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Direct Electrochemistry of Polyphenol Oxidase

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The electrochemistry of banana tissues on a carbon paste electrode modified with multi-walled carbon nanotubes (MWCNTs) is presented. Cyclic voltammetry is applied to investigate the direct electrochemistry of banana tissues *i.e.* a source of polyphenol oxidase (PPO). A redox couple with an anodic and counterpart cathodic peak is obtained. The influence of various parameters such as pH, scan rate of potential and heating on the electrochemical properties of polyphenol oxidase in banana tissues were examined. For apple tissues, a same signal is observed on a carbon paste electrode (CPE) modified with multi-walled carbon nanotubes. It was found that presence of carbon nanotubes (CNTs) is essential to observe the electrochemical activity of polyphenol oxidase in banana and apple tissues. In this paper, the electrochemistry of fruits was described. We have shown that multi-walled carbon nanotubes can enhance the direct electron transfer between the electroactive center of polyphenol oxidase in banana tissues and the underlying electrode.

Keywords: Multi-walled carbon nanotubes, Direct electrochemistry, Cyclic voltammetry, Banana, Polyphenol oxidase, Modified carbon paste electrode

INTRODUCTION

Many of the copper-containing redox proteins and enzymes show efficient direct electron transfer. Making the bio-electrochemistry of these enzymes is especially interesting for further study due to their ability to correlate direct electron transfer with common electrode materials (naked and surface modified carbon, gold, platinum, *etc.)* with their 3D-structure [1].

Direct electrochemistry of redox protein or enzyme is of great importance both for studying the intrinsic redox behaviors of proteins and fabricating biosensor without the addition of mediators. Since the first reports on the direct electron transfer between cytochrome *c* and bipyridyl-modified gold [2] or tin doped indium oxide [3] electrodes were communicated in 1977, a number of papers have been published giving detailed information on electrochemical reaction mechanisms of redox proteins and enzyme film at various types of electrode [1,4-6]. Many investigations were done by modified electrodes based on PPO such as; biosensors [7-11], biofuel cell [12], electrocatalytic oxygen reduction [13].

To observe electrochemical behavior, the existence of communication between the electrode and electro-active centre is necessary, but this phenomenon is not easily possible in the case of plant tissues, as their structure does not allow an electroactive centre to get close enough to the electrode surface to exchange electrons. Finding a way to establish this communication is essential to solve this problem.

The electrode material may play a vital role in the production of a bio-electrochemical device able to access the desired information. Therefore, the use of CNTs can be an efficient approach to achieve the communication between electrode and electro-active centre. CNTs are self-assembled nanoscale tubular structures made up of carbon atoms. There are two types of carbon nanotubes: single-walled and multi-walled. Single-walled carbon nanotubes

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(SWCNTs) are the simplest of these nanostructures, being a single plane rolled into a thin tube. The MWCNTs are composed of concentric cylinders of various single-walled carbon nanotubes [14]. The properties of nanotubes, as well as their applications as biosensors [15-18] and sensors [19-22] have been the subject of many studies since the re-discovery of CNTs by Ijima [23], thanks to their special mechanical and electronic properties, conductance and large surface area [24-26].

The main objective of this paper is to investigate the electrochemistry of fruit tissues as an example and to elucidate their electrochemical behavior. In our opinion, this study can provide a path for construction of electrochemical sensors using whole plant tissues. However, it seems to achieve this goal, more studies are required to identify the nature of observed electrochemical behavior and effective parameters on observed electrochemical activity.

MATERIAL AND METHODS

Materials

Bananas and apples were purchased at commercial maturity from a local store. The solvent used for the electrochemical studies was double distilled water. Buffer solutions in the 3.00-7.00 pH range were prepared from orthophosphoric acid and its salts (Merck, Germany). High viscosity paraffin (density = 0.88 g cm^{-3}) from Fluka (Switzerland) was used as the pasting liquid for the carbon paste electrode. Graphite powder (particle diameter = 0.1 mm) from Merck (Germany) was used as the working electrode (WE) substrate. MWCNTs (from Nanotab, USA, 95%), were added to solution containing sulfuric acid and nitric acid in a 3:1 (v/v) ratio for 24 h with stirring. Then, the solution was filtered and the precipitate was thoroughly washed with distilled water. The precipitate was allowed to dry at room temperature. Sulfuric acid (Merck, Germany) and nitric acid (Merck, Germany) were used as received. All other reagents were of analytical grade.

