Use of an Electrochemically Etched Platinum Microelectrode for Ascorbic Acid Mapping in Oranges

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A positionable platinum microelectrode fabricated by electrochemical etching was used to monitor the concentration of ascorbic acid in fruits and vegetables. Studies carried out with ascorbate oxidase confirmed the suitability of the amperometric sensor to measure selectively the ascorbic acid content. The results obtained with the proposed method for ascorbic acid determination in orange juices compared well with those found by iodimetry with coulometrically generated iodine. The standard deviation calculated by measuring limiting current values in voltammograms was found to be 3% ($n = 150$). The sensor allowed the evaluation of the spatial distribution of ascorbic acid concentration in oranges by in-situ measurements. Ascorbic acid concentration maps show that in a perpendicular cut the concentration is higher near the peel to the center of the fruit. In a parallel cut, the concentration increases with the distance to the stem. A correlation between the ripening stage and the ascorbic acid concentration was also observed from electrochemical measurements, the content being higher in mature fruits.

KEYWORDS: In-situ measurements; ascorbic acid; microelectrodes; fruit quality

INTRODUCTION

Citrus fruits and vegetables are recognized as major sources of ascorbic acid (vitamin C), a compound that reduces the risk of degenerative diseases such as cancer and cardiovascular and neurological diseases (*1−3*). Hence, the consumption of vitamin C is strongly encouraged; the recommended dietary allowance of ascorbic acid ranges from 30 to 120 mg.

Brazil is one of the largest producers of oranges in the world with 18,256,500 metric tons of orange production, representing 29% of world orange production of the fruit (*4*). Because citrus fruits are one of the largest suppliers of dietary vitamin C, investigations on the factors that affect ascorbic acid levels are continuously desired. For instance, the literature (*5*) reports some parameters that influence the concentration of ascorbic acid such as climate conditions, maturity stage, position of the fruit on the tree, type of citrus fruit, conditions used for fruit processing into different products, type of container for holding the processed product, handling, and storage.

Methods for measuring the amount of ascorbic acid in fruits and vegetables usually are based on chromatographic, spectrophotometric, and titrimetric measurements (*6−8*). Particular attention is needed to restrain the activity of degradative enzymes and to maintain the reducing power of ascorbic acid by using metal chelators and controlling the solution pH during the determinations (*5*). These precautions are especially important in studies in which the tridimensional distribution of the compound in the citrus fruit is desired, the typical procedure involving the collection of very small fractions of the fruit for further analysis. Accordingly, some studies on the distribution of ascorbic acid in potatoes (*6*), pears (*7*), and tomatoes (*8*) are reported in the literature. These procedures are time-consuming and may yield misleading information as they depend on considerable sample manipulation.

Microelectrodes possess some unique features that make their use advantageous as electrochemical sensors over their counterparts, that is, enhanced rates of mass transport of electroactive species to the electrode surface, reduced double-layer capacitance, and less susceptibility to ohmic losses (*9*). These characteristics make it possible to perform analysis with enhanced sensitivity on short time scales (*10*, *11*) under time-independent conditions.

The present work reports our efforts to fabricate an amperometric sensor to provide chemical information in the form of a spatially resolved image. The sensor microelectrode was used to give in-situ information on the ascorbic acid concentration in oranges and to complement the available information on this subject.

EXPERIMENTAL PROCEDURES

Chemicals. All solid reagents were of analytical grade and were used without further purification. Ascorbic acid, potassium ferricyanide, and potassium chloride were obtained from Merck (Darmstadt, Germany). Solutions were prepared by dissolving the reagents in deionized water processed through a water purification system (Nanopure Infinity, Barnstead).

Fruits. Oranges (*Citrus sinensis*), white guavas (*Psidium guajava* L.), and tomatoes (*Lycopersicon esculentum*) were obtained in a market. Oranges were used at two stages of maturity, the degree of fruit maturity being determined from the analysis of the fruit surface color: green

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Figure 1. Optical microscope image of a platinum microelectrode before (**A**) and after (**B**) the coating process.

Figure 2. Linear sweep voltammograms recorded using a platinum microelectrode in 10 mmol L^{-1} ascorbic acid at different solution compositions: 0.1 mol L^{-1} NaOH (**A**); phosphate buffer, pH 7.0 (**B**); Ac$^-$/HAc buffer, pH 5.6 (**C**); 0.01 mol L^{-1} HCl (**D**). Scan rate = 50 mV s^{-1}.

