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Sweet potato peel peroxidase-modified graphene oxide electrodes for detection of hydrogen peroxide via electrochemical sensing

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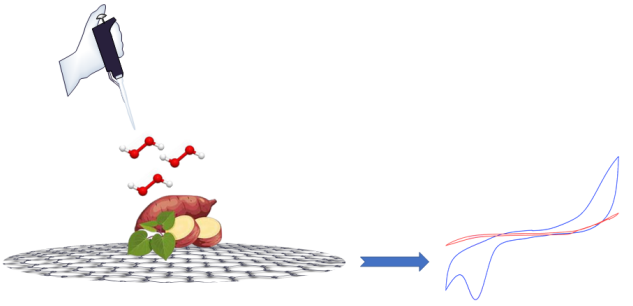
Graphical abstract



Electrochemical detection of H_2O_2 by sweet potato peroxidase

Abstract

The development of efficient and sensitive detection methods for hydrogen peroxide (H_2O_2) is crucial for various applications in biology, medicine, and environmental monitoring. Here, we present a novel approach utilizing sweet potato peel peroxidase (BPP) extract-modified screen-printed graphene oxide electrodes (SPGOE) for the electrochemical sensing of H_2O_2. The SPP was characterized as having a specific activity of 478 U mg^{-1}, an optimum pH of 8.0 and a thermostability at 60 °C with a K_{inact} of $7.02x10^{-3}$ min^{-1}. In this study, we systematically investigate the fabrication process of the peroxidase batata-modified SPGOE and characterize their electrochemical performance using cyclic voltammetry technique. Scanning electron microscopy (SEM) was used to analyze the surface morphology of the modified electrodes, revealing successful enzyme immobilization with a marked increase in surface roughness and visible enzyme clusters compared to the bare SPGOE. This surface modification supports efficient electron transfer, which contributes to the sensor's enhanced electrocatalytic performance. The SPP-SPGOE demonstrates outstanding electrocatalytic performance for the reduction of H_2O_2, showing a linear response across the 250 µM to 5 mM concentration range and a detection limit of 4.6 mM. This novel sensor, created by incorporating SPP onto the GO electrode, offers a promising electrochemical detection system for measuring H_2O_2 in real-world samples, which has significant biomedical and environmental applications. Overall, this study presents a versatile and efficient strategy for electrochemical sensing of H_2O_2 using SPP-SPGOE, paving the way for advanced analytical methodologies with broad applications in biology and beyond.

***Keywords**: Sweet potato peel; Peroxidase; Cyclic voltammetry; Sensing; Hydrogen peroxide.*

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Electrodos de óxido de grafeno modificados con peroxidasa de cáscara de batata para la detección de peróxido de hidrógeno mediante sensado electroquímico

Resumen

El desarrollo de métodos eficientes y sensibles para la detección de peróxido de hidrógeno (H_2O_2) es crucial para diversas aplicaciones en biología, medicina y monitoreo ambiental. En este trabajo, presentamos un enfoque novedoso que utiliza electrodos serigrafiados de óxido de grafeno (ESOG) modificados con extracto de peroxidasa de cáscara de batata (PCB) para la detección electroquímico de H_2O_2. La PCB presentó una actividad específica de 478 U mg^{-1}, un pH óptimo de 8,0 y una termoestabilidad a 60 °C con un K_{inact} de 7,02x10^{-3} min^{-1}. En este estudio, investigamos de manera sistemática el proceso de fabricación de los ESOG modificados con PCB y caracterizamos su desempeño electroquímico mediante la técnica de voltamperometría cíclica. Se utilizó microscopía electrónica de barrido (SEM) para analizar la morfología superficial de los electrodos modificados, revelando una inmovilización exitosa de la enzima con un marcado incremento en la rugosidad de la superficie y la presencia de cúmulos enzimáticos visibles en comparación con los ESOG sin modificar. Esta modificación superficial favorece una transferencia de electrones eficiente, lo que contribuye al desempeño electrocatalítico mejorado del sensor. El PCB-ESOG exhibió un desempeño electrocatalítico sobresaliente para la reducción de H_2O_2, mostrando una respuesta lineal en el rango de concentración de 250 µM a 5 mM y un límite de detección de 4,6 mM. Este novedoso sensor, creado mediante la incorporación de PCB en el electrodo de óxido de grafeno, ofrece un sistema prometedor para la detección electroquímica de H_2O_2 en muestras reales, con aplicaciones significativas en los campos biomédico y ambiental. En conjunto, este estudio presenta una estrategia versátil y eficiente para la detección electroquímico de H_2O_2 utilizando PCB-ESOG, abriendo el camino para metodologías analíticas avanzadas con amplias aplicaciones en biología y otros campos.

