidzein in 10% ethanol and 90% 1X PBS solution.

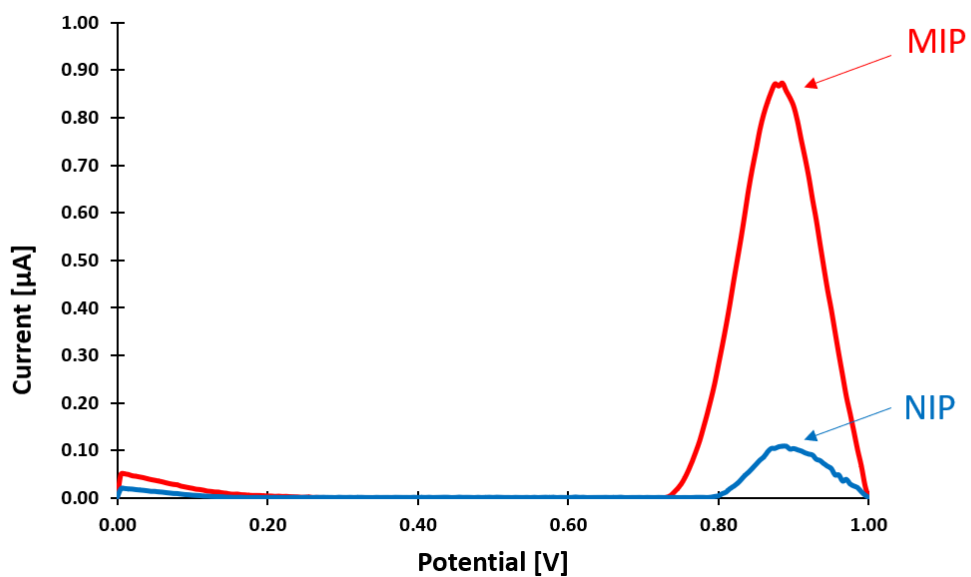


Figure S14. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 10 ppm of 7-hydroxyflavone in 10% ethanol and 90% 1X PBS solution.

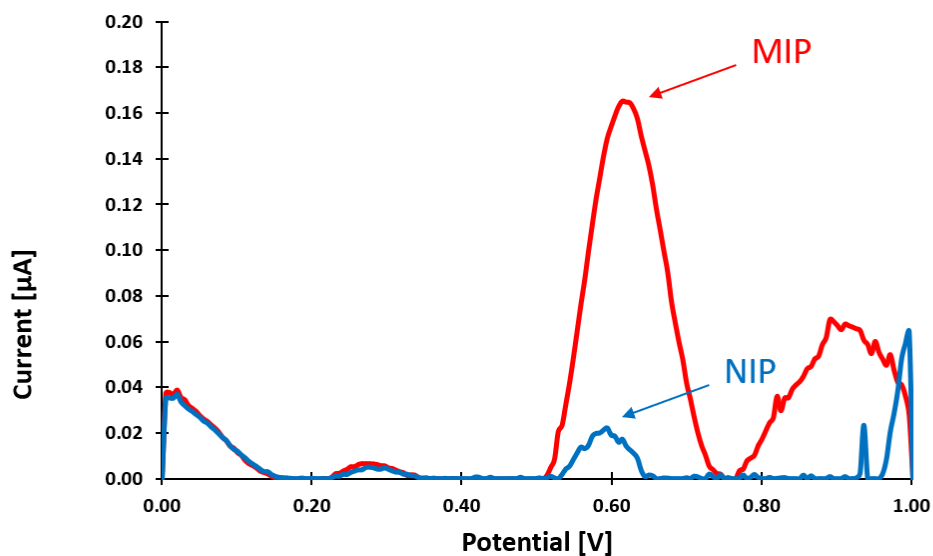


Figure S15. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 10 ppm of quercetin and 10 ppm of genistein in 10% ethanol and 90% 1X PBS solution.