(immature) or yellow-orange (mature). After purchase, fruits and vegetables were stored at 5 °C.

Electrodes and Instrumentation. An Autolab PGSTAT 30 (Eco Chemie) bipotentiostat with data acquisition software made available by the manufacturer (GPES 4.8 version) was used for electrochemical measurements. A homemade Ag/AgCl (saturated KCl) electrode was used as reference electrode.

Microelectrode Fabrication. An etching setup reported by Song et al. (*12*) was used to fabricate the microelectrode. An AC transformer (Luka Trafos) with 5 V of efficacy was used to etch the platinum wire (50 μm radius), instead of a function generator or a more expensive device (*13*, *14*). A sufficient length of the Pt wire was inserted in a diphase system electrolyte (1:1 CaCl$_2$ saturated/acetone) and an insulating liquid (CCl$_4$). Counter electrodes of graphite were mounted in a circular arrangement of 10 mm on the Pt wire. Etching occurs only on the part of the wire in contact with the CaCl$_2$/acetone phase. A continuous 60 Hz alternating current voltage was applied to the electrodic system. The etching process was terminated when the lower part of the Pt wire dropped off in the electrochemical cell (∼15−16 min using 5 V). More information about the mechanism of the process can be found in the literature (*15*). The result of the etching process is shown in **Figure 1A**. After the etching procedure, the microelectrode was coated with a plastic rubber paint (Plasti Film, Quimatic) and left to dry for 30 min. The exposed electrode surface was electrochemically cleaned by using a potential sweep program [square wave voltammetry, 0−2.0 V (50 Hz), 10 min] in a 0.05 mol L^{-1} sulfuric acid solution. The performance of the platinum microelectrode was evaluated by recording voltammograms in a 1 mmol L^{-1} potassium ferricyanide solution containing 0.5 mol L^{-1} potassium chloride as supporting electrolyte.

Enzymatic Pretreatment (Selective Oxidation of Ascorbic Acid). To investigate possible matrix interferences in amperometric measurements, the selective destruction of ascorbic acid was accomplished by using cucumber (*16*), *Cucumbis sativus*, a natural source of the enzyme ascorbate oxidase. Cucumber was cut in small pieces (peel was previously removed), and ∼1 g was added to 10.0 mL of the juice (pH ∼5−6). The mixture was kept under magnetic stirring for 15 min, and the remaining solution was filtered prior to analysis.

Coulometry Experiments. Validation of the results for the ascorbic acid content measured in commercial samples was performed by coulometric titration with electrogenerated iodine (*17*). A Metrohm E 211A coulometer was used to generate the titrant, and platinum electrodes were used as anode (a gauze cylinder) and cathode (a wire spiral). The working solution consisted of 50 mL of 1 mol L^{-1} HAc/0.1 mol L^{-1} Ac$^-$ buffer + 0.1 mol L^{-1} KI, pH 3.7. Starch was used as end point indicator in the titrations.