***Palabras clave**: Cáscara de batata; Peroxidasa; Voltamperometría cíclica; Detección; Peróxido de hidrógeno.*

Eletrodos de óxido de grafeno modificados com peroxidase da casca de batata-doce para a detecção de peróxido de hidrogênio via sensoramento eletroquímico

Resumo

O desenvolvimento de métodos eficientes e sensíveis para a detecção de peróxido de hidrogênio (H_2O_2) é crucial para diversas aplicações em biologia, medicina e monitoramento ambiental. Neste estudo, apresentamos uma abordagem inovadora utilizando eletrodos impressos de óxido de grafeno (SPGOE) modificados com extrato de peroxidase da casca de batata-doce (SPP) para o sensoramento eletroquímico de H_2O_2. A SPP foi caracterizada com uma atividade específica de 478 U mg^{-1}, pH ótimo de 8,0 e estabilidade térmica a 60 °C com um K_{inact} de 7,02x10^{-3} min^{-1}. Investigamos sistematicamente o processo de fabricação dos SPGOE modificados com peroxidase de batata-doce e caracterizamos seu desempenho eletroquímico utilizando a técnica de voltametria cíclica. A microscopia eletrônica de varredura (SEM) foi empregada para analisar a morfologia da superfície dos eletrodos modificados, revelando a imobilização bem-sucedida da enzima, com um aumento marcante na rugosidade da superfície e a presença de aglomerados enzimáticos visíveis em comparação ao SPGOE não modificado. Essa modificação de superfície promove uma transferência de elétrons eficiente, contribuindo para o desempenho eletrocatalítico aprimorado do sensor. O SPP-SPGOE demonstrou desempenho eletrocatalítico excepcional na redução do H_2O_2, exibindo uma resposta linear na faixa de concentração de 250 µM a 5 mM e um limite de detecção de 4,6 mM. Este sensor inovador, desenvolvido pela incorporação da SPP no eletrodo de óxido de grafeno, oferece um sistema promissor de detecção eletroquímica para a medição de H_2O_2 em amostras reais, com aplicações significativas nas áreas biomédica e ambiental.

Este estudo apresenta, portanto, uma estratégia versátil e eficiente para a detecção eletroquímica de H_2O_2 utilizando o SPP-SPGOE, abrindo caminho para metodologias analíticas avançadas com amplas aplicações em biologia e áreas afins.

***Palavras-chave:** Casca de batata-doce; Peroxidase; Voltametria cíclica; Sensoramento; Peróxido de hidrogênio.*

Introduction

Hydrogen peroxide (H_2O_2) detection plays a pivotal role across various domains including biomedical diagnostics, environmental monitoring, and industrial processes [1,2]. The accurate and sensitive measurement of H_2O_2 is crucial for ensuring safety, health, and operational efficiency. Electrochemical sensing has emerged as a versatile technique for H_2O_2 detection due to its inherent advantages such as simplicity, rapid response, and high sensitivity [3]. Among the plethora of electrochemical sensing platforms, graphene oxide (GO) electrodes have garnered considerable attention owing to their remarkable properties, including high surface area, excellent electrical conductivity, and facile surface functionalization [4-7].

Peroxidases (PODs), specifically classified as EC.1.11.1.x enzymes, are essential enzymes that break down H_2O_2 while oxidizing a wide range of substrates, both phenolic and non-phenolic compounds (AH) [8-10]. The catalytic mechanism can be observed in Figure 1. These enzymes are found ubiquitously in nature, present in bacteria, fungi, algae, plants, and animals [11].

The POD binds to its substrate, typically a peroxide compound like H_2O_2. The enzyme reacts with the peroxide substrate, leading to the formation of an intermediate compound known as Compound I. This step involves the oxidation of the heme iron at the active site. Compound I, now an active oxidizing agent, reacts with the substrate, causing the oxidation of the substrate. This step is crucial for the peroxidase's function in breaking down or transforming specific substrates. After substrate oxidation (AH), Compound I is reduced to Compound II. This reduction involves the transfer of electrons within the enzyme. The enzyme is then restored to its original state through the introduction of an external reducing agent. This process often involves reducing Compound II back to the enzyme's native state, thereby concluding the catalytic cycle [12].