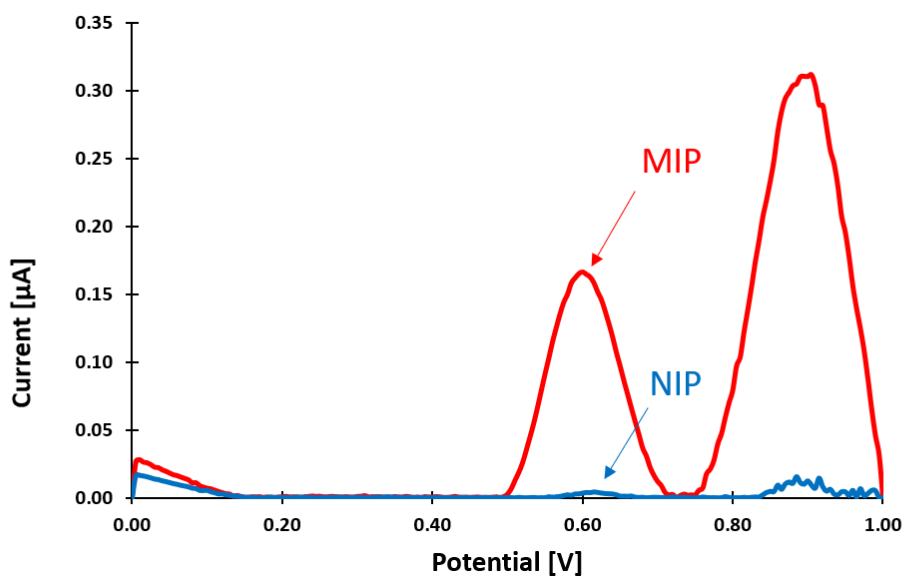


Figure S16. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 10 ppm of 7-hydroxyflavone and 10 ppm of genistein in 10% ethanol and 90% 1X PBS solution.

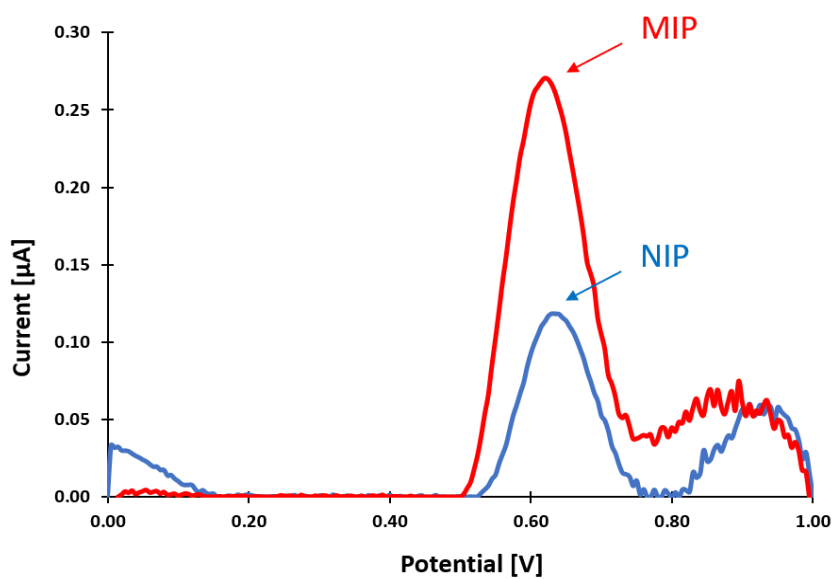


Figure S5. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 10 ppm of daidzein and 10 ppm of genistein in 10% ethanol and 90% 1X PBS solution.