RESULTS AND DISCUSSION

Preliminary studies were performed to investigate the electrochemical behavior of ascorbic acid at the platinum microelectrode surface. **Figure 2** shows linear voltammograms
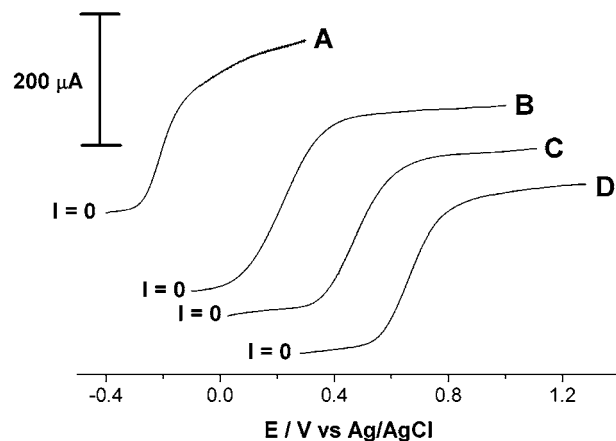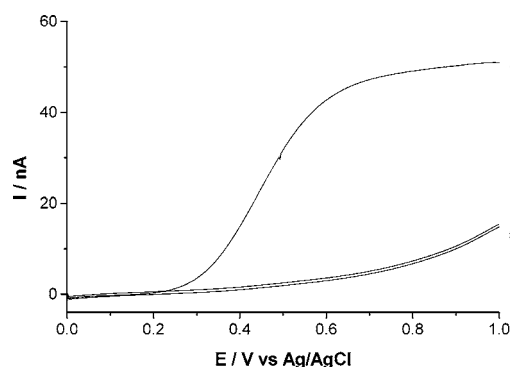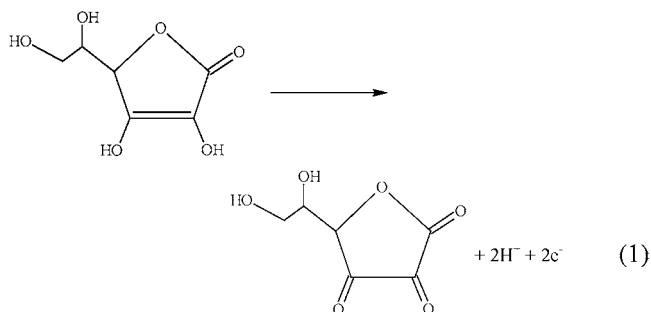

Figure 3. Linear sweep voltammograms recorded using a platinum microelectrode before (**A**) and after (**B**) addition of cucumber in an orange juice. Curve C was recorded in a phosphate buffer solution (pH 7.0). Scan rate = 50 mV s^{-1}.

recorded in four different solutions with increased pH values changing from 2.0 to 13.0. The wave corresponding to the anodic oxidation of ascorbic acid is shifted toward less positive potentials as the pH is increased; hence, protons are expected to participate in the electron-transfer step. The electrode process was carefully investigated by Rueda et al. (*18*), who reported that at gold surfaces the electron transfer occurs in two defined steps, their extent depending on the pH. The overall process involves the formation of dehydroascorbic acid with the release of two protons and two electrons, as described by eq 1:


$$+ 2H^+ + 2e^- \qquad (1)$$

To investigate the influence of the matrix on the response of the microelectrode sensor for ascorbic acid, initial studies were carried out using orange juice samples. A linear sweep voltammogram recorded in the orange juice is depicted in **Figure 3A**, and the obtained curve resembles those shown in **Figure**

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Table 1. Results of the Standard Addition–Recovery Experiment Using Three Different Orange Juices Spiked with 1 mmol L^{-1} Ascorbic Acid (Final Concentration in Solution)

|	ascorbic acid, mmol L^{-1}		
orange juice	added	found	recovery value, %
1	1.00	0.98	98
2	1.00	0.95	95
3	1.00	1.02	102

2. The orange juice was then mixed with cucumber according to the procedure described under Experimental Procedures, and a new voltammogram was recorded. Curve **B** in **Figure 3** clearly indicates the effectiveness of ascorbate oxidase on ascorbic acid elimination from the solution, as indicated by eq 2:
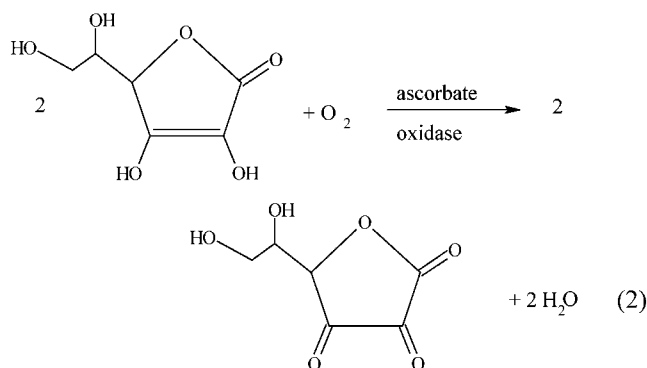
From the above-mentioned results one can also conclude that ascorbic acid is the only electroactive species present in the juice at the potential range studied, suggesting the validity of using the platinum microelectrode as a sensor for measuring selectively ascorbic acid in oranges. Standard addition studies were also performed in an orange juice to further investigate matrix effects problems. Accordingly, orange juice samples were spiked with a standard ascorbic acid solution, and current values were used to calculate the unknown concentration. Recovery values varied between 95 and 102% (**Table 1**), therefore reinforcing the usefulness of the proposed ascorbic acid sensor and the absence of matrix effects.