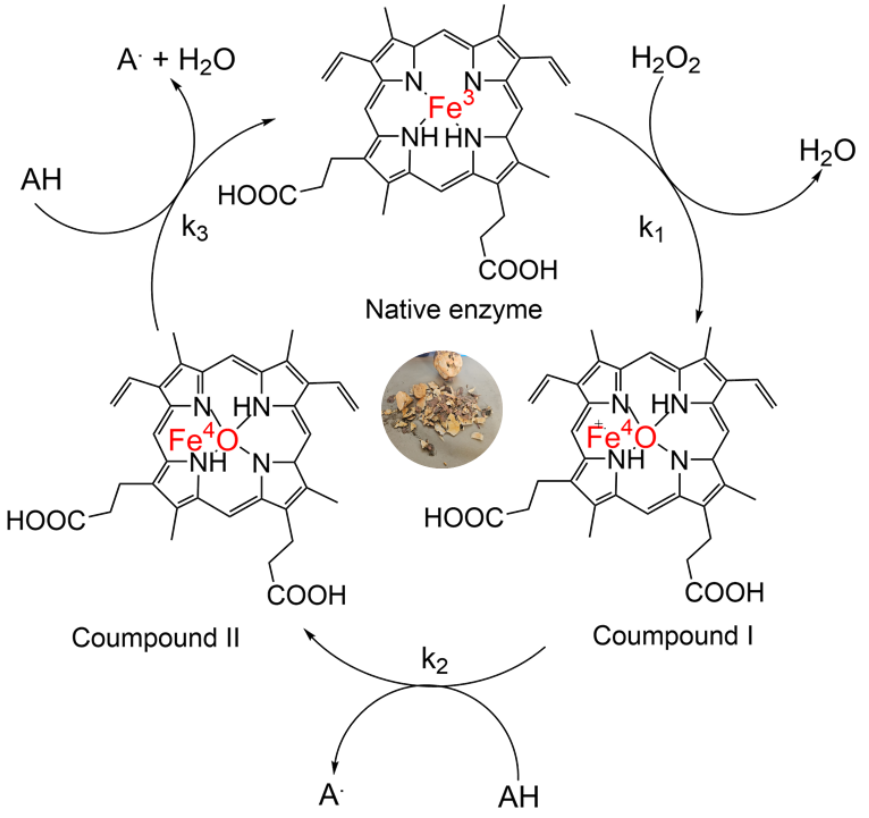

Figure 1. Mechanism of the catalytic cycle of plant PODs.

In recent years, there has been a growing interest in the development of eco-friendly and cost-effective catalysts to enhance the sensitivity and selectivity of electrochemical sensors [13,14]. In the realm of electrochemical sensing, horseradish peroxidase (HRP) has long been a staple enzyme due to its catalytic prowess [15]. However, the conventional use of HRP in electrochemical sensing applications is not without its drawbacks. One significant limitation is its susceptibility to denaturation under harsh conditions, (high concentrations of H_2O_2 and extreme pHs and temperature) leading to a rapid loss of enzymatic activity and compromising the sensor's performance over time [16-18]. Furthermore, the extraction and purification processes required for obtaining HRP are often labor-intensive, involving multiple steps and specialized equipment. This not only increases the cost of production but also introduces potential environmental concerns associated with the use of chemical solvents and reagents. Additionally, the relatively high cost of HRP and its limited stability further hinder its widespread adoption, particularly in resource-constrained settings. These challenges underscore the need for alternative bio-catalysts that offer comparable or superior performance while addressing the limitations associated with HRP. In this context, exploring natural sources such as plant extracts for enzymatic catalysts presents an attractive avenue towards developing sustainable and cost-effective solutions for electrochemical sensing applications. Natural sources, particularly plant extracts, offer a promising avenue for obtaining bio-catalysts due to their abundance, biocompatibility, and enzymatic activities. SPP (sweet potato) has emerged as a notable candidate in this regard, thanks to its intrinsic peroxidase activity and availability from agricultural by-products [19-21].

The utilization of SPP-modified SPGOE for electrochemical H_2O_2 detection presents a novel approach. Immobilizing SPP onto GO electrodes creates a synergistic platform that harnesses the unique properties of both materials, thereby enhancing the catalytic activity towards H_2O_2 reduction. This study encompasses a comprehensive investigation into the characterization of SPP, the fabrication and optimization of SPP-modified GO electrodes, and the electrochemical performance for H_2O_2 sensing. The integration of SPP with SPGOE not only offers enhanced sensing performance but also presents a sustainable and eco-friendly approach to electrochemical sensing applications. This study contributes to the advancement of bio-inspired sensing platforms and holds promise for diverse applications, ranging from biomedical diagnostics to environmental monitoring.

Materials and methods

Chemicals and Instrumentation

All chemicals were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany). All solutions were prepared with ultra-pure water (18.2 MΩcm, Milli-Q purification device, Millipore Corporation, Billerica, MA, USA) unless otherwise stated.

Electro-analytical measurements were conducted using an Autolab PGSTAT101 device (Echo Chemie, Utrecht, the Netherlands) operated with the NOVA 1.10.1.9 software (Metrohm, Filderstadt, Germany). Screen-printed graphene oxide electrodes (dimensions: 33 mm length x 10 mm width x 0.5 mm height), modified with graphene oxide (SPGOE, 110GPHOX), were sourced from DropSens (Oviedo, Spain). These electrodes, which include a working electrode with a diameter of 4 mm, a silver rod pseudo-reference electrode, and an auxiliary carbon electrode, are disposable and modified with graphene and a single layer of GO, respectively. The BPP-modified electrodes were placed in a 5 mL methacrylate electrochemical cell from DropSens (Oviedo, Spain). Cyclic voltammetry (CV) experiments were performed at 27 °C in various solutions. Prior to each experiment, the solutions were degassed with N_2 and stirred magnetically for 30 seconds to remove oxygen bubbles.

