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Electronic Detection of *Drechslera* sp. Fungi in Charentais Melon (*Cucumis melo* Naudin) Using Carbon-Nanostructure-Based Sensors

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ABSTRACT: The development of chemical sensor technology in recent years has stimulated an interest regarding the use of characteristic volatiles and odors as a rapid and early indication of deterioration in fruit quality. The fungal infestation by *Drechslera* sp. in melons is a severe problem, and we demonstrate that electronic sensors based on carbon nanostructures are able to detect the presence of these fungi in melon. The responses of sensor conductance G and capacitance C at 27 kHz were measured and used to calculate their ΔG and ΔC variation over the full melon ripening process under shelf conditions with proliferation of *Drechslera* sp. fungi. The sensor response showed that these fungi can be electronically identified in charentais melon, constituting an effective and cheap test procedure to differentiate between infected and uninfected melon.

KEYWORDS: *Melon ripening, sensors, carbon nanotubes, carbon nanocoils, fungus*

INTRODUCTION

There is an enormous increase in the demand for applications of sensors in the detection of different chemical species. Several sensors based on conjugated polymers, carbon nanostructures, and related composites are attracting attention because of their potential low cost, high sensitivity, and rapid response to stimuli.[1]

These devices show an electrical response that is dependent upon both the nature of the molecular species and their concentration. When such sensors are exposed to a gaseous analyte, they interact with at least one of the volatile compounds, thereby influencing the electronic transport in the composite.[1−3]

Carbon nanocoils and nanotubes have similar structural characteristics, because they are both made of carbon and have a nanometric radius. These structures possess π-electron orbitals, which are responsible for charge transport and show high electron density at the carbon structure surface. Moreover, considering the high surface area/volume ratio of these structures, molecular adsorbates can strongly affect their intra- and inter-carbon nanotube electronic transport properties.

In recent years, technological advances have allowed for chemical sensors to be exploited to study agricultural products, for example, to assess the quality and the ripening stage in fruits.[4] Fruit ripening is associated with the physical characteristics of fruit and is a programmed process that culminates in dramatic changes in color, released species, firmness, texture, flavor, and aroma of the fruit flesh, rendering the fruit acceptable for consumption.[5−10]

During ripening, charentais melon (*Cucumis melo* Naudin) generates a characteristic aroma and intense, volatile precursors are generated. Approximately 240 compounds have been identified as volatile constituents of melon and melon products.[11,12] These include (*Z*)-3-hexanal, hexanal, (*Z*)-3-octen-1-ol, ethanol, acetaldehyde, 3-methyl-2-butenyl acetate, methyl heptanoate, and 1-pentanol, which have been shown to be the most odor-active aroma volatiles in fresh melon.[13−15] The liberation of these volatile chemical species may quantitatively and qualitatively vary during the different ripeness stages.[6,7] Thus, chemical sensors based on composites, which are known to have a response dependent upon analyte nature and concentration, could in principle also respond differently to the different ripeness stages of the melon.[11,16]

The charentais melon belongs to the cantaloupensis group.[17] The main fruit crop is grown in the northeast region of Brazil.[12,18−20] This fruit is classified as a berry, has a variable shape, size, and color, contains between 200 and 600 seeds, and has a central cavity and edible portion.[12]

Because melons have a short postharvest storage time frame, careful planning is required to modulate production and demand. The fruits may require transport at various ripening stages prior to consumption.[21] However, during transport and storage the presence of microorganisms may cause deterioration of the melon quality. To monitor the presence of microorganisms (mostly fungi) during transport and storage, sensors can play a vital role in early stage warning for logistics planning, timing, and commercial strategies.[22−24] A specific chemical sensor marker with adequate reproducibility to detect early spoilage would help

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prevent major losses as a result of infestation of the stored melon by fungi because of poor storage management.[14,23] Fungi commonly produce volatile compounds as they start colonizing the nutrient-rich substrate inside melons.[13−15] The major volatile compounds that result from this process are 3-methyl-1-butanol, 1-octen-3-ol, and other 8-carbon ketones and alcohols.[15,25]

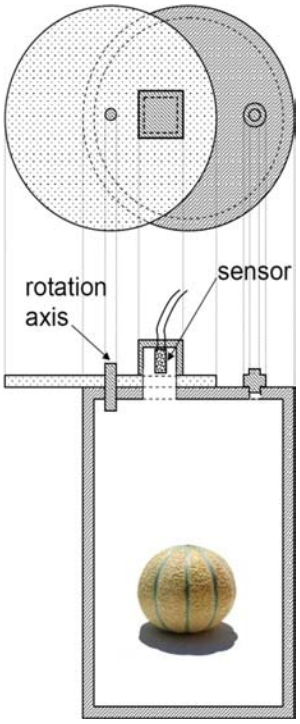

Figure 1. Sealed chamber (7.8 L) with inserted charentais melon.