The amount and distribution of nutrients in fruits and vegetables are dependent on environmental and cultural practices, as well as maturity. For instance, the literature reports that the concentration of ascorbic acid increases with the degree of maturation in some fruits such as black mulberry, strawberry, and raspberry (*19*) and oranges, araxa, and acerola (or Barbados cherry) (*20*). As already stated, some efforts were devoted to evaluate the distribution of ascorbic acid in potatoes (*6*), pears (*7*), and tomatoes (*8*), but in all cases measurements were performed by analyzing small pieces collected in the fruit or vegetable. To avoid these time-consuming steps, the possibility of using the microelectrode sensor to measure the concentration of ascorbic acid in situ was investigated by insertion of the platinum microelectrode in an orange. **Figure 4** shows a photograph of the electrodes (working and reference) inserted in the orange (5 mm depth) as well as the linear sweep voltammogram recorded in situ. The curve shown in this figure exhibits the characteristic sigmoidal response observed for microelectrodes, the radial diffusion being the primary mode of mass transport under this experimental condition.

Further studies were performed to investigate the influence of the ohmic drop in the voltammetric measurements. This was accomplished by modifying the position of the reference
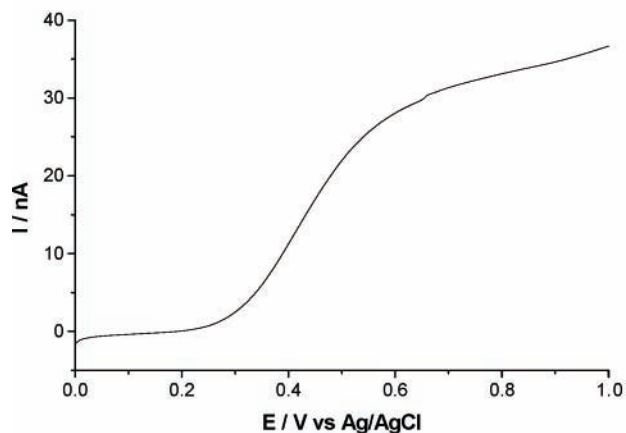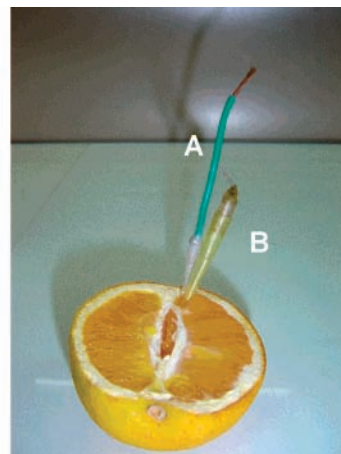
Figure 4. In-situ linear sweep voltammogram recorded in an orange using a platinum microelectrode. The positions of the microelectrode (**A**) and the reference electrode (**B**) in the orange are shown in the photograph. Scan rate = 50 mV s^{-1}.

Table 2. Measurements of Resistance, $E_{1/2}$ and I_L, by Changing the Distance between the Microelectrode and Reference Electrodes Inserted in an Orange

distance between electrodes in the orange, mm	resistance, $k\Omega$	$E_{1/2,}{}^a$ mV	$I_L,{}^a$ nA
1.0	25.2	431 ± 9	29.0 ± 0.9
10.0	37.2	438 ± 8	27.5 ± 0.7
19.0	48.2	436 ± 8	28.7 ± 0.8
33.2	55.6	437 ± 9	27.3 ± 0.8
57.0	76.2	436 ± 8	28.0 ± 0.8

^{a} Standard deviation was calculated between three measurements.

electrode in relation to the working electrode. Resistance measurements in the orange were carried out by using two tips connected to a potentiometer, and they indicated the increase of resistance as the separation between both tips was increased, as shown in **Table 2**. However, analysis of limiting current and $E_{1/2}$ values obtained in these experiments confirms that changes in resistance are not significant, especially because of the reduced effects of the ohmic drop when electrochemical measurements are performed with electrodes of small area. Notwithstanding, further in-situ measurements were carried out by inserting both working and reference electrodes in the fruit as close as possible.