Partial Purification and Enzymatic Activity of Batata Peel Peroxidase

Sweet potato (*Ipomea batata*) peels were acquired in a local market located in Santander, Colombia. The peels were washed and extracted in 10 mM borate buffer at pH 8.0. The POD solution obtained was processed using a two-phase system of polyethylene glycol (PEG) and ammonium sulfate ($(NH_4)_2SO_4$) to remove colored compounds. The aqueous phase containing BPP was concentrated via ultracentrifugation. SPP activity was assessed using spectrochemical methods. In this procedure, 10 µL of the enzyme solution was added to 2.5 mL of 30 mM phosphate-buffered saline (PBS), pH 8.0, containing 10 mM guaiacol and 4.4 mM H2O2 as substrates. The reaction was carried out at 25 °C, and the change in absorbance was monitored

at 470 nm (ε_{470} = 26000 M^{-1} cm^{-1}). One unit of enzyme activity (U) was defined as the quantity of enzyme required to oxidize 1 µmole of substrate per minute. The specific activity was calculated as units of activity per milligram of protein, with protein concentrations determined using the PierceTM BCA protein assay kit [22].

pH and Thermostability

The pH stability of SBPP was study using a 10 mM universal buffer (CH_3COOH, H_3PO_4, H_3BO_3–NaOH). In a separate experiment, the thermal stability of BPP was assessed as previously described [9]. A 990 µL solution of 10 mM Tris–HCl (pH 8.0) was heated to 65 °C, after which 10 µL of SPP was added and maintained at the same temperature. At various time intervals, 10 µL aliquots of the enzyme solution were taken, quickly mixed with 90 µL of the same buffer, and incubated for 30 minutes at 25 °C to recover any activity lost due to reversible inactivation. The enzyme activity was then measured.

Immobilization of Sweet Potato Peel Peroxidase onto Screen-Printed Graphene Oxide Electrodes

Initially, the bare electrodes were cleaned with ethanol and water, followed by thorough drying under a nitrogen stream. Next, 5 µL of BSPP solution was applied to the surface of the screen-printed electrodes and allowed to air dry for approximately 10 hours. To stabilize the SPP film and prevent interferences, 2 µL of 0.5 % Nafion was added as a binder over the dried film. The modified electrodes were then incubated at 4 °C for 4 hours. Following incubation, the electrodes were gently rinsed with 10 mM phosphate buffer at pH 7.0 to remove any unbound SPP.

Scanning electron microscopy characterization of the screen-printed graphene oxide electrodes modified with the batata peel peroxidase

The surface morphology of the SPGOE modified with SPP was analyzed using scanning electron microscopy (SEM). Prior to imaging, the modified electrodes were dried under a nitrogen stream to remove residual moisture. Samples were mounted onto aluminum stubs using conductive carbon tape and, when necessary, coated with a thin layer of gold to enhance conductivity. SEM images were captured at magnifications ranging from 200 to 1000 x to observe the distribution and integration of POD onto the graphene oxide surface.

Electrochemical Analysis of Screen-Printed Electrodes Modified with Sweet Potato Peel Peroxidase

CV measurements were conducted on both modified and unmodified electrodes in 10 mM PBS at pH 7.8 to assess the redox behavior of BPP. The bio-electrocatalytic activity of SPP in the reduction of H_2O_2 was investigated using CV. These experiments were performed at a scan rate of 50 mV/s and 25 °C.

Results and Discussion

The results corresponding to the partial purification of SPP are summarized in Table 1. The pigments from the crude extract were removed by using an aqueous biphasic system composed of PEG-ammonium sulfate. The initial crude extract, containing 60 mg of protein, exhibited a total POD activity of 14,980 U. Following ammonium sulfate precipitation with remotion of pigments using PEG, the specific activity increased to 343 U/mg, representing a 1.4 purification fold with an 83 % yield.

The final step, ultrafiltration, significantly improved the specific activity to 478 U/mg, achieving a two-fold purification with a 51 % yield. This step effectively concentrated the enzyme, reducing the total protein to 16 mg while retaining a substantial portion of the enzymatic activity.

Enzyme stability is significantly influenced by pH as it can alter the ionization states of amino acid side chains, affecting their activity [9,23-26]. Extreme pH levels can lead to alterations in the ionization state of these residues, which can disrupt the hydrogen bonding, ionic interactions, and overall tertiary structure necessary for catalytic function. It is well-known that POD activity is pH-dependent. Therefore, the stability of SPP was tested across various pH levels at room temperature. The pH stability experiment for SPP revealed significant variations in activity across different pH levels. The enzyme showed minimal activity at pH 2 and 10, indicating that extreme acidic or basic conditions are detrimental to its function. A gradual increase in activity was observed as the pH moved towards neutrality, with notable enhancements at pH 3, 4, and 5. The enzyme's activity peaked at pH 8, achieving 100 % activity, which suggests this as the optimal pH for its stability and function. At pH 7, the enzyme maintained high activity (89.64 %), while a significant drop was noted beyond the optimal pH 8, with activities at pH 9 and pH 10 declining sharply. These findings indicate that the