The interaction of these volatiles with carbon structure−polyvinyl alcohol (PVA) composites could result in a significant modification of the electric properties of the composite material-based devices. This study reports on the investigation of the response of devices based on composites made of PVA and a carbon nanostructure to the ripeness stages of charentais melon and the detection of the presence of fungi that attack the melon.

EXPERIMENTAL PROCEDURE

Two types of carbon nanostructures, multiwalled carbon nanotubes, (MWCNTs), and carbon nanocoils (CNCs), were used to make composites with PVA as the host polymer. The preparation of the MWCNT- and CNC-based composites is reported in detail elsewhere.[1] A dispersion of a MWCNT−PVA or a CNC−PVA composite in 1,2-dichlorobenzene was used in the preparation of the sensors (0.5% weight content of MWCNTs or CNCs in PVA).[1,2] The sensors were prepared by depositing the composite solution on top of a 8×9 mm^2 glass-fiber-printed circuit board, by sequentially dropping 20, 30, and 35 μL volumes of the dispersion with a time interval of 60 min. The printed board contained 9 pairs of 7.5 mm long tin-coated copper electrodes with a gap of 0.3 mm between the electrode pairs, as described elsewhere.[1,2]

Electrical measurements were made using an Agilent 4284A LCR meter, applying an alternating current (AC) signal with amplitude of 0.5 V and a frequency of 27 kHz. This frequency was selected because, in this range, the noise/signal ratio is not significant and allows for good sensitivity for capacitance, C, and conductance, G.[2] The variation in the device conductance (ΔG) and capacitance (ΔC) upon exposure to charentais melon fruit and *Drechslera* sp. fungi was measured. All experiments were carried out at room temperature in air in the dark.

For the measurements, the sensor was placed inside a sealed glass chamber with a volume equal to 7.8 L (see Figure 1) and the change in capacitance and conductance was monitored. The sensor was connected to the electronic equipment using crocodile clips, and the sensor was exposed to either charentais melon with or without *Drechslera* sp.

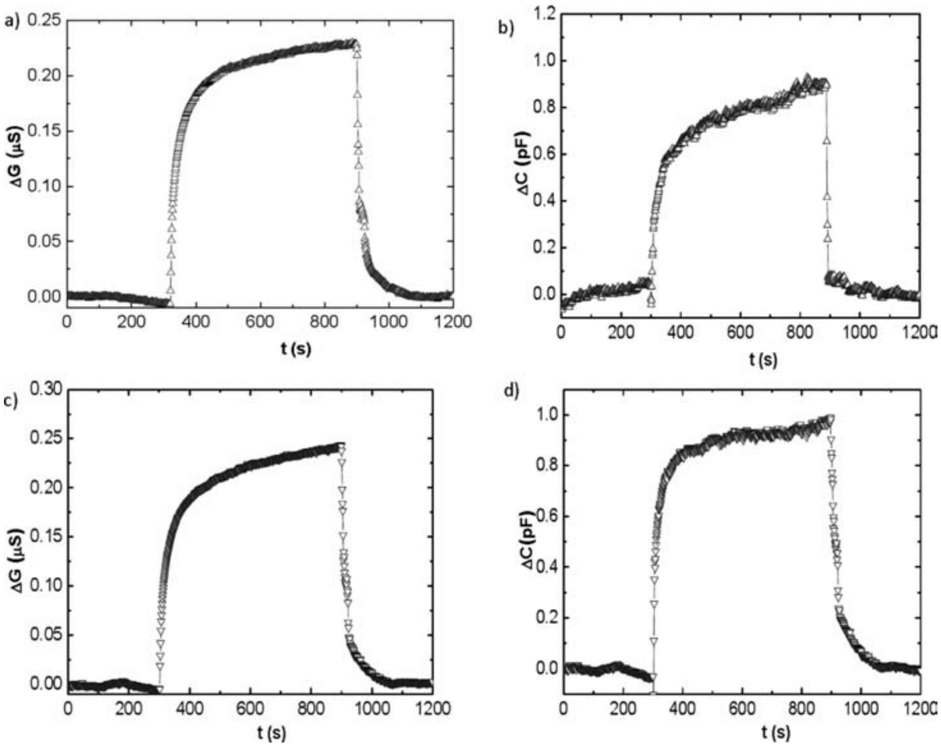

Figure 2. Response ΔG and ΔC of composite-based chemical sensors of (a and b) MWCNT−PVA and (c and d) CNC−PVA to a ripe charentais melon after the third day.

fungi (inside the chamber) or air (outside the chamber) by a simple 180° rotation of the top screw, as depicted in Figure 1.

