INTERNATIONAL JOURNAL OF Electroactive Materials

Int. J. Electroactive Mater. 4 (2016) 27 – 32
www.electroactmater.com

Distinguishing Between Organic and Pesticide Treated Strawberries Using a Chemical Sensor Array

Márcia W. C. C. Greenshields[1], Rafael Rodrigues[1], Mireli T. de Souza[2], Neil J. Coville[3], Maria A. C. Zawadneak[2], Ivo A. Hümmelgen[1,*]

[1]Departamento de Física, Universidade Federal do Paraná, Caixa Postal 19044, 81531-980 Curitiba, Brazil.
[2]Departamento de Patologia Básica, Entomologia Agrícola e de Microbiologia e Biologia Molecular-LabMicro, Universidade Federal do Paraná, Curitiba, Brazil.
[3]DST-NRF Centre of Excellence in Strong Materials and Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, PO Wits, 2050, Johannesburg, South Africa.
*E-mail Address: iah@fisica.ufpr.br.

Received: 18 November 2016 / Accepted: 14 December 2016 / Published: 19 December 2016

ABSTRACT: We report on the application of a chemical sensors array to distinguish organic strawberries from pesticide treated strawberries. The sensors were prepared dispersing multiwalled carbon nanotubes (undoped, nitrogen doped or boron doped multiwalled carbon nanotubes) by an aqueous solution using a surfactant assisted method and depositing the dispersion onto interdigitated electrodes. The conductance of the sensors was taken as sensor response and tristimulus analysis was applied as a pattern recognition method. We investigated strawberries sprayed with a specific pesticide, lambda-cyhalothrin (synthetic pyrethroid), comparing the sensors array response with that produced by organic strawberries. We demonstrate that the use of this simple 3-sensors array and tristimulus analysis strategy is an effective method for the distinction of organic strawberries from pesticide treated ones.

Keywords: Chemical sensors array, carbon nanotubes, tristimulus analysis, *Fragaria* x *ananassa*, synthetic pyrethroids

1. Introduction

Strawberries (*Fragaria* x *ananassa* Duch.) have a good commercial appeal due to their characteristic taste and flavor [1]. An observed increase in the productivity of the strawberries in the current agricultural production systems is directly related to the intensive use of synthetic plant protection products, such as fertilizers and pesticides. The pests are usually controlled by the use of insecticides such as pyrethroids that can damage the environment and contaminate food [2-5].

In some cases the appearance of pesticides in fruits [6] and vegetables [7] has been of concern to consumers about possible diseases related with these chemicals. Thus, methods to determine the presence of pesticide residues in the fruits need to be developed. One option that can be used to determine traces of the pesticides is the use of chemical sensors. Chemical sensors are easy to use and have advantages such as their non-destructive character, low cost and relatively fast analysis response times. Ortiz et al [8] developed an electronic nose using 16 sensors based on metal oxides to determine organochloride compounds, reporting high device stability.

Chemical sensors based on carbon nanostructure were shown to have good sensitivities for volatile organic compounds (VOCs) [9], but concomitantly present the problem of low specificity that needs to be overcome using additional tools like pattern recognition methods. In carbon nanotubes π-electron orbitals that are responsible for the charge transport show high electronic density at their outer face. Additionally, due to the high surface area/volume ratio of carbon nanotubes (CNT), molecular adsorbates can strongly affect the intra- and inter-CNT electronic transport properties. Despite the fact that all molecules adsorbed on the CNTs or interacting with CNT defects may disturb the π-electronic system affecting the charge transport properties, different molecules disturb the system in different ways. The intensity of the disturbance depends on molecular electronic density distribution and stereochemical characteristics of the VOCs, as well as characteristics of the CNTs [9]. The VOC (in many cases more than one) interaction with the CNTs is very complex and a strategy that avoids requiring an understanding of details of this interaction is more effective in practical problems.

We use a set of three unspecific chemical sensors based on multiwalled carbon nanotubes (MWCNTs), nitrogen doped multiwalled carbon nanotubes (N-MWCNTs) and boron doped multiwalled carbon nanotubes (B-MWCNTs), collecting their conductance data when exposed to the fruit containing environment. The set of responses is mathematically treated using the tristimulus analysis approach [10]. The analysis of VOCs exhaled by fruits is usually complex because the number of exhaled substances is large and varies depending on the ripening stage [11]. For this reason, a strategy like tristimulus analysis is necessary because it constitutes a data reduction procedure that allows representing a complex situation using a two-dimensional coordinates representation. As a consequence, the described procedure can be used in practical situations, like monitoring of strawberries in a market.