Working Electrode Preparation

Banana-MWCNTs modified carbon paste electrodes and apple-MWCNTs modified carbon paste electrodes were prepared in the following way: for the preparation of banana-MWCNTs-CPE (banana: 5%, MWCNTs: 10%) 0.01 g of banana and 0.02 g of MWCNTs were mixed with paraffin oil in a mortar followed by incorporation of graphite powder (0.2 g) with a subsequent mixing for 10 min. For apple-MWCNTs-CPE, the desired amount of apple was added instead of banana. The resulting paste was then inserted in the bottom of a glass tube. The electric contact was established through a copper wire. The surface of the paste electrode was smoothed on a weighing paper. Unmodified carbon paste electrode was prepared in a similar way, but without MWCNTs and banana or apple.

Instrumentation

Electrochemical studies were carried out using a computer-controlled potentiostat/galvanostat (Autolab, Netherlands). Electrochemical measurements were performed in a three-electrode cell with a banana-MWCNTs-CPE, as working electrode, a Pt wire as counter electrode, and saturated calomel electrode (SCE) as reference electrode. Thus all potentials are referenced to the SCE.

RESULTS AND DISCUSSION

Characterization of Banana-MWCNTs-CPE with Cyclic Voltammetry

Cyclic voltammetry was used as a valuable tool to investigate electrochemistry of banana-MWCNTs-CPE. Cyclic voltammograms of unmodified CPE, CPE-MWCNTs (5% w/w), CPE-banana (5% w/w) and banana-MWCNTs-CPE (MWCNTs: 5% w/w, banana: 5% w/w) in phosphate buffer solution (NaH_2PO_4 0.06 M/Na_2HPO_4 0.04 M, pH = 7.00) are shown in Fig. 1. As Fig. 1 demonstrates, no peak current is seen in curves a, b and c. Thus, direct electrochemistry of enzyme cannot be observed at the surface of unmodified carbon paste electrode. However, after modifying the CPE with MWCNTs in the presence of banana, a couple of well-defined redox peaks (with E_{pa} = 501 mV and E_{pc} = 249 mV *vs.* SCE) with a peak-to-peak separation ($\Delta E_p = E_{pa} - E_{pc}$) of 252 mV at scan rate of potential (υ) 100 mV s^{-1} is appeared (curve d of Fig. 1), that is ascribed to electrochemical behavior of polyphenol oxidase. The obtained ΔE_p is greater than of 59/n mV expected for a reversible system; this result suggests the enzyme redox centre (copper) does not act as a reversible

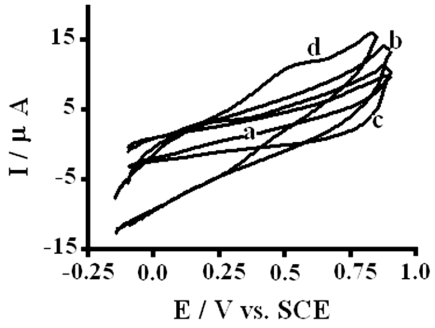

Fig. 1. Cyclic voltammograms of a) CPE, b) CPE-MWCNTs, c) CPE-banana, d) banana-MWCNTs-CPE in phosphate buffer solution (0.1 M phosphate buffer + 0.1 KCl solution, pH = 7.00) at scan rate 100 mV s^{-1}.

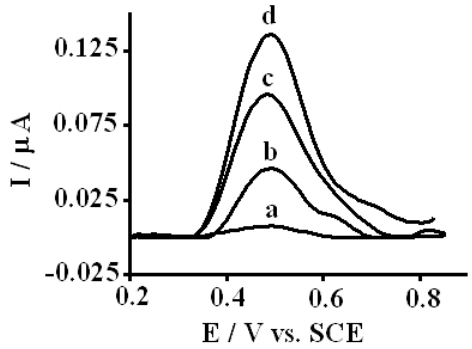

Fig. 2. Differential pulse voltammograms of banana-MWCNTs-CPE in phosphate buffer (0.1 M phosphate buffer + 0.1 KCl solution, pH = 7.00) in the presence of : a. 0.0; b. 4×10^{-3}; c. 7×10^{-3} and d. 9×10^{-3} M of polyphenol oxidase at scan rate of potential 20 mV s^{-1}. The pulse amplitude and pulse time are 25 mV and 5 s, respectively.