Melon fruits were inserted into the sealed closed chamber every 24 h, and the measurements were started 5 min after fruit insertion. Measurements were made using three different sensors based on each one of the two composites; i.e., a total of six sensors were used. After measurement, the fruit was removed from the chamber and shelf-stored at room temperature under natural illumination conditions and the chamber was left open to the atmosphere. The procedure was repeated every 24 h to follow the time evolution of the electrical response of the sensor during the melon-ripening process in the presence and absence of the fungi.

Before each insertion into the chamber, the charentais melon was sequentially washed with neutral soap (Ypê clear) and abundant water. The fruit was soaked for 3 min in 2% (w/w) chlorinated water solution, washed with ethanol (92%), washed with abundant water, and dried with a paper towel. The same procedure was repeated after finishing each session of measurements. The presented experimental results are averages of four repetitions performed each day.

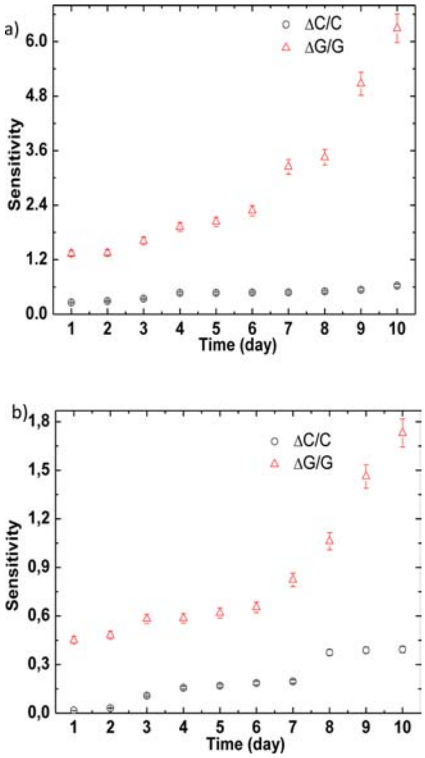

Figure 3. Response of chemical sensor-based (a) MWCNT−PVA composites and (b) CNC−PVA composites of the charentais melon for 10 days. The error bar is approximately 5% of the measure and refers to three measurements under the same conditions.

■ RESULTS AND DISCUSSION

Figure 2 shows a typical response of the MWCNT−PVA and CNC−PVA composite sensors during measurement of the volatile organic compounds generated from the charentais melon fruit. Each curve represents either the conductance G or the capacitance C of each sensor against time. The measured G and C were related to the variation ΔG and ΔC for the charentais melon fruit during the measurement period.

The conductance and capacitance of the two sensors increased sharply after the sensors were exposed to the volatile organic compounds released by the charentais melon fruit in a chamber for 600 s. This occurred after an initial period of low conductivity and capacitance, corresponding to sensor exposure to the outside environment. Regardless of the difference in crystallinity of the two carbon structures, both sensors responded similarly, from a qualitative point of view (Figure 2). After exposure to the volatile organic compounds of the charentais melon fruit, both sensors respond very rapidly, and moreover, once the sensors are removed from the chamber, the response returns rapidly to the baseline position, rapidly indicating complete sensor recovery. This is an important property in practical applications, because it allows for sensor reuse.

To determine the response of the two sensors, experiments were conducted for 10 consecutive days (results shown in Figure 3). On the sixth day, fungi appearance near the melon stem region was observed, and for this reason, the fruit was cut, as shown in Figure 4a. Figure 4b shows the internal view of the removed part, where fungi proliferation can be observed. The removed part was put back in the melon, and the same fruit was used in the measurements until the 10th day. For both sensors, the $\Delta G/G_0$ measurements rapidly increased after the seventh day, but $\Delta C/C_0$ measurements only showed slow increments during the 10 days of measurements, except for a stepwise increment on the eighth day in the case of the CNC-based sensor (the subscript "0" denotes the baseline value). Moreover, the $\Delta G/G_0$ measurement of the MWCNT−PVA composite-based sensor is found to be more sensitive than that of the CNC−PVA composite-based sensor (Figure 3). In both cases, the $\Delta C/C$ measurement was less sensitive throughout the test.