In our study, we used lambda-cyhalothrin (Karate Zeon 50 CS®) (see **Figure 1**), a pesticide that belongs to the chemical group of synthetic pyrethroids, commonly used in the control of agricultural pests of the strawberry. However, the application of an insecticide results in undesirable side effects because it affects adversely the natural enemies, resulting in population increments of other crop pests [12]. It is derived from the pyrethrum plants is called Chrysanthemum cinerariaefolium [12, 13]. Lambda cyhalothrin has a relative density of 1.33 g/cm^3 at 25 °C [13] and presents a molecular structure of $C_{23}H_{19}ClF_3NO_3$, molecular mass of 449.9 g/mol and its water solubility is 0.005 mg/L. It is soluble in other solvents like acetone, methanol, toluene, hexane [11,13] and presents a mild odor and negligible equilibrium vapor pressure at 20 ºC with b.p. of 499 ºC [3, 14, 15].

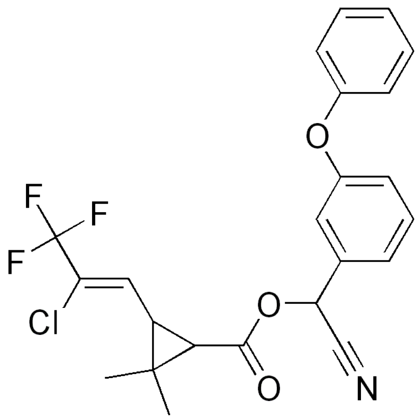

Figure 1: Lambda-cyhalothrin structure

The main objective of this study is to present a simple strategy based on simple and inexpensive sensors to detect pesticide residues in strawberries and contribute to the development of tools to allow distinguishing pesticide treated strawberries from organic strawberries.

2. Experimental

Sensors were prepared by the deposition of dispersed carbon nanostructures onto interdigitated electrodes. Interdigitated electrodes were purchased from Micropress SA. Each one has 9 pairs of 7.5 mm long ENIG (Electroless Nickel Immersion Gold) stripes with a gap of 0.3 mm between them, deposited onto a FR4 circuit board substrate, as reported before [16].

2.1 Materials and sensor preparation

Carbon nanoparticles, in general, present poor dispersion in water and to overcome this problem we used a previously reported route of dispersion in deionized water assisted by an ionic surfactant, hexadecyltrimetilammonium bromide (CTAB) [17], which was purchased from Sigma Aldrich and used without purification. MWCNTs were synthesized using the procedure reported by Mhlanga et al [18], based on the decomposition of the acetylene (C_2H_2). MWCNTs presented an inner diameter of ∼10 nm and outer diameter of around 20–30 nm [17, 19].

The synthesis of N-MWCNTs and B-MWCNTs are similar to that for MWCNTs except that acetonitrile (CH_3CN) and trimethyl borate ($(CH_3O)_3B$) were used as nitrogen and boron sources, respectivelly. The synthesis and characterization of the nanotubess were previously reported [20-22]. The nitrogen concentration of the N-MWCNTs was determined by CN elemental analysis to be 2.2%; the relative atomic concentration of boron in the B-MWCNTs was found by XPS to be 1.6% [19].

CTAB (6 mg/mL^{-1} in water) and either MWCNTs, N-MWCNTs or B-MWCNTs (4 mg/mL^{-1}in water) were mixed and ultrasonicated at 60 °C for 30 min, followed by a further 30 min ultrasonication at ~0 °C. The aqueous dispersion is kept for 4 days at ~0 °C to keep the solution free of micelles (the Kraft temperature of CTAB is 25 °C [23, 24]) and to give time to precipitate all the excess of surfactant in form a crystalline phase. This process ensured that a minimum amount of surfactant was used to stabilize the particles that were well dispersed in water. After this period, 20μL of the 50% upper volume of the supernatant dispersion of either MWCNT, N-MWCNT or B-MWCNT is deposited by drop casting onto the interdigitated electrodes and annealed at 60 °C for 1 h for water evaporation [18, 25]. **Figure 2(a)** shows a picture of a finalized sensor.