An important feature of a sensor regarding its long-term stability is the repeatability of measurements. For instance, a continuous loss of signal has been reported in ascorbic acid

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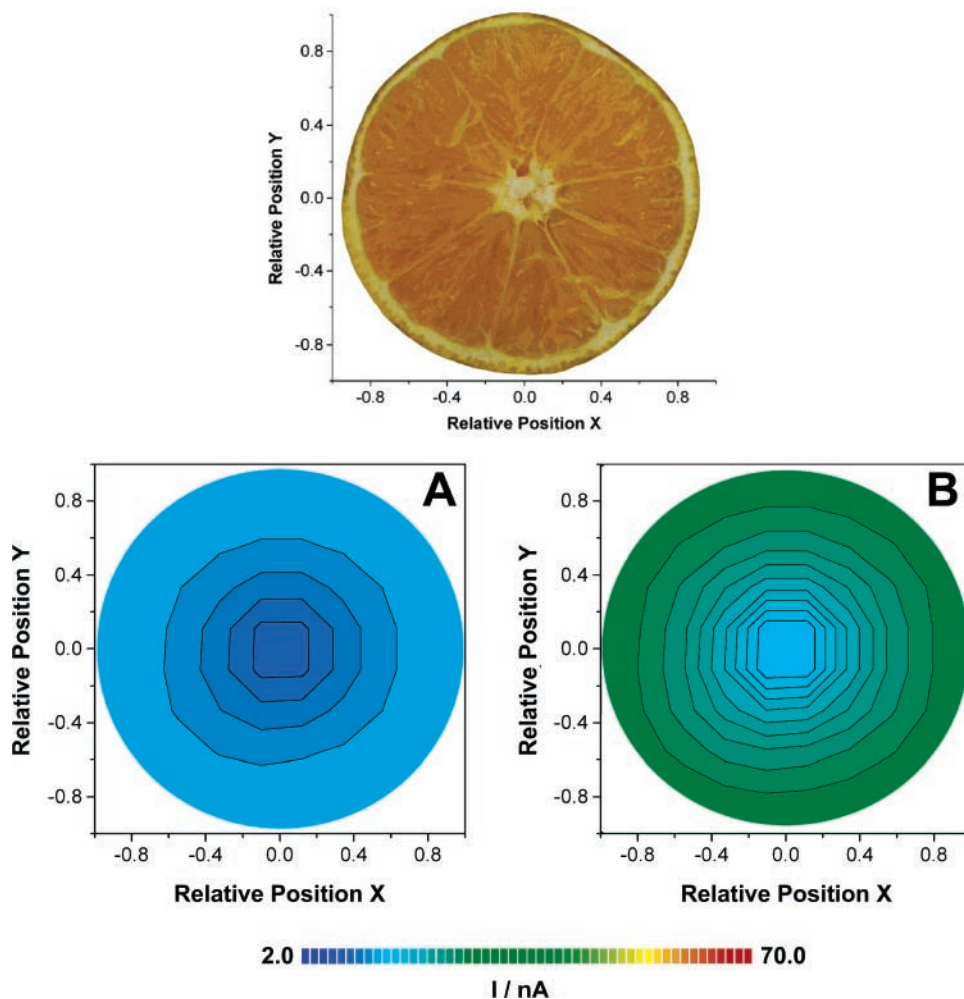
Figure 5. 2D graphs of the limiting current measured in voltammograms recorded in an orange using a platinum microelectrode. The bidimensional representation was plotted as a function of the microelectrode position (*XY*, see the upper graph). **A** and **B** represent 2D graphs for an immature and a mature orange, respectively. Scan rate = 50 mV s^{-1}.

determinations performed with gold electrodes (*21*). In the present work the repeatability was evaluated by recording 150 consecutive linear voltammograms in in-situ measurements performed in an orange. The standard deviation calculated by measuring limiting current values in the voltammetric curves was found to be 3%, and these results confirm the absence of problems associated with electrode surface fouling.