system. The half-wave potential, $E_{1/2}$, ((Epa + Epc)/2) of the polyphenol oxidase redox centre is estimated +0.375 V *vs.* SCE, which is in agreement with the reported one (+0.36 V *vs.* SCE) for copper ions of tyrosinase at pH 7.00 [27]. The observed feature reveals that MWCNTs facilitate the electron transfer of polyphenol oxidase redox center. Polyphenol oxidase or tyrosinase are enzymes with a binuclear copper centre, present in plant and animal tissues [28], which catalyze the *o*-hydroxylation of monophenols to *o*-diphenols [29]. They can also catalyze the oxidation of *o*-diphenols to produce *o*-quinones. The electrochemistry of tyrosinase was investigated at the surface of a polished graphite electrode using cyclic voltammetry [27]. The main difference of our study with [18] is using whole banana tissues as a source of enzyme rather than extracted and purified enzyme. To demonstrate that the observed signal at the surface of banana-MWCNTs-CPE is due to redox behavior of polyphenol oxidase we performed another experiment as follows: first, a differential pulse voltammogram of banana-MWCNTs-CPE was obtained in phosphate buffer (pH = 7.00) (Fig. 2, curve a), and then various concentrations of polyphenol oxidase were added to the solution and its electrochemical behavior was studied with differential pulse voltammetry. As shown in Fig. 2, the oxidation peak current is increased with respect to polyphenol oxidase concentration (curves b, c and d), that demonstrates the observed signal at banana-MWCNTs-CPE is due to polyphenol oxidase redox activity.

To investigate the stability of the prepared modified electrode, its continuous cyclic voltammetry was performed in pH 7.00 buffer solution (Fig. 3). The stability was suitable; however, the obtained peak current for different experiment was not exactly the same between and within different studies. One possible explanation could be that, the banana used in the experiments was not the same and this resulted in electrodes with different enzyme contents.

Influence of WMCNTs Percentage

Banana-MWCNTs-CPE with two different percentages of MWCNTs (w/w): 5% and 10% was prepared to probe the influence of the percentage of MWCNTs on the observed electrochemical signal. As shown in Fig. 4 with 10% MWCNTs, the increase in the peak current and decrease in peak potential separation was enough for further investigation. As expected, with an increasing percentage of MWCNTs, the peak current and peak potential separation increased and decreased, respectively. This result indicates the electron transfer of polyphenol oxidase redox center is more facilitated on a banana-MWCNT-CPE with a higher amount of MWCNT.

Probing Influence of Banana Percentage

The effect of banana percentage on the observed

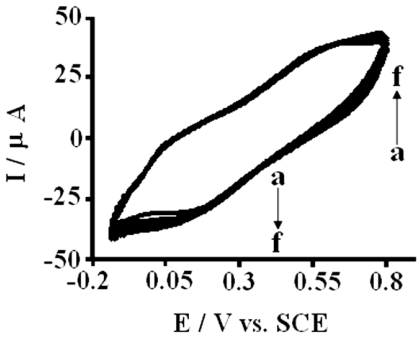

Fig. 3. Continuous cyclic voltammograms of banana-MWCNTs-CPE in buffer solution (0.1 M phosphate buffer + 0.1 KCl solution, pH = 7.00) at scan rate of 100 mV s^{-1} (a → f is first to sixth cycles).

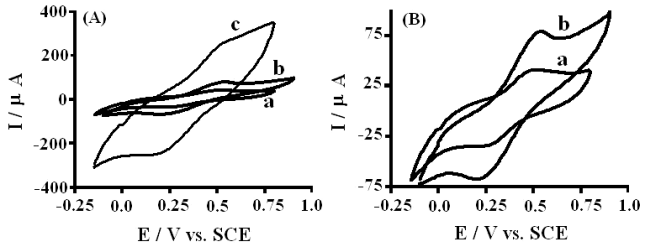

Fig. 5. Cyclic voltammograms of banana-MWCNTs-CPE with various percentage of Bananas (A): a. 5%; b. 10%; c. 20% in buffer solution (0.1 M phosphate buffer + 0.1 KCl solution, pH = 7.00) at scan rate 100 mV s^{-1}. (B) Just includes curves "a" and "b" for better illustration.