2.2 Measurement apparatus and procedure

An Agilent 4284A LCR meter was used in the conductance measurements. The devices were characterized at 27 kHz due to the large response and low noise to signal ratio observed at this frequency [20].

The sensors array was mounted in the cap of a sealable chamber, in a way that each device can be connected with the LCR meter to measure its conductance. The temperature and humidity of the chamber was recorded using a Minipa MT-241 hygrometer. The humidity of the chamber, with the fruits inside, was around 90% and the temperature around 18 °C.

The strawberries shown in **Figure 2** were harvested in a strawberry experimental farm locate in the Pinhais municipality, Paraná state, Brazil (25º25'S, 49º08'W) and were selected due to absence of physiological defects, size and color (mature strawberries). After harvest, the fruits were sequentially washed in water, in ethanol (50%) for 1 min., in 1% sodium hypochlorite for 30 s and in sterile water for 1 min., to remove any microorganisms present in the fruits. After the cleaning, one of the organic strawberries was placed inside the glass recipient shown in **Figure 2(c)** and inserted in the sealed chamber (volume of 3.2 L) in whose cap the 6 sensors (2 sensors based in each one of the three carbon nanostructures) were placed, on a way that all the sensors were exposed to the volatiles exhaled by the fruit. After 15 min (time enough to estabilize the response of the device) the conductance of each chemical sensor was measured again and the response $\Delta G/G_0 = (G - G_0)/G_0$ was calculated (G_0 is the initial conductance and G is the saturation conductance of the

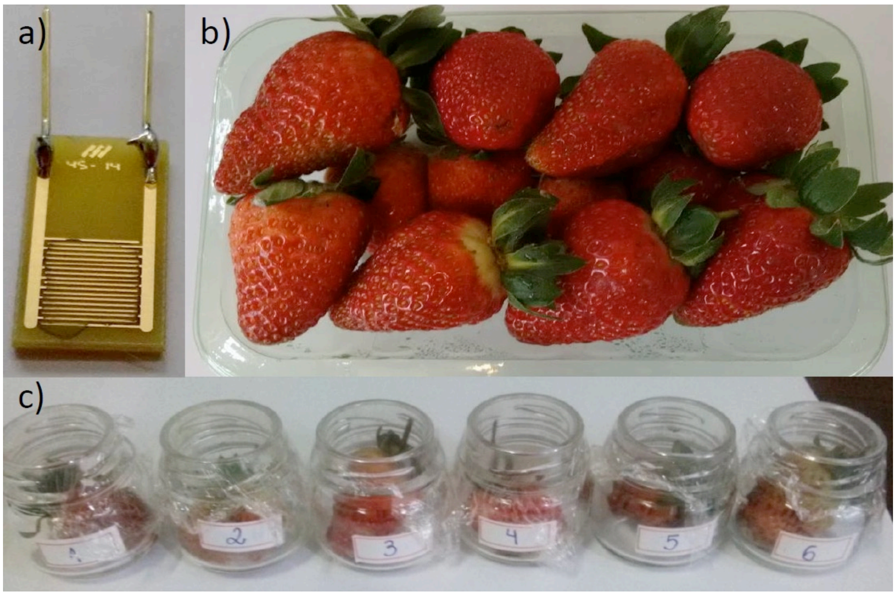

Figure 2: (a) Interdigitated electrodes sensor; (b) organic strawberries without pesticide and; (c) strawberries treated with lambda-cyhalothrin pesticide

sensor after it was exposed to the fruit). The same procedure was repeated with the six organic strawberry fruits. After these measurements, the six strawberry fruits, still inside the recipients shown in **Figure 2(c)**, were sprayed with the pesticide, lambda-cyhalothrin. Each strawberry was sprayed with 0.2 mL lambda-cyhalothrin suspension using an airbrush coupled to a compressor (Sagyma, model ASW 186) set at 15 $lb.pol^{-2}$. A 40 µg/mL dose, determined following the technical recommendation information of the commercial pesticide to the control of *Caetosiphon fragaefolli* (Hemiptera: Aphididae) in strawberry was used.