After the 10th day of measurements, part of the fungi was removed and separated for identification and multiplication for use in further experiments.

Sensors, including electronic nose sensor arrangements, have been used for various procedures such as food process monitoring,

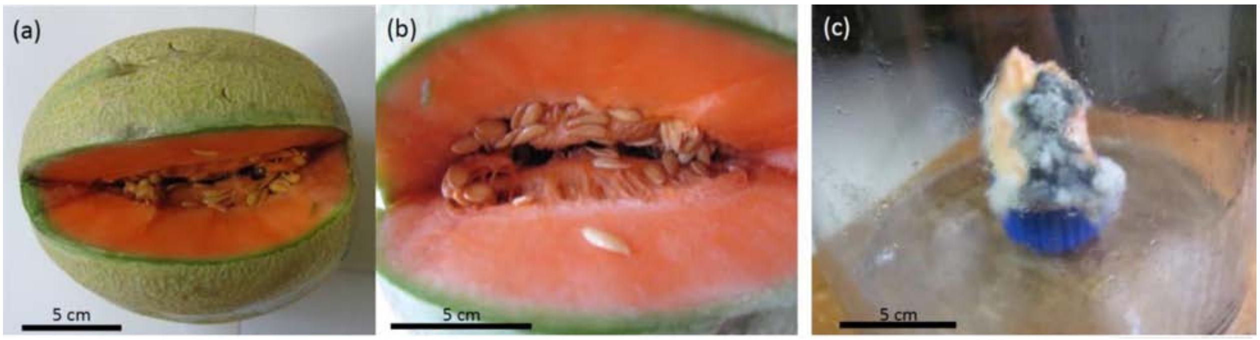

Figure 4. Major changes during charentais melon fruit development and ripening. (a) Charentais melon, (b) appearance of the fungi on the charentais melon, and (c) fungi and charentais melon within a sealed container of 7.8 L.

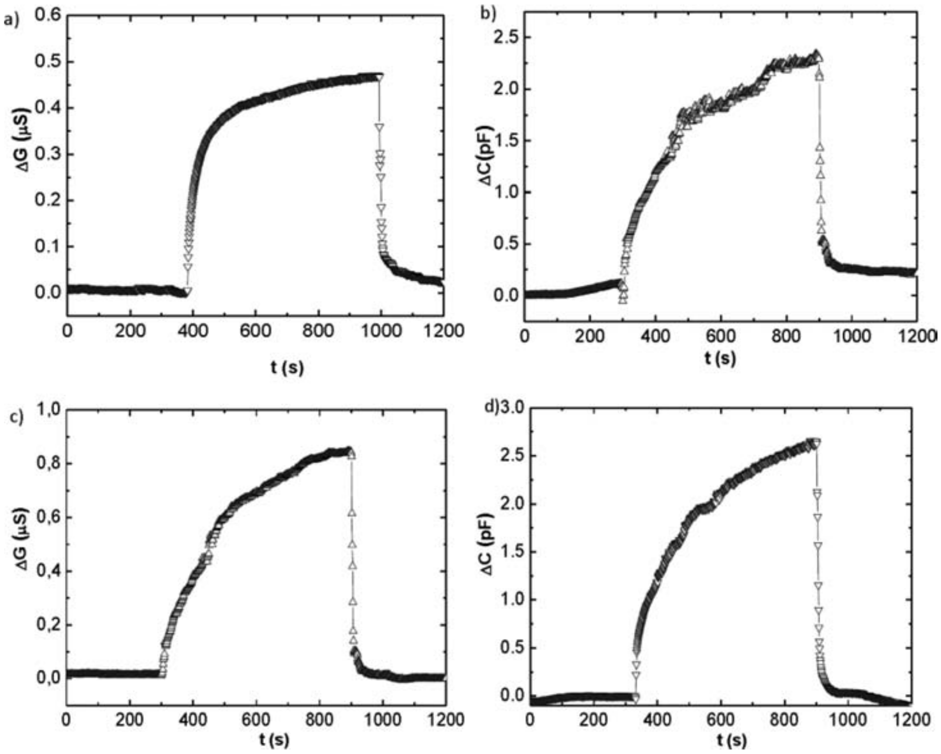

Figure 5. Response of ΔG and ΔC of the sensor based on (a and b) MWCNT−PVA composite and (c and d) CNC−PVA composite after the introduction of the colony of *Drechslera* sp. fungi into the chamber.