enzyme is most stable and active in a slightly basic environment (pH 7-8), which is typical for many biological enzymes [3,27,28]. The sharp decline in activity at pH levels beyond this range underscores the enzyme's sensitivity to pH extremes, which can lead to denaturation or conformational changes affecting its catalytic efficiency. Similar pH-stability profiles have been reported for HRP [15], peroxidase from Royal palm tree (RPP) [18] and guinea grass peroxidase (GGP) at 25, 70 and 66 °C [22], respectively.

Table 1. Purification steps for batata peel peroxidase.

Step	Total volume (mL)	Protein (mg)	Total activity (U)	Specific activity (Umg^{-1})	Yield (%)	Purification fold
Initial extraction	230	60	14,980	249	100	1.0
Elimination of pigments	170	36	12,379	343	83	1.4
Ultrafiltration	40	16	7,653	478	51	2.0

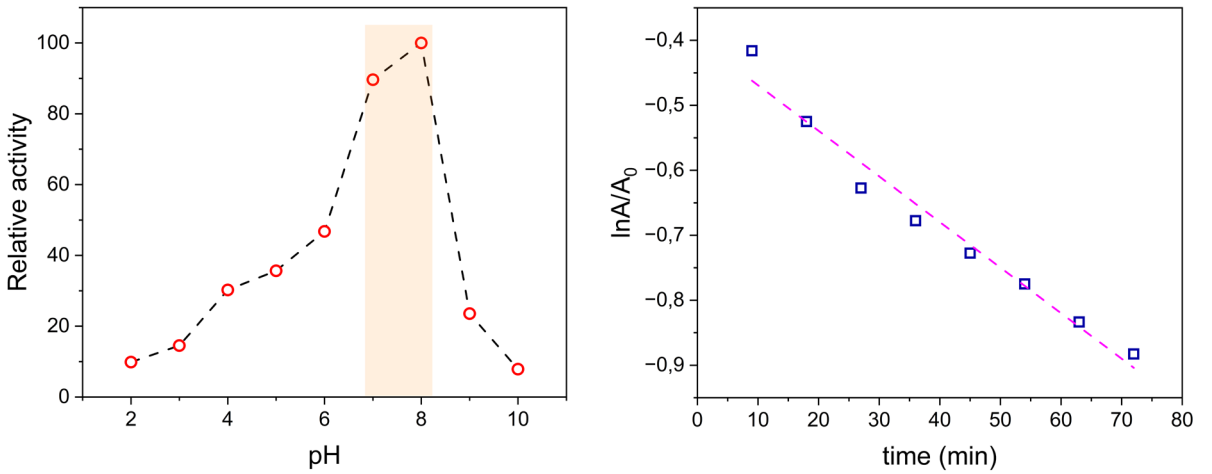

Figure 2. Effect of pH (A) and temperature (B) on activity from sweet potato (*Ipomea batata*).

Enzyme activity generally increases with temperature up to an optimal point, beyond which it decreases rapidly due to denaturation. This relationship is often explained by the Arrhenius law, which describes how reaction rates increase with temperature due to higher kinetic energy, leading to more frequent and forceful collisions between molecules. SPP retains 75 % of its enzymatic activity after 1 hour of incubation at 65 °C and pH 8.0. A similar result was showed by GGP at the same conditions [22]. Under similar conditions, peroxidases from other sources are rapidly inactivated. For instance, tobacco peroxidase is inactivated at 65 °C after 1 hour of incubation [29]. Likewise, peroxidase from pepper fruit retains only 25 % of its total activity after 1 hour at 60 °C. As illustrated in Figure 2B, the thermal inactivation of BPP follows a first-order kinetic model, with a rate constant (k_{inact}) of $7.02x10^{-3}$ min^{-1}. HRP, in comparison, has considerably lower thermostability, with a k_{inact} of $1.6×10^{-2}$ min^{-1} at around 64 °C, inactivating approximately twice as fast. The high stability of SPP is likely due to its strong thermal resistance, which can be attributed to the carbohydrate domain commonly found in many plant PODs [30-32]. This stability, combined with the plentiful availability of raw materials for enzyme purification, creates new opportunities for future analytical applications.

The SEM analysis of the SPGOE, both bare and modified with SPP, reveals distinct differences in surface morphology, indicating successful enzyme immobilization. As is shown in Figure 3A, the bare electrode surface appears relatively smooth and uniform, with few prominent features, suggesting a clean and even distribution of the graphene oxide

layer. In contrast, the electrode modified with SPP exhibits noticeable changes, with an increased surface roughness and the appearance of clustered structures. These clusters are likely attributed to the POD deposited onto the graphene oxide surface, which integrates with the electrode material, resulting in a more heterogeneous morphology. The increase in surface texture and roughness due to the enzyme modification could enhance the active surface area, potentially improving the electrode's sensitivity to H_2O_2 detection. The visible differences between the bare and modified surfaces confirm the successful attachment of SPP to the electrode, which aligns with previous studies demonstrating enzyme immobilization on graphene oxide-based substrates.