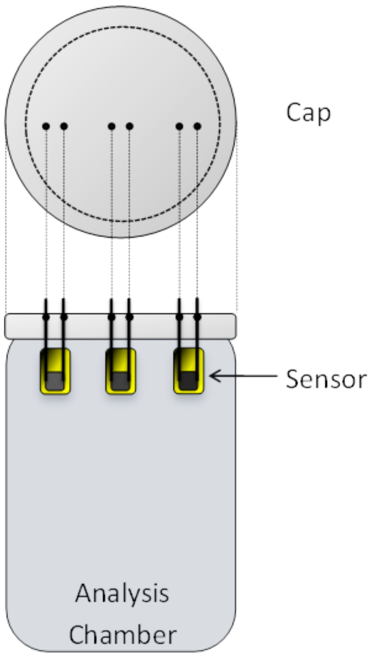

Figure 3: Measurement chamber, showing (for clarity) 3 of the 6 sensors

After spraying, recipients containing the sprayed strawberries were then sequentially placed again one by one inside the sealed chamber (see **Figure 3**) for 15 min., when the sensors array in the cap interacted with the volatiles exhaled by the sprayed strawberries. The final conductance for each one of the sprayed strawberries was recorded and the relative conductance variation was calculated. All the sprayed fruits were measured separately in sequence, taking the average value of the six sprayed fruits. The procedure was repeated daily for a period of 6 days with the set of strawberries sprayed with pesticides, to investigate if the response of the sprayed fruits changed with the time. After each daily measurement, the glass recipients were stored open, with the fruits inside, in an exhaustion chamber.

3. Results and discussion

After all the sensor conductance measurements with strawberries in the chamber were concluded, the chamber was cleaned and dried in the same manner as mentioned before and pure lambda-cyhalothrin was sprayed in one glass recipient, which was then inserted in the chamber, and the conductance was measured in the presence of this pesticide.

The data of the $\Delta G/G_0$ responses of the chemical sensors was used as input in a pattern recognition method (tristimulus analysis), which was employed to facilitate the visualization of data and evaluate tendencies potentially useful for the distinction between organic and pesticide treated strawberries. Tristimulus analysis uses the responses of three sensors, i. e.:

$$X_G \equiv \left[\frac{\Delta G}{G_0}\right]_{MWCNTs} \quad (1)$$

$$Y_G \equiv \left[\frac{\Delta G}{G_0}\right]_{N-MWCNTs} \quad (2)$$

$$Z_G \equiv \left[\frac{\Delta G}{G_0}\right]_{B-MWCNTs} \quad (3)$$

where the subscript denotes the carbon nanostructure used in the specific sensor) to construct the tristimulus vector $\vec{r}=X_G\hat{x}+Y_G\hat{y}+Z_G\hat{z}$. Each sensor response is the magnitude of one component of the vector [16,18,20], as shown in **Figure 4**. If the response of the sensors depends linearly on the volatiles concentration, different amounts of volatiles exhaled by the fruit only change the magnitude of the vector preserving the spatial orientation. The coordinates where the vector crosses the unitary plan given by $x_G + y_G + z_G = 1$, are given by;

$$x_G = \frac{X_G}{(X_G+Y_G+Z_G)}, \quad (4)$$

$$y_G = \frac{Y_G}{(X_G+Y_G+Z_G)} \quad (5)$$

$$z_G = \frac{Z_G}{(X_G+Y_G+Z_G)}. \quad (6)$$

This point is characteristic of the set of sensitivities, which in turn depend on the volatiles composition. For this reason, the coordinates of the point can be seen as a signature of the volatiles present in the environment. Since $x_G + y_G + z_G = 1$, two of the (x_G, y_G, z_G) coordinates are enough to characterize the point (the other coordinate can be calculated) and a bi-dimensional graph with orthogonal axes corresponding either to the *xy*, *yz* or *zx* projection can be used as representation, without loss of information [20].

Figure 5 shows the response of a MWCNT based sensor when exposed either to air, to an organic strawberry, to a pesticide treated strawberry or to pesticide, demonstrating that in all the cases the signal to noise ratio of the response is adequate for quantitative evaluations, since noise cannot be observed in the curves shown in **Figure 5**.