Analysis of $E_{1/2}$ values in voltammetric curves recorded with different oranges may also provide information on the extent of the ripening process. Accordingly, $E_{1/2}$ values were found to be 370 and 465 mV for a mature and an immature orange, respectively. To make the comparison possible, both measurements were carried out by inserting the microelectrode in identical positions of the mature and the immature fruit. Taking into account the $E_{1/2}$ dependence on pH for the anodic oxidation of ascorbic acid, these results indicate that the mature fruit is less acidic (pH 5.9) than the immature one (pH 4.7), at least in the positions where the sensor was inserted. For instance, $E_{1/2}$ measurements obtained by inserting the microelectrode at other positions yielded lower pH values, in agreement with those established in the literature (*22*), where pH values between 3.1 and 4.1 are reported in orange juices.

The steady-state current in voltammograms recorded in ascorbic acid solutions was found to vary linearly with the concentration of the electroactive species in the range from 1 to 10 mmol L^{-1} ($I/10^{-7}A = 0.015 + 0.208C$/mmol L^{-1}, $R^2 = 0.99945$). This relationship between current response and

concentration was used to monitor the ascorbic acid concentration in different locations of various oranges. **Figure 5** shows the distribution of ascorbic acid in oranges with different degrees of maturation, that is, an immature and a mature orange. Twenty-four triplicate measurements in homogeneously distributed locations were performed at the surface of the oranges (5 mm depth), which were cut perpendicular to the longitudinal axis (see photograph in **Figure 5**). A similar experiment was repeated with two other oranges, but in these cases measurements were done after the fruits had been cut parallel to the longitudinal axis (**Figure 6**). By analyzing the distribution diagrams shown in **Figures 5** and **6**, two main conclusions may be pointed out:

1. Ascorbic acid seems to be symmetrically distributed within the fruit by looking at data shown in **Figure 5**, a different situation being observed if measurements are done in the surface parallel to the longitudinal axis (**Figure 6**). These results are supported by some previous studies (*21*, *23*) which demonstrated that the concentration of ascorbic acid in the proximity of the peel is higher than that in the center of the fruit for apples and oranges. A possible justification is based on the fact that younger cells capable of producing ascorbic acid are located in the outer cortex tissue (*24*). **Figure 6** also shows that ascorbic acid is present at lower amounts at the stem of the oranges. This has also been observed for potatoes (*6*) and oranges (*20*), but we have no definite response to explain this distribution profile.

2. There is a noticeable difference in ascorbic acid concentration if a comparison is done between mature and immature fruits.
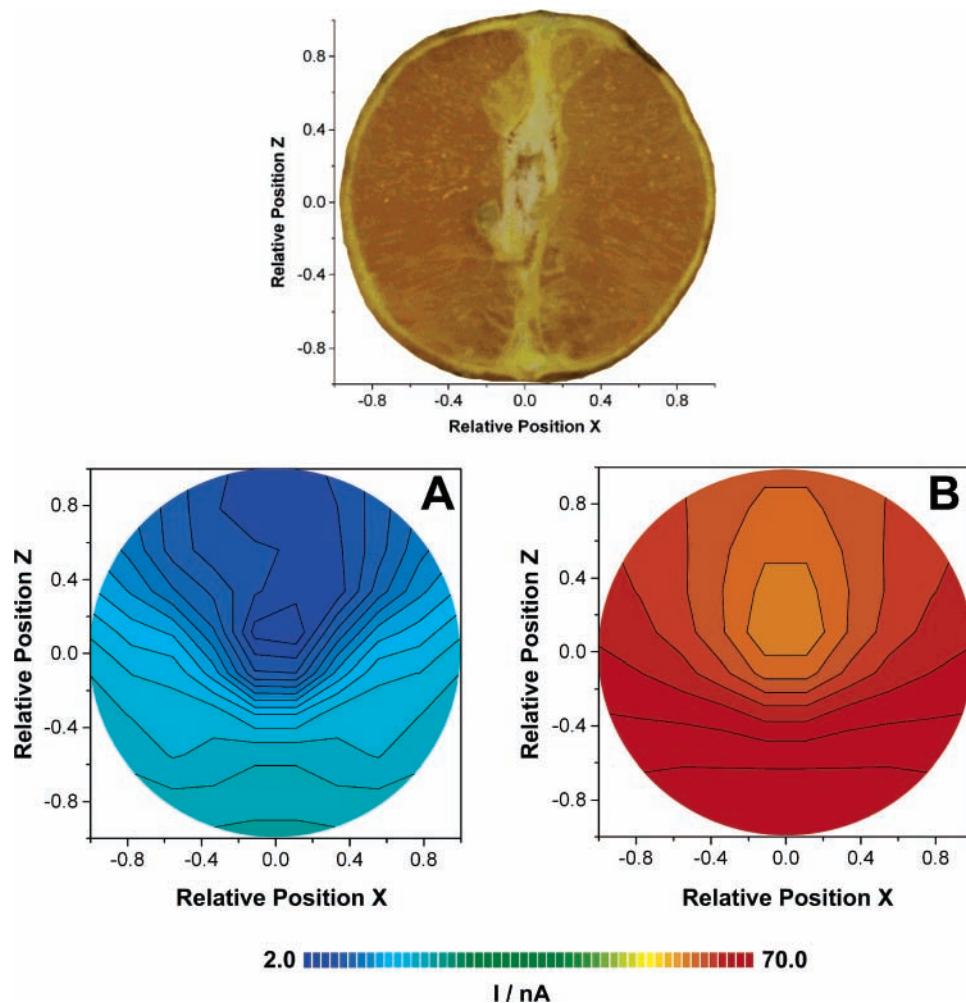
Figure 6. Bidimensional representation of limiting current values as a function of the microelectrode position in oranges (**A**, immature; **B**, mature) cut parallel to the longitudinal axis. Other conditions were as in **Figure 5**.