To study the reversibility of the redox electron transfer process, we used $[Fe(CN)_6]^{4-}$, a well-known redox probe (), to evaluate the electrochemical response of the electrode after modification with BSPP. $[Fe(CN)_6]^{4-}$ is widely used as an electrochemical redox probe due to its well-defined and reversible redox properties. In biosensing, it acts as a mediator to facilitate electron transfer between the electrode surface and electroactive species. When applied in CV or other electrochemical techniques, $[Fe(CN)_6]^{4-}$ can serve as a sensitive indicator of the electron transfer efficiency of modified electrode surfaces. Figure 3A, shows the CV of the couple ferrocyanide/ferricyanide process in the presence and absence of BPP. The presence of SPP on the surface electrode can significantly enhance the reversibility of the redox reaction, the ferrocyanide/ferrocyanide redox couple exhibited a well-defined symmetric oxidation and reduction peaks, indicating a fast electron transfer process. This results in a closer approach to the ideal Nernstian behavior, with the peak-to-peak separation being smaller, suggesting high reversibility (). In contrast, in the absence of SPP, $[Fe(CN)_6]^{4-}$ showed and irreversible behavior, characterized by larger peak-to-peak separation and asymmetric peaks.

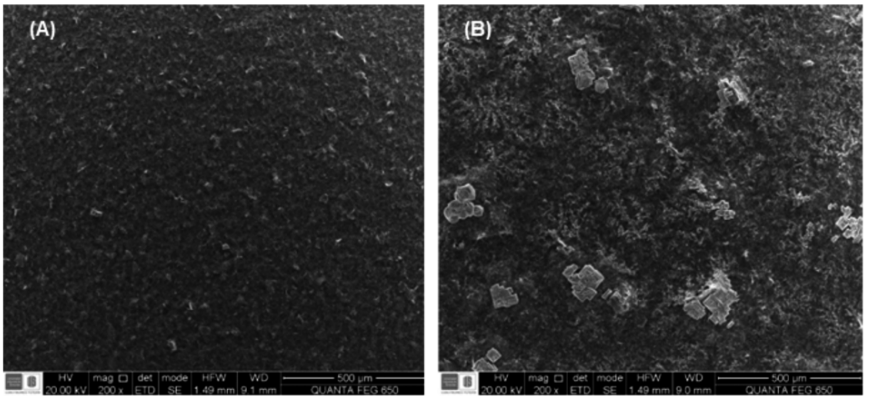

Figure 3. (A) SEM of the unmodified SPGOE and (B) SPGOE modified with the peroxidase from sweet potato.

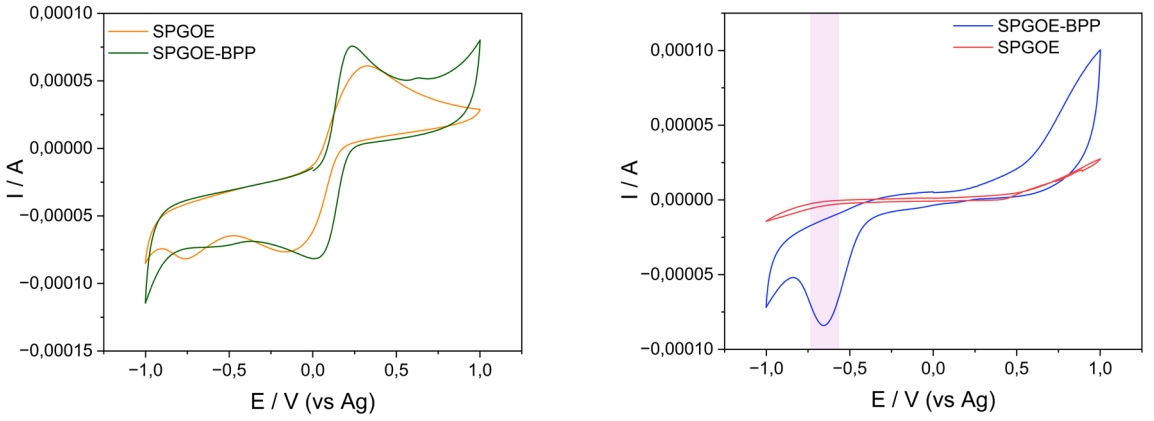

Figure 4. (A) Cyclic voltammograms of bare SPGOE (orange) and, SPP-SPGOE (green) in the presence of 10 mM $[Fe(CN)_6]^{4-}$ containing 0.1 M KCl, scan rate of 50 mV s^{-1}. (B) Cyclic voltammograms of SPP-SPGOE in the absence (red) and in the presence (blue) of 4 mM of H_2O_2, scan rate of 100 mV s^{-1}.