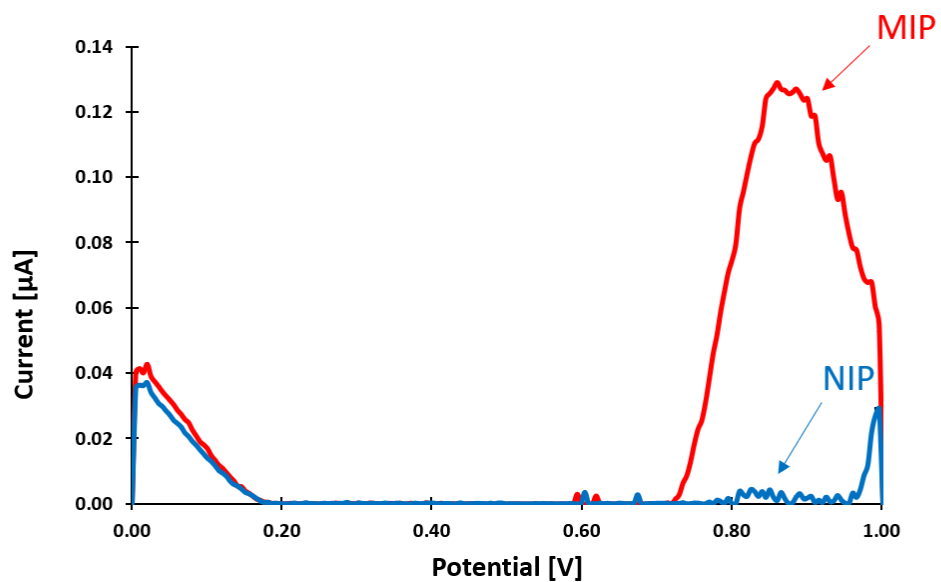


Figure S6. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 200 ppm of tyrosine in 10% ethanol and 90% 1X PBS solution.

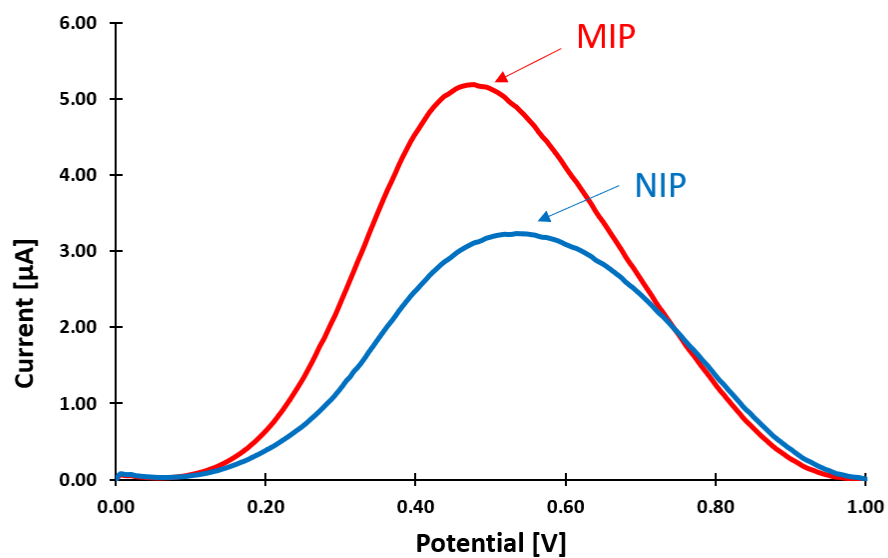


Figure S19. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 200 ppm of vitamin C in 10% ethanol and 90% 1X PBS solution.

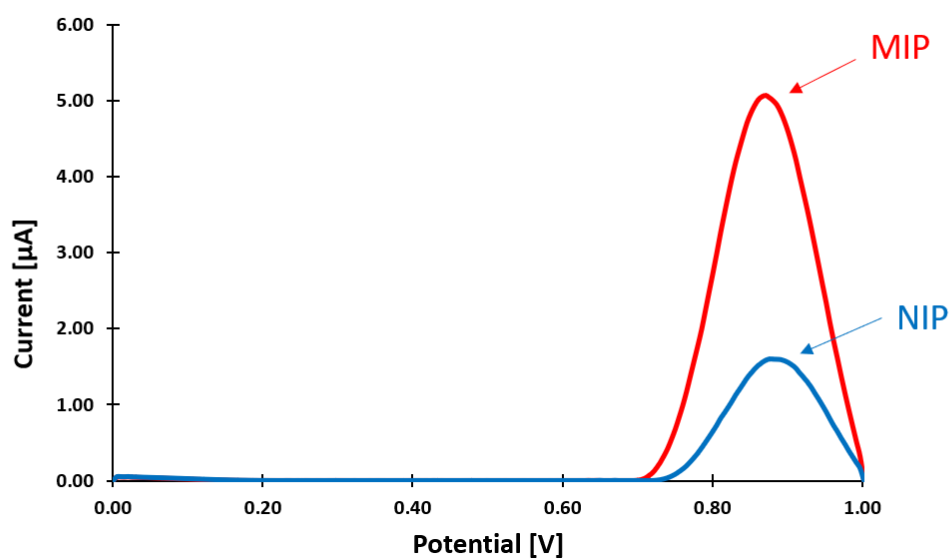


Figure S20. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 200 ppm of tryptophan in 10% ethanol and 90% 1X PBS solution.