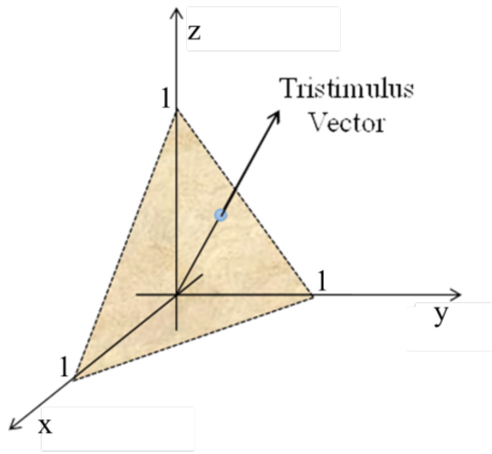

Figure 4: Graphical representation of the tristimulus vector and the interception point in the unitary plane

In **Figure 6** we show the (z_G,y_G) coordinates calculated using the results of the previously described experiments. It can be seen that the points corresponding to the six organic strawberries form a compact cluster, indicating that the results variation between different strawberry fruits is very small and can be well distinguished from the other clusters.

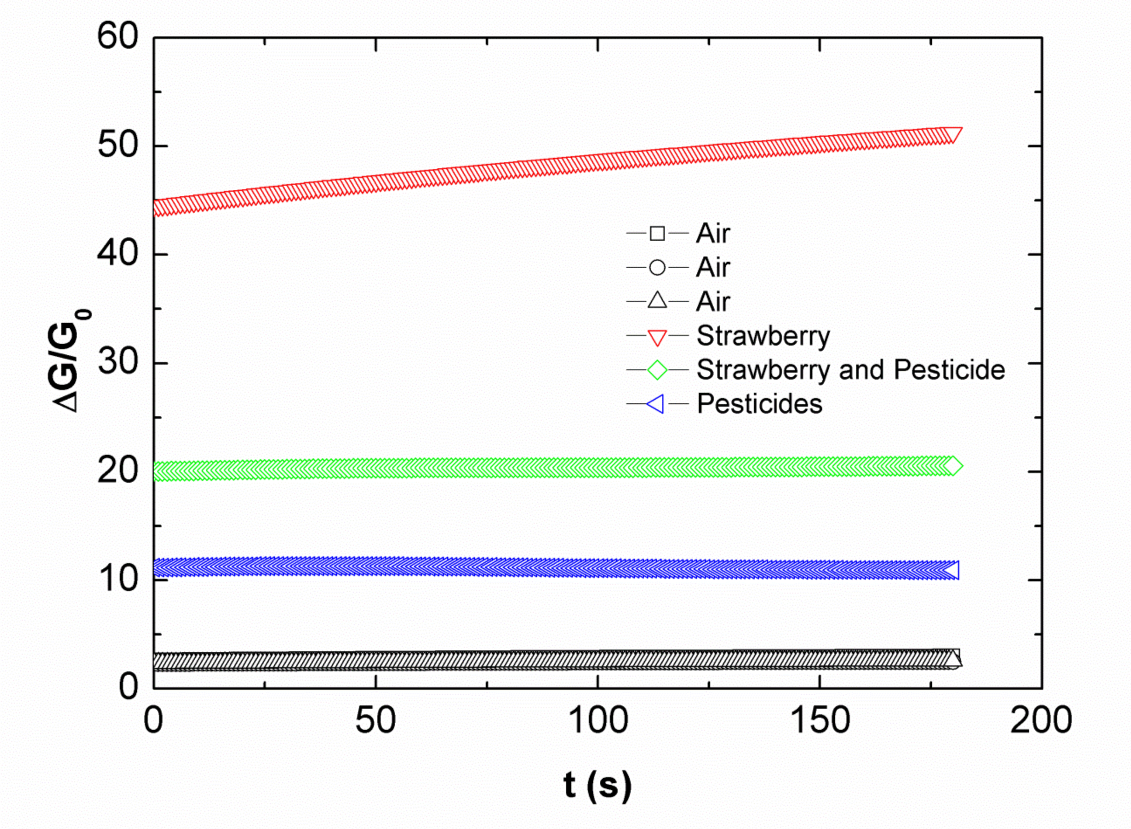

Figure 5: Response variation of a MWCNT based sensor when exposed to air, to a strawberry, to a pesticide treated strawberry and to only pesticide. The data for the three air samples all give a similar low value