To investigate the electrocatalytic activity of SPP for the reduction of H_2O_2, CV was employed. The modified electrodes with SPP exhibited significantly enhanced electrocatalytic activity in comparison to the unmodified electrodes towards reduction of H_2O_2. This enhancement is attributed to the catalytic properties of the SPP. As is shown in Figure 3A, the CV profiles of the modified electrodes demonstrated a notable increase in the peak current density, indicating a higher rate of electron transfer [16]. This behavior was not observed in the unmodified electrodes, which showed minimal catalytic activity under similar conditions. The modified electrodes exhibited a well-defined redox peak at -650 mV, characteristic of efficient catalytic processes, thereby confirming the successful immobilization of BPP onto the electrode surface. Similar to many other plant PODs and based on earlier studies [24,31,33], the mechanism by which SPP immobilized on SPGOE can reduce H_2O_2 through the formation of compound-I, as is shown in Figure 1. In comparison, the unmodified electrodes showed negligible redox activity under the same experimental conditions. The presence of clear and distinct redox peaks in the SPP-modified electrodes confirms the successful integration of the peroxidase enzyme into the electrode surface, facilitating efficient electron transfer processes [34]. The effect of scan rate is crucial for understanding the kinetics and mechanisms of electron transfer processes [35]. Scan rate variation can reveal important information about the redox behavior, stability, and efficiency of the modified electrodes. Figure 4 illustrates CVs and the linear relationship between the square root of the scan rate and the anodic and cathodic peaks, suggesting a characteristic surface-controlled quasi-reversible process of the electroactive species migrating to the electrode surface [36,37].

To further evaluate the electrocatalytic activity of the SPP-modified electrodes, CV experiments were conducted in the presence of varying concentrations of H_2O_2. The results demonstrated a linear increase in the reduction peak current with increasing H_2O_2 concentrations, indicating a strong catalytic response. This behavior suggests that the SPP-modified electrodes can effectively catalyze the reduction of H_2O_2, which is a crucial reaction in various biosensing applications [24].

In contrast, the unmodified electrodes showed only a slight increase in current with increasing H_2O_2 concentrations, reinforcing the superior catalytic properties of the SPP-modified electrodes. A linear response range of the modified electrode towards different concentrations of H_2O_2 was from 250 µM to 5 mM, and the linear equation regression where $I= 4.56[H_2O_2] – 42.55$, with a correlation coefficient of 0.98. the limit of detection (LOD) was calculated based on the concept given by the International Union of Pure and Applied Chemistry (IUPAC) ($C_{LOD} = 3.3\ S_b/m$, where S_b is the standard deviation of the blank and m the slope of the calibration curve). Sensitivities were calculated from the slope of the calibration curve obtained from current (µA) versus H_2O_2 concentration (mM) (Figure 5B).

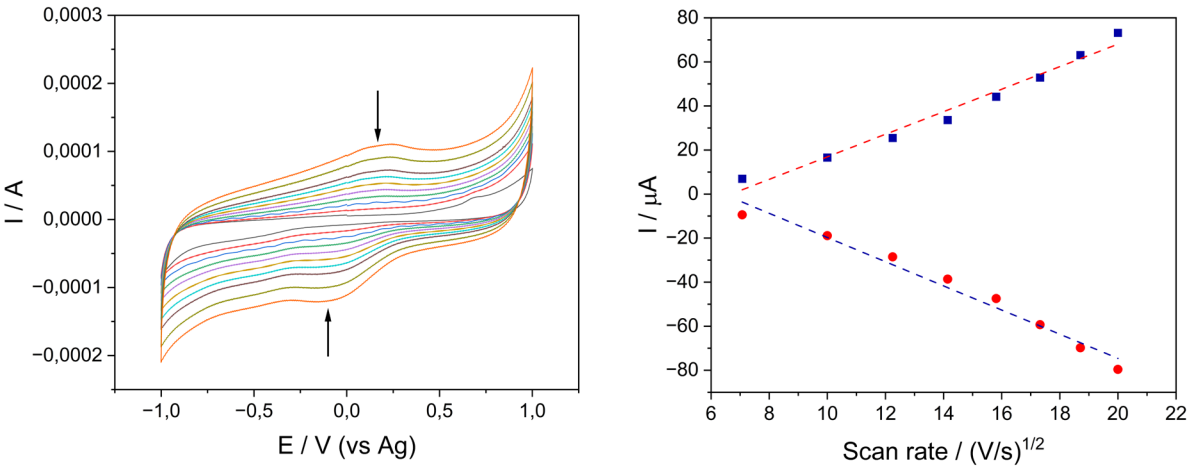

Figure 5. Cyclic voltammograms (A) and scan rate root vs cathodic and anodic currents (B) SPP-SPGOE in 10 mM phosphate buffer pH 7.8 at different scan rates (50, 100, 150, 200, 250, 350, 400, 450, 500 mV s^{-1}).