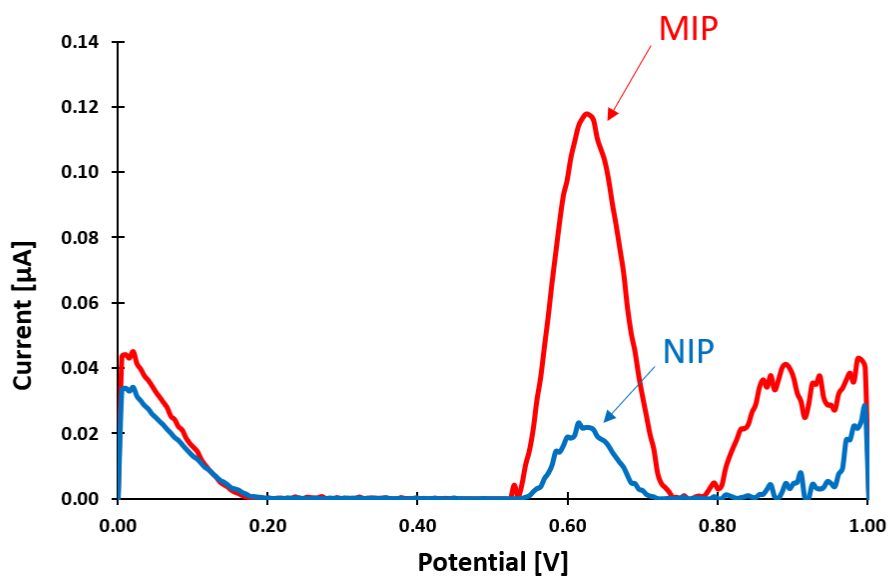


Figure S21. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 200 ppm of tyrosine and 10 ppm of genistein in 10% ethanol and 90% 1X PBS solution.

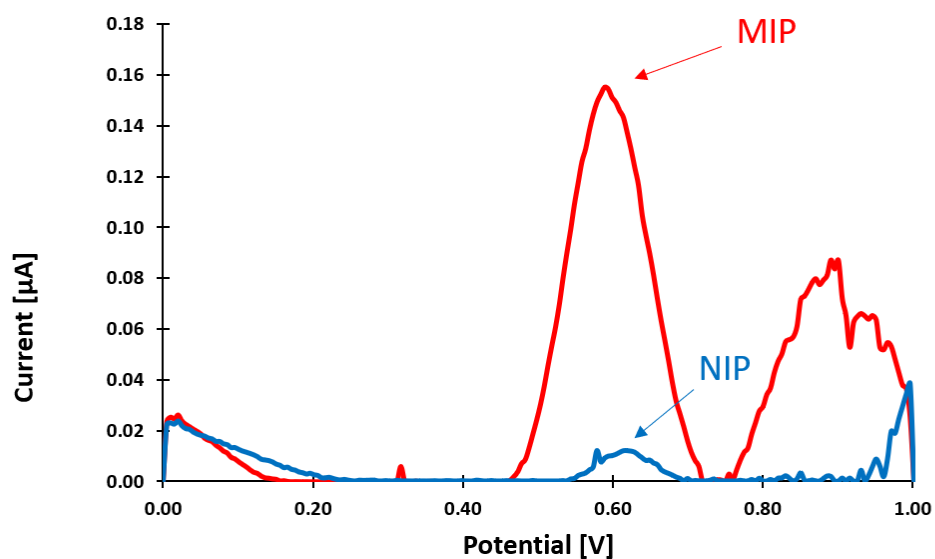


Figure S22. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 100 ppm of vitamin C and 10 ppm of genistein in 10% ethanol and 90% 1X PBS solution.