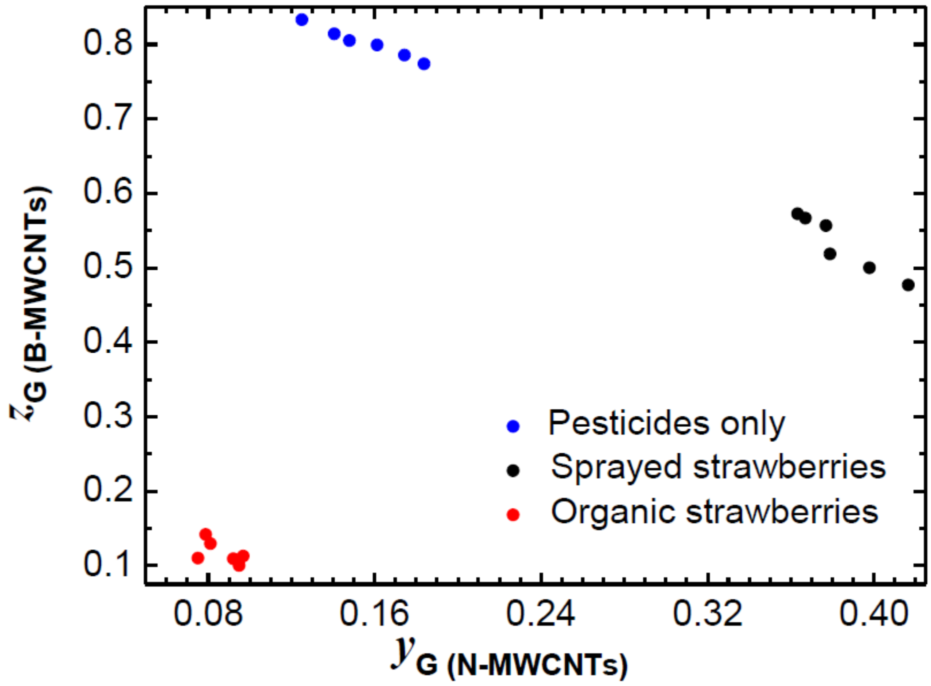

Figure 6: *ΔG/G₀* responses plotted using tristimulus representation graphs. Red squares: 6 organic strawberries; black triangles: pesticide sprayed strawberries measured once a day during six days (each point corresponds to the average of the six strawberries at one day) and; blue circles: six days measurement of a glass recipient sprayed with the pesticide

The point corresponding to the average value calculated taking the six responses of the six strawberry fruits sprayed with pesticide shows a small displacement, dependent on the day of the measurement (measurements were made once a day, over six days). The same happens with the points that correspond to the glass recipient sprayed with pesticide. But the displacement is small in all cases, so that all clusters can be easily distinguished.

These results demonstrate that it is possible to distinguish organic strawberries from strawberries treated with lambda-cyhalothrin applying tristimulus analysis to the conductance variation of the set of these three carbon nanostructure based chemical sensors. The distinction is clearly possible even six days after the strawberries were sprayed with lambda-cyhalothrin. As a consequence, the described procedure can be seen as a practical tool to certify that strawberries sold as organic ones did not receive lambda cyhalothrin treatment. This is important because pesticide treated strawberries may be dishonestly offered as organic ones, in order to take advantage of the higher commercial value of the latter. It is also important to mention that the methodology is highly sensitive to lambda cyhalothrin presence, since as mentioned before, this pesticide presents a negligible equilibrium vapor pressure at the temperature in which measurements were performed. As a consequence, it is expected that the number of molecules of lambda-cyhalothrin that interact with the carbon nanotube, modifying their conductance, is very small. Our experiments allow the detection of the pesticide in strawberries, although at this stage the method does not allow the quantification of the process.

4. Conclusion

We used a set of three resistive chemical sensors based on three distinct carbon nanostructures, either undoped, nitrogen doped or boron doped multiwalled carbon nanotubes, to detect the volatiles exhaled by either organic or lambda-cyhalothrin pesticide treated strawberries. Applying tristimulus analysis to the set of sensors responses, it is possible to distinguish both types of strawberries, even a few days after pesticide treatment. In spite of application to a specific pesticide, this method is general and can in principle be applied to other cases of pesticide use to distinguish organic strawberries from pesticide treated ones.