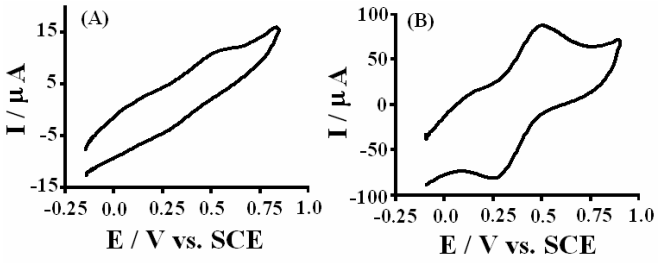

Fig. 4. Cyclic voltammograms of banana-MWCNTs-CPE with various percentage of MWCNTs (A): 5%, (B) 10% in buffer solution (0.1 M phosphate buffer + 0.1 KCl solution, pH = 7.00) at scan rate 100 mV s^{-1}.

electrochemical current was also examined. As expected, increasing banana percentage in preparation of banana-MWCNTs-CPE resulted in an increased peak current (Fig. 5). However, with a higher percentage of bananas, the quality of the carbon paste was decreased, so we investigated the influence of banana percentages set at 5%, 10% and 20%.

Investigation of pH Effect

The cyclic voltammetry of the banana-MWCNTs-CPE was performed at various pH values ($3.00 \leq pH \leq 7.00$) to examine the effect of pH on its electrochemical behavior. These studies revealed that while pH variation does not have noticeable effect on ΔE_p and peak position, better current is observed at pH = 7.00. Therefore, we use this pH for later studies. Some papers record the effect of pH on the enzyme activity and report that pH = 7.00 or 6.00 is optimum value [30,31].

Effect of Scan Rate

The effect of the potential scan rate on the electrocatalytic property of banana-MWCNTs-CPE was studied by cyclic voltammetry at various scan rates and then oxidation peak current (I_{pa}) was plotted *vs.* υ and $\upsilon^{1/2}$ (Fig. 6). As can be seen, I_{pa} increases linearly with $\upsilon^{1/2}$, which demonstrates a diffusion controlled electrochemical process.

Heating Effect on Electrochemical Response of Banana-MWCNTs-CPE

It is generally accepted that the enzyme activity decreases extremely with heating [32-34]. For this reason we also examined the effect of heating on the electrochemical activity of the banana-MWCNTs-CPE. A desired amount of banana was first weighed and heated at 50 °C in oven and then added to MWCNTs-CPE. Figure 7A shows the cyclic voltammograms of banana-MWCNTs-CPE without heating (curve a) and with heating (curve b). As can be seen, the peak currents decrease enormously after heating, which is expectable for an enzyme. Moreover, the heating effect after electrode fabrication was also studied which led to a same result. As can be seen in Fig. 7B, heating the fabricated electrode decrease the redox peak