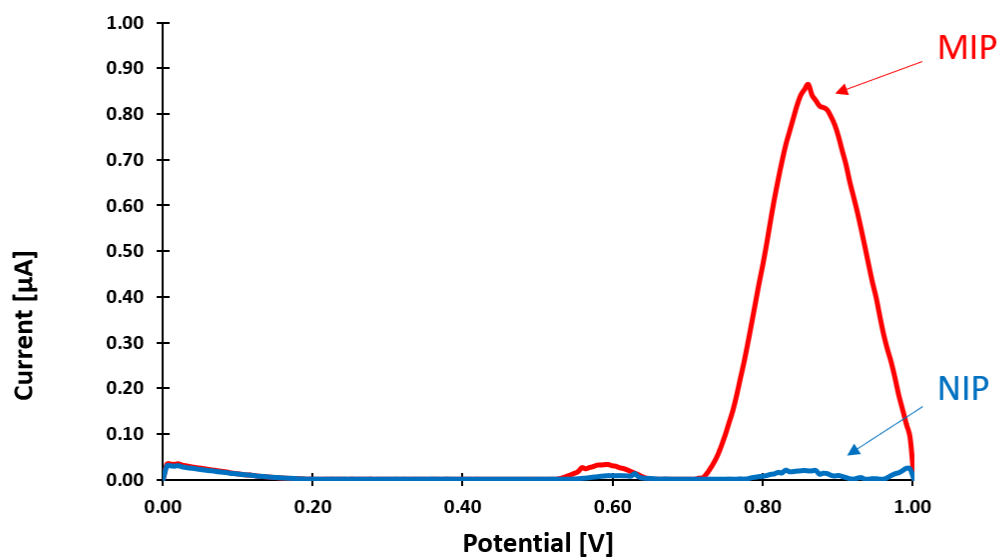


Figure S23. Differential pulse voltammetry recorded for MIP (red) and NIP (blue) in 100 ppm of tryptophan and 10 ppm of genistein in 10% ethanol and 90% 1X PBS solution

6. Food Ingredients

Table S1. List of ingredients in each of the tested food samples.

Food	# of Ingredients	Ingredients
Soy Milk	8	Water, Soybeans, Cane sugar, Sea salt, Carrageenan, Natural flavor, Calcium, Vitamin D3
Non-soy Cookie	9	Flour, Baking soda, Baking powder, Salt, Butter, Sugar, Egg, Vanilla extract, Chocolate
Caramel Soy Sauce	6	Sugar, Lemon, Heavy cream, Vanilla bean, Butter, Soy sauce
Soy Cookie	15	Flour, Baking soda, Baking powder, Salt, Butter, Sugar, Egg, Vanilla extract, Chocolate, Sugar, Lemon, Heavy cream, Vanilla bean, Butter, Soy sauce
Ginger Bok Choy	9	Baby bok choy, Canola oil, Garlic, Ginger, Chicken stock, Butter, Lemon juice, Salt, Pepper
Red Pepper Hummus	9	Chickpeas, Tahini, Canola oil, Red peppers, Sea salt, Lemon juice, Citric acid, Garlic, Lemon oil
Golden Italian Dressing	12	Soybean oil, Vinegar, Sugar, Salt, Garlic, Xanthan gum, Onion, Red bell pepper, Lemon juice, Beta-carotene, Paprika, Natural flavor
Egg Salad	16	Eggs, Canola oil, Honey, Vinegar, Salt, Lemon juice, Celery, Scallions, Mustard seed, White wine, Citric acid, Turmeric, Tartaric acid, Dextrose, Maltodextrin, White pepper
Chicken Salad	10	Chicken, Canola oil, Eggs, Honey, Vinegar, Salt, Lemon juice, Celery, Nisin, Black pepper
Gingerbread	16	Flour, Niacin, Iron, Ascorbic Acid, Thiamine Mononitrate, Riboflavin, Folic Acid, Sugar, Vegetable Oil, Molasses, Water, Cinnamon, Sodium Bicarbonate, Ginger, Salt, Caramel Color.
Soy Muffin	7	Soy flour, Soy milk, Baking powder, Egg, Salt, Sugar, Vegetable oil
Non-soy Muffin	7	Flour, Rice milk, Baking powder, Egg, Salt, Sugar, Canola oil

REFERENCES

- Losito, I., Palmisano, F., Zambonin, P. G. (2003). *Analytical Chemistry*, 75(19), 4988–4995. doi: 10.1021/ac0342424
- Monti, P., Bacciu, A., Arrigo, P., Marceddu, S., Migheli, Q., Serra, P. A., & Rocchitta, G. (2020). Chronoamperometry as effective alternative technique for electro-synthesis of ortho-phenyldiamine permselective films for biosensor applications. *Journal of Applied Polymer Science*, e49172. doi: 10.1002/app.49172