Acknowledgement

The authors would like to thank CNPq and PNPD/CAPES(Brazil) and IBSA and NRF (South Africa) for research grants.

References

1. M. A. Pombo, H. G. Rosli, G. A Martínez, P. M. Civello. Postharvest Biol. Tec. 59 (2011) 94-102.
2. R. Naravaneni, K. Jamil. J. Biochem. Mol. Toxic.19 (2005) 304-310.
3. M. Zhao, Y. Zhang, W. Liu, C. Xu, L. Wang , J. Gan. Environ. Toxico.l Chem. 27 (2008) 1194–1200.
4. C. Han, C. Lederer, M. McDaniel, Y. Zhao. J. Food Sci. 70 (2005) S172-S178.
5. G. Rogmanazzi, E. Feliziani, M. Santini, L. Landi. Postharvest Biol. Technol. 75 (2013) 24-27.

6. J. Ortiz, O. Gualdron, C. Duran. Aust. J. Basic Appl. Sci. 10 (2016)107-113.
7. A. Dogan, N. Selcuk, M. Erkan. Scientia Horticulturae 207 (2016) 104–116.
8. G. Neri, Chemosensors 3 (2015) 1-20.
9. M. W. C. C. Greenshields, I. A. Hümmelgen, M. A. Mamo, A. Shaikjee, S. D. Mhlanga, W. A. L. van Otterlo, N. J. Coville, J. Nanosci. Nanotechnol. 11 (2011) 10211–10218.
10. R. Rodrigues, I. A. Hümmelgen, J. Solid State Electrochem. 20 (2016) 1295-1301.
11. M. W. C. C. Greenshields, B. B. Cunha, M. A. Mamo, N. J. Coville, I. A. Hümmelgen, Int. J. Electroactive Mater. 3 (2015) 10 – 14.
12. M. Poletti, L. P. Collette, C. Omoto, BioAssay 3 (2008) 1-14.
13. A. Dogan, S. Nurten, M. Erkan. Scientia Horticulturae 207 (2016) 104-116.
14. R. Naravaneni, K. Jamil. J. Biochem. Mol. Toxic.19 (2005) 304-310.
15. J. Liu, X. Lü, J. Xie, Y. Chu, C. Sun, Q. Wang. Environ. Sci. Pollut. Res. 16 (2009) 414–422.
16. M. Kopjar, V. Piližota, J. Hribar, M. Simčič, E, Zlatič, N. Tiban. Food Eng. 87 (2008) 341-350.
17. R. Rodrigues, M. A. Mamo, N. J. Coville, I. A. Hümmelgen. Mater. Res. Express 1 (2014) 015605.
18. S. D. Mhlanga, K. C. Mondal, R. Carter, M. J. Witcomb, and N. J. Coville, S. Afr. Chem. 62 (2009) 67- 76.
19. M. W. C. C. Greenshields, B. B. Cunha 1, N. J. Coville, I. C. Pimentel , M. A. C. Zawadneak, S. Dobrovolski, M. T. Souza, I. A. Hümmelgen. Chemosensors 4 (2016) 19-28.
20. M. A. Mamo, A. O. Sustaita, Z. N. Tetana, N. J. Coville, I. A. Hümmelgen. Nanotechnol. 24 (2013) 125203.
21. Z. N. Tetana, . D. Mhlanga, G. Bepete, N. J. Coville, R. W. M. Krause, S. Afr. Chem. 65 (2012) 39-49.
22. M. W. C. C. Greenshields, M. A. Mamo, N. J. Coville, I. C. Pimentel, J. G. Destro, M.V. Porsani, A. Bozza, I. A. Hümmelgen. Sensors Actuators B Chem. 188 (2013) 378-384.
23. S. Dölle, B. D. Lechner, J. H. Park, S. Shymura, J. P. F. Lagerwall, G. Scalia, Angew. Chem. Int. Ed. 51 (2012) 32547.
24. B. B. Cunha, M. W. C. C. Greenshields, M. Mamo, N. J. Coville, I. A. Hümmelgen. J. Mater. Sci. Mater. Electron. 26 (2015) 198–4201.
25. W. H. Duan, Q. Wang, F. Collins. Chem. Sci. 2 (2011) 1407-1413.