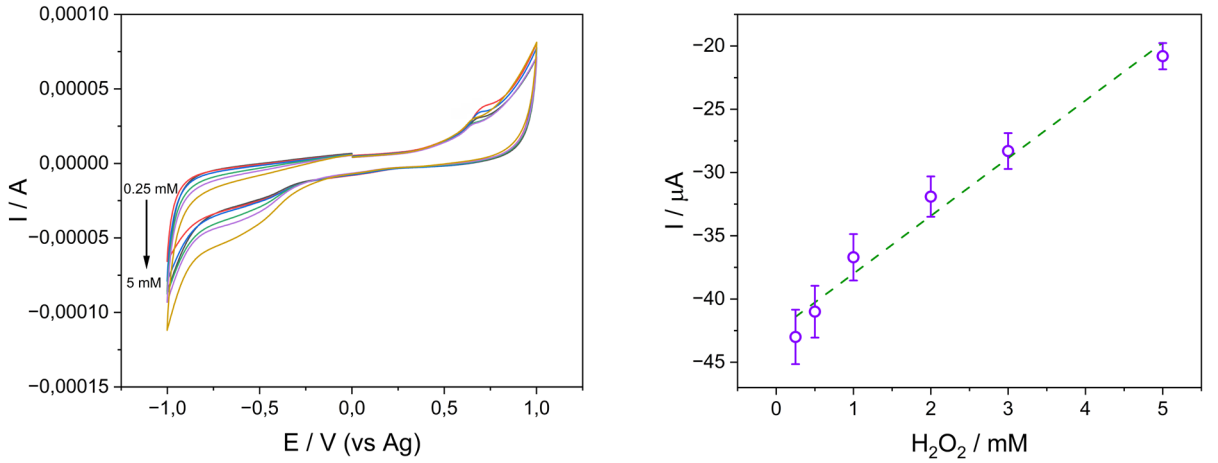

Figure 6. Cyclic voltammograms (A) and calibration curves of BPP-SPGOE (B) at different concentrations of H_2O_2, scan rate (50 mV s^{-1}).

These findings highlight the potential of SPP as an effective biocatalyst for enhancing the electrochemical performance of screen-printed electrodes.

The SPP biosensor exhibits a sensitivity of 1.08 µA/mM, which is lower than that of the Guinea grass (6.29 µA/mM) and Royal palm (4.55 µA/mM) biosensors. This indicates that while the SPP biosensor is responsive, it may require higher concentrations of H_2O_2 to achieve a comparable signal change. The LOD for the batata peel POD biosensor is 4.6 mM, which is the highest among the biosensors listed. This suggests that the batata peel biosensor is less capable of detecting very low concentrations of H_2O_2 compared to its counterparts. When compared with other biosensors in the table, the batata peel peroxidase-based biosensor shows a balanced performance, with strengths in its fast response time and a reasonable linear range. However, its relatively lower sensitivity and higher LOD suggest that there might be trade-offs in using this biosensor for applications demanding high sensitivity and the detection of low H_2O_2 concentrations. The choice of GO as the electrode material contributes to the fast response time and decent linear range, reflecting the material's excellent conductivity and surface area, which enhance electron transfer processes. Nevertheless, further optimization could potentially improve its sensitivity and lower its detection limit, making it more competitive with other high-performing biosensors.

Table 2. Comparative analysis of plant peroxidase-based biosensors to detect H_2O_2: key analytical parameters.

Plant POD source	Electrode	Sensitivity (µA/mM)	Limit of detection (mM)	Linear range (mM)	Response time (s)	Reference
Guinea grass	Graphene	6.29	0.17	0.1-4	3	[22]
Horseradish	Carbon paste	-	0.05	0.05-10	10	[38]
Soybean	Glassy carbon	-	0.02	0.02-0.1	8	[35]
Royal palm	Graphene/ chitosan /glutaraldehyde	4.55	0.87	0.1-5	5	[16]
Sweet potato peel	Graphene oxide	1.08	4.6	0.25-5	3	This study

Conclusions

The study focused on developing efficient and sensitive method for detecting H_2O_2, essential for applications in biology, medicine, and environmental monitoring. We introduced a novel approach using SPP-SPGOE for electrochemical sensing of H_2O_2. The BPP exhibited a specific activity of 478 U mg^{-1}, an optimal pH of 8.0, and thermostability at 60 °C with a K_{inact} of $7.02x10^{-3}$ min^{-1}.The SPP-SPGOE demonstrated excellent electrocatalytic activity for H_2O_2 reduction, with a linear response from 250 µM to 5 mM and a detection limit of 4.6 mM. This innovative sensor, incorporating BPP onto the GO electrode, offered a promising electrochemical detection system for H_2O_2 in real-world samples, with significant biomedical and environmental implications.

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