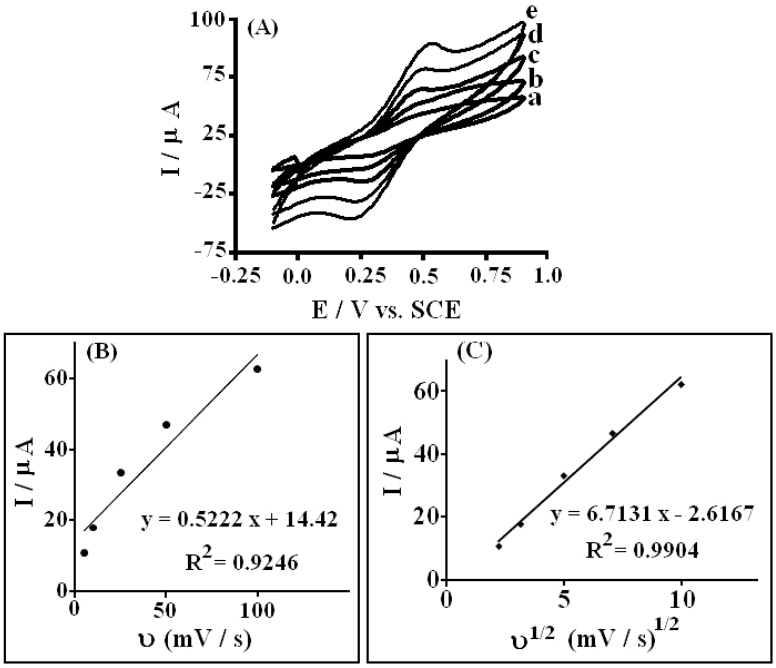

Fig. 6. (A) Cyclic voltammogram of banana-MWCNTs-CPE in phosphate buffer solution (0.1 M phosphate buffer + 0.1 KCl solution, pH = 7.00) at various scan rates of potentials: a. 5; b. 10; c. 25; d. 50 and e. 100 mV s^{-1}. The plots of oxidation peak current *vs.* υ (B) and *vs.* $υ^{1/2}$ (C).

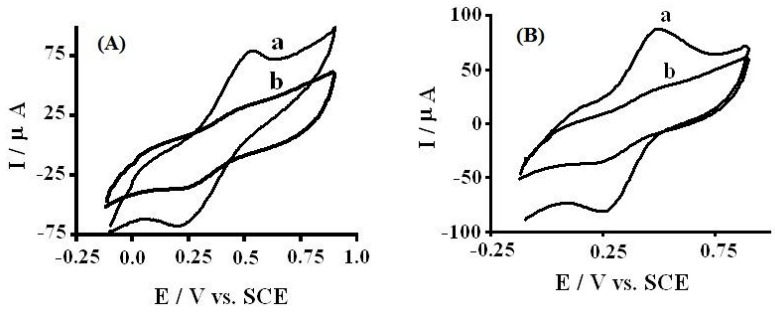

Fig. 7. Cyclic voltammogram of banana-MWCNTs-CPE prepared: (A) (a) without heating, (b) with heating the babana tissue; (B) (a) without heating, (b) with heating the electrode after fabrication , in phosphate buffer solution (0.1 M phosphate buffer + 0.1 KCl solution, pH = 7.00) at scan rate 100 mV s^{-1}.

current.

Modification MWCNTs-CPE with Apple

In order to be more confident that the proposed method is applicable to other fruits, we also used apple instead of banana as another modifier which is believed to contain polyphenol oxidase as its redox center [35-37]. Thus, a working electrode was modified with 10% MWCNTs and 5% apple then its electrochemical behavior was studied by cyclic voltammetry (Fig. 8). The potentials of the two peaks are the same, indicating that the peaks obtained are related to the same redox centers. Peak current for apple (curve a)

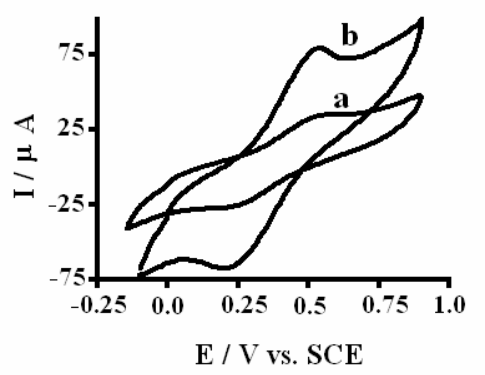

Fig. 8. Cyclic voltammograms of (a) app-MWCNTs-CPE, (b) banana-MWCNTs-CPE in phosphate buffer solution (pH = 7.00) at scan rate 100 mV s^{-1}.

is less than that for banana (curve b); possible explanations for that could be lower content of polyphenol oxidase as an electroactive center in apple or differences in the diffusion coefficient between the different fruits.

CONCLUSIONS

In this paper, the electrochemistry of fruits was described for the first time. We have shown for the first time that MWCNTs can enhance the direct electron transfer between the electroactive center of polyphenol oxidase in banana tissues and the underlying electrode. It was found that presence of carbon nanotubes is essential to observe the electrochemical activity of polyphenol oxidase in banana and apple tissues. The obtained peaks' current for banana tissues was greater than that of apple tissue, which could be ascribed to a lower enzyme content of apple. The observed electrochemical activity was found to be sensitive to heating, as expected for enzyme activity.

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