Even though there is some disagreement in the literature on this subject (*25*), results shown in **Figures 5** and **6** are very convincing and confirm that higher concentrations of ascorbic acid are found in mature fruits. Curiously enough, by looking at **Figure 6** it is possible to conclude that the ascorbic acid gradient is more pronounced when the fruit is immature; that is, ripening favors a more homogeneous distribution of ascorbic acid in the fruit.

The ascorbic acid content in other fruits was also evaluated by in-situ measurements of limiting current with the microelectrode sensor. By taking into account the data shown in **Table 3**, it is possible to conclude that higher concentrations of ascorbic acid are found in guava. This table also presents information on the concentration of ascorbic acid in orange samples. The presented values were calculated by using the calibration plot obtained with an orange juice and considering that the density of an orange juice at 11.8 °Brix is 1.044 g cm^3 (*26*) (to convert concentration to milligrams of ascorbic acid per 100 g of orange juice). The results obtained are in good agreement with those reported in the literature (*22, 27*).

To validate our measurements, the ascorbic acid concentration in an orange juice was determined with the microelectrode sensor, and the result was compared with the one obtained from the use of a standard coulometric procedure [titration of ascorbic acid with electrogenerated iodine (*17*)]. The good agreement between results obtained with the use of the proposed voltammetric method (30.9 ± 0.9 mg of ascorbic acid/100 g of orange juice) and the standard method (29 ± 1 mg of ascorbic acid/

Table 3. Determination of Ascorbic Acid Concentration in Different Fruits[a]

fruit	limiting current in situ, nA	concentration, mg 100 g^{-1}
oranges		
1	36.5	32.3
2	31.5	27.9
3	30.2	26.7
4	70.0	61.9
5	22.2	19.6
tomato	5.2	
guava	58.5	

[a] Literature values for ascorbic acid concentration in fruits (*22, 25*): orange, 30–90 mg 100 g^{-1}; tomato, 17 mg 100 g^{-1}; and guava, 100–430 mg 100 g^{-1}.

100 g of orange juice) confirms the effectiveness of the microelectrode sensor as a practical device to monitor the ascorbic acid concentration in orange samples. Further investigations will be directed to establish a correlation between limiting current signals and ascorbic acid concentration in other fruits and vegetables having compositions that are not essentially liquid as is the case of oranges.

In conclusion, a positionable platinum disk microelectrode has been proved to be a powerful tool to spatially resolve ascorbic acid measurements in fruits and vegetables. The results of our measurements correlate well with those obtained independently with a standard procedure and with the expectations on

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the ascorbic acid distribution in mature and immature oranges. The long-term stability of the device, the repeatability of the measurements, and the good correlation of the results with those obtained by using an independent method indicate that this device may be used as a practical sensor for ascorbic acid in horticultural practice and industry. Furthermore, the ability to monitor in real time and with high precision the distribution of ascorbic acid at a cellular level may be valuable in studies involving the participation of this compound in metabolic processes.

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