freshness evaluation, shelf-life investigation, authenticity determination, and product traceability.[26−28] More importantly, sensors can be exploited to screen microbial contamination of food by analyzing the pattern of volatile compounds produced by microbial metabolism.[29,30] Therefore, to test our sensor, we deliberately contaminated the charentais melon by introducing into the chamber the fungi extracted as described above, identified as *Drechslera* sp. fungi. These fungi were then placed near a charentais melon fruit that did not show any visible evidence of fungi contamination. In this case, the two sensors quickly responded to the volatile organic compounds, similar to what was observed for the sample without fungi (Figure 5). However, when Figures 2 and 5 are compared, it can be noted that the presence of fungi in the chamber enhanced the sensor response in ΔG and ΔC between 60 and 70% at 600 s with respect to that without fungi. This result indicates that this large response in ΔG and ΔC is due to the presence of *Drechslera* sp. fungi in the chamber.

A conductance G response dependence upon frequency was conducted over 6 days. During this time, the frequency was scanned from 0.1 to 1 MHz for the sensor-based composites (MWCNT−PVA and CNC−PVA) when exposed to volatile organic compounds of charentais melon with and without a colony of *Drechslera* sp. fungi. In the first 3 consecutive days, the test was only conducted on charentais melon. During this time, the G response was relatively small and its variation was negligible. However, on the fourth and fifth days, after a colony of *Drechslera* sp. fungi was introduced into the chamber, both sensors responded differently. For a MWCNT−PVA composite-based sensor, the response was strong and more distinguishable than that without a colony of *Drechslera* sp. fungi over most of the entire frequency range. Interestingly, when the colony of *Drechslera* sp. fungi was removed from the chamber, the response dropped to the same level as it was on the third day (before fungi insertion). A very interesting response aspect for the MWCNT−PVA composite-based sensor is that, even at

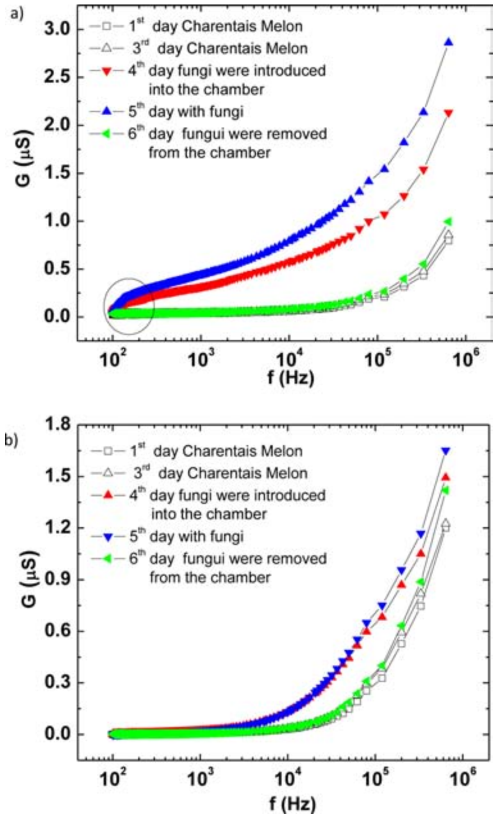

Figure 6. Response G of chemical sensors based on (a) MWCNT−PVA composites and (b) CNC−PVA composites into the charentais melon and the colony of *Drechslera* sp. fungi.

a low frequency, this sensor allows for distinguishing between an environment with and without fungi (see Figure 6). This aspect is very important for practical applications. In the case of the CNC−PVA composite-based sensor, there are distinct responses between 100 and 500 kHz after the introduction of the colony of *Drechslera* sp. fungi. Generally, the *G* response of the CNC−PVA composite-based sensor is weak, especially at lower and higher frequencies, where the *G* response with and without a colony of *Drechslera* sp. fungi was indistinguishable.

In summary, we have demonstrated that electronic sensors based on carbon nanostructures are able to detect the presence of *Drechslera* sp. in charentais melon. The sensor response showed that these fungi can be electronically identified in the charentais melon and constitute an effective and cheap test procedure to differentiate between infected and uninfected melon.

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Notes

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